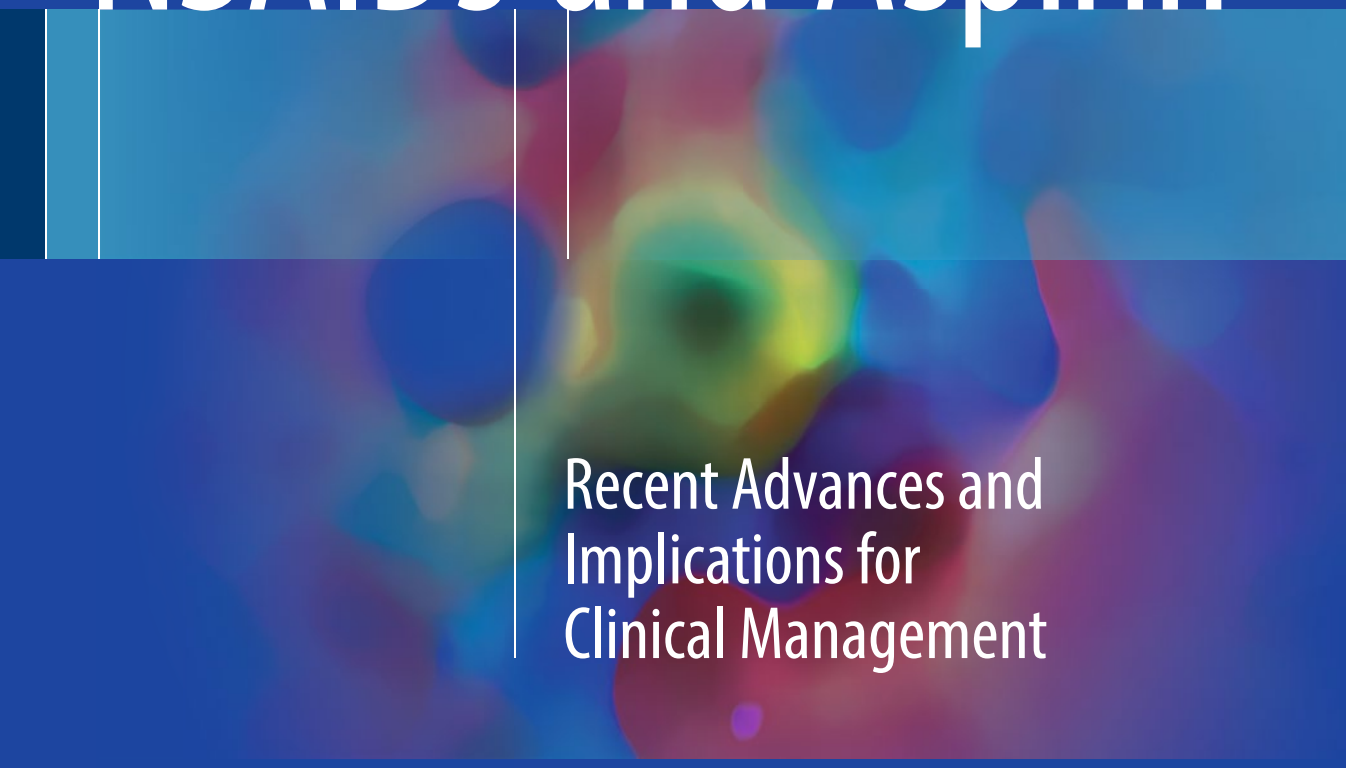
Angel Lanas
Editor

NSAIDs and Aspirin



Recent Advances and Implications for Clinical Management

Springer

NSAIDs and Aspirin

Angel Lanas
Editor

NSAIDs and Aspirin

Recent Advances and Implications for Clinical Management

Springer

Editor
Angel Lanas
Universidad de Zaragoza
IIS Aragón, CIBER Enfermedades Hepáicas
y Digestivas (CIBERehd)
Zaragoza, Spain

Service of Digestive Diseases
University Hospital Lozano Blesa
Zaragoza, Spain

ISBN 978-3-319-33887-3 ISBN 978-3-319-33889-7 (eBook)
DOI 10.1007/978-3-319-33889-7

Library of Congress Control Number: 2016945854

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

Preface

NSAIDs are one of the most widely prescribed drugs around the world to treat pain and inflammation. Prescribers of these drugs include a wide range of medical specialties, including general practitioners, rheumatologists, oncologists, orthopedists, and trauma and internal medicine specialists. Gastroenterologists have a special interest on these compounds based on the gastrointestinal adverse events derived from their use and the recent data on colorectal cancer prevention with aspirin and NSAIDs.

The field has underwent great changes and outstanding new advances in the last 10 years, which have changed the prescription habits, guidelines, and new restrictions and recommendations made by international regulatory agencies such as the FDA or the EMA. The knowledge and advances have been produced essentially since the development of the new class of drugs that inhibited selectively the COX-2 isozyme. The clinical use of these new compounds uncovered adverse effects that had been hidden to the eyes of investigators. In this way, today we know that the use of either COX-2 selective inhibitors or traditional NSAIDs is associated with adverse events not only from the upper GI tract but from the lower GI tract and the CV system. Advances on this area have proved that COX-1 and COX-2 products are involved not only in pain and inflammation but in cancer development as well. In fact, most outstanding advances in the field where discovered when these drugs were tested to prevent gastrointestinal cancer. These advances and knowledge cannot be separated today from the effects of aspirin on the cardiovascular system and on cancer prevention and treatment. In addition aspirin is still being used for the short-term treatment of cold, fever, and pain.

This book provides a comprehensive state-of-the art review of all these aspects and will serve as reference book for the clinician and those who look for an update and summary of the recent advances of the field in the last 10 years. The book will provide practical recommendations for a safe prescription of NSAID based on the most recent knowledge. I expect these recommendations will last for some time since no new relevant advances are expected on this topic in the next few years. The book includes also chapters specifically focused on aspirin and the cardiovascular system and cancer, hot topics that are evolving rapidly in the last few years and that are closely linked to the NSAID field and cannot be left out in a book of this type. In fact, the last part of this book is dedicated to the impact of NSAIDs and especially

of aspirin on cancer prevention and treatment. This makes the book comprehensive in most aspects related with NSAIDs and aspirin use and goes from basic science to practical clinical recommendations.

All chapters have been written by worldwide renowned and outstanding specialists in the field. All authors had investigated extensively on this area and have a tremendous clinical experience. I had had the privilege of working with most of them and/or had scientific discussion and interaction with all of them for years. Here, I need to express my most sincere gratitude to them for being able to write these chapters despite of having very busy agendas. I am in debt with them and hope their work will be recompensed by the interest the book will arouse among the readers.

Zaragoza, Spain Angel Lanas

Acknowledgments

The editor needs to express here his most sincere gratitude to all authors for writing exceptional chapters and all the work done to make this book possible. In particular to Sara Calatayud, Juan Vicente Esplugues, Bibiana Rius, and Joan Claría for the first section of the book on pharmacology and mechanisms of action of NSAIDs. To Marc Hochberg, Lee Simon, Sunny H. Wong, Francis K.L. Chan, Carmelo Scarpignato, Corrado Blandizzi, Mohammad Yaghoobi, and Richard Hunt for the section on clinical effects and safety of NSAID treatments. To Karsten Schrör, Michael Voelker, Dennis McCarthy, Ruben Casado-Arroyo, Pedro Brugada, Katsunori Iijima, Sara Wood, and Charles Hennekens for the section on pharmacodynamics, pharmacokinetics, and all clinical aspects related with aspirin use. Finally, my most sincere thanks to Anna Lisa Bruno, Melania Dovizio, Paola Patrignani, Elena Piazuelo, Paul J. Lochhead, Andrew T. Chan, Elizabeth Half, Ahmad Fokra, and Nadir Arber for their exceptional work done on the impact and effects of NSAIDs and aspirin on colorectal cancer prevention and treatment.

Contents

Contributors

Nadir Arber, M.D., M.Sc., M.H.A. Department of Gastroenterology, Integrated Cancer Prevention Center, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Integrated Cancer Prevention Center, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Corrado Blandizzi, M.D., Ph.D. Division of Pharmacology, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

Pedro Brugada, M.D., Ph.D. Cardiovascular Division, Heart Rhythm Management Center, Cardiovascular Center, Free University of Brussels (UZ Brussels) VUB, Brussels, Belgium

Annalisa Bruno, M.D., Ph.D. Department of Neuroscience, Imaging and Clinical Science, Section of Cardiovascular and Pharmacological Sciences, School of Medicine, CeSI-MeT, "G. d'Annunzio" University, Chieti, Italy

Sara Calatayud, Ph.D. Department of Pharmacology and CIBERehd, Faculty of Medicine, University of Valencia, Valencia, Spain

Ruben Casado-Arroyo, M.D., Ph.D. Department of Cardiology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium

Andrew T. Chan, M.D., M.P.H. Clinical and Translational Epidemiology Unit, Massachusetts General Hospital, and Harvard Medical School, Boston, MA, USA

Division of Gastroenterology, Massachusetts General Hospital, Boston, MA, USA

Channing Division of Network Medicine, Department of Medicine, Harvard Medical School, Boston, MA, USA

Francis K.L. Chan, M.D., D.Sc. Institute of Digestive Disease, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Science, and Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China

Joan Clària, Ph.D. Department of Biochemistry and Molecular Genetics, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona University School of Medicine, Barcelona, Spain

Melania Dovizio, M.D., Ph.D. Department of Neuroscience, Imaging and Clinical Science, Section of Cardiovascular and Pharmacological Science and CeSI-MeT, School of Medicine, G. d'Annunzio University, Chieti, Italy

Juan Vicente Esplugues, M.D., Ph.D. Department of Pharmacology and CIBERehd, Faculty of Medicine, University of Valencia, Valencia, Spain

Ahmad Fokra, M.D. Integrated Cancer Prevention Center, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

Elizabeth Half, M.D. Institute of Gastroenterology, Rambam Health Care Campus, Haifa, Israel

Charles H. Hennekens, M.D., Dr.P.H. Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL, USA

Marc C. Hochberg, M.D., M.P.H., M.A.C.P., M.A.C.R. Department of Medicine, University of Maryland School of Medicine, Baltimore, MD, USA

Richard H. Hunt, F.R.C.P., F.R.C.P.Ed., F.R.C.P.C. Division of Gastroenterology and Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada

Katsunori Iijima, M.D. Department of Gastroenterology, Akita University Graduate School of Medicine, Akita, Japan

Angel Lanas, M.D., Ph.D., A.G.A.F., A.C.G.F. Universidad de Zaragoza. IIS Aragón, CIBER Enfermedades Hepáticas y Digestivas (CIBERehd), Zaragoza, Spain

Service of Digestive Diseases, University Hospital Lozano Blesa, Zaragoza, Spain

Paul J. Lochhead, M.B.Ch.B., Ph.D. Clinical and Translational Epidemiology Unit, Division of Medicine, and Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA, USA

Harvard Medical School, Boston, MA, USA

Denis M. McCarthy, M.D., Ph.D., F.A.C.P., F.R.C.P.I. University of New Mexico School of Medicine, & Veteran's Administration Medical Center-111, Albuquerque, NM, USA

Paola Patrignani, Ph.D. Department of Neuroscience, Imaging and Clinical Science, Section of Cardiovascular and Pharmacological Sciences and CeSI-MeT, "G. d'Annunzio" University School of Medicine, Chieti, Italy

Elena Piazuelo, M.D., Ph.D. Instituto Aragones de Ciencias de la Salud, Zaragoza, Spain

IIS Aragón, Zaragoza, CIBER Enfermedades Hepáticas y Digestivas (CIBERehd), Universidad de Zaragoza, Zaragoza, Spain

Bibiana Rius, M.D. Department of Biochemistry and Molecular Genetics, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona University School of Medicine, Barcelona, Spain

Carmelo Scarpignato, M.D., D.Sc., Pharm.D., M.P.H. Clinical Pharmacology and Digestive Pathophysiology Unit, Department of Clinical and Experimental Medicine, Maggiore University Hospital, University of Parma, Cattani Pavillon, Parma Italy

Karsten Schrör, M.D. Institut für Pharmakologie und Klinische Pharmakologie, Universitäts Klinikum, Heinrich-Heine Universität Düsseldorf, Düsseldorf, Germany

Lee S. Simon, M.D., F.A.C.P., F.A.C.R. SDG LLC, Cambridge, MA, USA

Michael Voelker, M.D. Bayer AG, Consumer Health Division, Global Medical Affairs, Leverkusen, Germany

Sunny H. Wong, M.B.Ch.B. (Hons.), D.Phil. Institute of Digestive Disease, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Science, and Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, Hong Kong, China

Sarah K. Wood, M.D. Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL, USA

Mohammad Yaghoobi, M.D., M.S. (Epi.), A.F.S. Division of Gastroenterology, McMaster University, Hamilton, ON, Canada

Abbreviations

3′UTR	3′ Untranslated region
4E-BP1	4E-Binding protein 1
ACCEPT	Assessing the Cardiovascular Risk Between CElecoxib and Nonselective Nonsteroidal Antiinflammatory Drugs in Patients With Rheumatoid Arthritis and Osteoarthritis Trial
ACP	American College of Physicians
ACS	Acute coronary syndrome
AF	Atrial fibrillation
AHA	American Heart Association
AKF	Acute kidney failure
AMA	American Medical Association
AMI	Acute myocardial infarction
APC	Adenoma Prevention with Celecoxib
APPROVe	Adenomatous Polyp Prevention on Vioxx™
APTC	Antiplatelet Trialists' Collaboration
AS	Ankylosing sponsilytis
DVT	Deep vein thrombosis
AAA	Aspirin for Asymptomatic Atherosclerosis
ACE	Angiotensin-converting enzyme
AChEI-NSAIDs	NSAID prodrugs with acetylcholinesterase inhibitory activity
ACF	Aberrant crypt foci
ADR	Adverse drug reaction
AE	Adverse event
AF	Atrial fibrillation
AFPPS	Aspirin/Folate Polyp Prevention Study
AMPK	AMP activated protein kinase
APACC	Association pour la Prevention par l'Aspirine du Cancer Colorectal
APC	Adenoma prevention with celecoxib (trial)
APHS	Aspirin analog 2-(acetoxy-phenyl)hept-2-ynyl sulfide

APPROVe	Adenomatous Polyp Prevention on Vioxx
ARRIVE	Aspirin to Reduce Risk of Initial Vascular Events
AS	Ankylosing spondylitis
ASA	Acetyl Salicylic Acid
ASPREE	Aspirin in Reducing Events in the Elderly
ATC	Antithrombotic Trialists' Collaboration
ATL	Aspirin-triggered lipoxin
AU	Adenylate- and uridylate
AUC	Area under the plasma-concentration time curve
BASDAI	Bath Ankylosing Spondylitis Disease Activity Index
BASFI	Bath Ankylosing Spondylitis Functional Index
BDAT	British Doctors Aspirin Trial
CaPP1	Colorectal Adenoma/Carcinoma Prevention Programme 1
CABG	Coronary artery bypass graft
cAMP	Cyclic AMP
CAST	Chinese Acute Stroke Trial
cGMP	Cyclic guanosine monophosphate
CHD	Coronary heart disease
CHF	Congestive heart failure
CHM	Commission on Human Medicines (UK)
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
CINOD	COX-inhibiting NO donators
CLASS	Celecoxib Long-term Arthritis Safety Study
CNT	Coxib and Traditional NSAID Trialist
C_{max}	Maximum plasma concentration
COGENT	Clopidogrel and Optimization of Gastrointestinal Events Trial
CONDOR	Celecoxib versus omeprazole and diclofenac in patients with osteoarthritis and rheumatoid arthritis (study)
COX	Cyclooxygenase
COXIB	COX-2 inhibitor
cPGES	Cytosolic PGE synthase
CRC	Colorectal cancer
CV	Cardiovascular
CVD	Cardiovascular disease
CSC	Cancer stem cells
DARE	Database of Abstracts of Reviews of Effects
DFMO	Difluoromethylornithine
DVT	Deep vein thrombosis
EDGE	Etoricoxib versus Diclofenac sodium Gastrointestinal Tolerability and Effectiveness trial
EET	Epoxyeicosatrienoic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
EMA	European Medicines Agency

EMT	Epithelial-mesenkymal transition
eNOS	Endothelial NO-synthase activity
EPA	Eicosapentaenoic acid
ERK	Extracellular signal-regulated kinase
ESCEO	European Society for Clinical and Economic Outcomes in Osteoarthritis
ETDRS	Early Treatment Diabetic Retinopathy Study
FAP	Familial adenomatous polyposis
GCS	Glucocorticoids
GI	Gastrointestinal
GP	Glycoprotein
GPCR	G-protein coupled receptor
GST	Glutathione S-transferase
GLUT4	Glucose transporter 4
HEAT	Helicobacter Eradication Aspirin Trial
5-HETE	5(*S*)-Hydroxyeicosatetraenoic acid
HO-1	Hemoxygenase-1
H-PGDS	Hematopoietic PGDS
HOT	Hypertension Optimal Treatment (study)
HTA	Health Technology Assessment
HTRA	Histamine-2 receptor antagonist
HTS	Hydrogen sulfide
HuR	Human antigen R
IFN	Interferon
ICAM-1	Intracellular adhesion molecule-1
IL	Interleukin
ISIS	International Study of Infarct Survival (trial)
IST	International Stroke Trial
JAPAD	Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes
KD	Knockdown
KO	Knockout
LC-MS/MC	Liquid chromatography-mass spectrometry
LDA	Low-dose aspirin
LPDGS	Lipocalin PGD synthase
LPS	Lipopolysacharide
LS	Lynch syndrome
LT	Leukotrienes
LTB_4	Leukotriene B_4
LX	Lipoxin
MAP	MYH-associated polyposis
MAPK	Mitogen-activated protein kinase
MEDAL	Multinational Etoricoxib Versus Diclofenac Arthritis Long-Term
MHRI	Medicines and Healthcare products Regulatory Agency (UK)
MI	Myocardial infarction

miRNA	microRNA
MRP4	Multidrug resistance-associated protein 4
mTOR	Mammalian target of rapamycin
NANSAID	Nonaspirin nonsteroidal anti-inflammatory drug
NF-kB	Nuclear factor-kB
NDGA	Nordihydroguaretic acid
NHS	Nurses' Health Study
NNT	Number needed to treat
NO	Nitric oxide
NQO	NDA(P)H:quinine oxireductase
NSAID	Nonsteroidal anti-inflammatory drug
NVAF	Nonvalvular AF
OA	Osteroarthritis
OR	Odds ratio
OTC	Over-the-counter
PAR	Protease-activated receptor
PC-NSAIDs	NSAIDs associated with phophatidylcholine
PDGF	Platelet-derived growth factor
PE	Pulmonary embolism
PG	Prostaglandin
PGEM	Prostaglandin E metabolite
PGES	Enzyme PGE synthase
PGHS	Prostaglandin endoperoxide synthase
PGI_2	Prostacyclin
PGIS	PGI-synthase
PHS	Physicians' Health Study
PIFA	Platinum-induced fatty acid
PIK3CA	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PKC	Protein kinase C

Part I

Pharmacology and Mechanisms

Chemistry, Pharmacodynamics, and Pharmacokinetics of NSAIDs

1

Sara Calatayud and Juan Vicente Esplugues

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) constitute a heterogeneous group of drugs used to treat inflammation, pain, and fever. Despite substantial differences in their chemical structure, they present a common mechanism of action consisting of the inhibition of the enzymes responsible for prostanoid synthesis, namely, cyclooxygenases (COX). However, since they are so heterogeneous, they exert other actions that can condition their therapeutic value.

Chemistry

NSAIDs are organized in subgroups according to their parental chemical structure (Table 1.1, Fig. 1.1). Most are organic acids with relatively low p*K*a (Table 1.2), and this acidic nature influences their pharmacodynamic and pharmacokinetic profiles (see below). The exceptions to this rule are paracetamol and pyrazolic derivatives (metamizole, propyphenazone), which are often excluded from the NSAID group because of their low anti-inflammatory activity, and also the diaryl heterocyclic compounds (coxibs).

S. Calatayud, Ph.D. (✉) • J.V. Esplugues, M.D., Ph.D.
Department of Pharmacology and CIBERehd, Faculty of Medicine, University of Valencia, Av. Blasco Ibáñez, 17, 46010 Valencia, Spain
e-mail: sara.calatayud@uv.es; juan.v.esplugues@uv.es

Pharmacodynamics

The therapeutic effects of NSAIDs are mediated chiefly through the inhibition of prostaglandin synthesis. Prostanoids are formed enzymatically through prostaglandin–endoperoxide synthases 1 and 2 (PGHS-1 and PGHS-2), which are also known as cyclooxygenases 1 and 2 (COX-1 and COX-2). PGHSs catalyze two different reactions at two sites that are physically distinct but functionally linked. The cyclooxygenase reaction provokes a bisoxygenation of arachidonic acid to generate prostaglandin G2 (PGG2), which is then transformed into PGH2 through a peroxidase reaction. These unstable intermediates are quickly transformed into different prostaglandins, prostacyclins, and thromboxanes by specific synthases. A major limiting factor of prostanoid formation is the availability of the substrate arachidonic acid, and this constraint usually determines a low rate of basal prostanoid formation. However, this synthetic pathway is greatly enhanced when phospholipase A2 is activated to release arachidonic acid from phospholipids (Fig. 1.2).

This arachidonic acid cascade is of great importance in inflammation, pain, and fever. Prostanoid synthesis is significantly elevated in inflamed tissues, where PGE2 and prostacyclin (PGI2) contribute to this response by increasing local blood flow, vascular permeability, and leukocyte infiltration [1–3]. These prostanoids also

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_1

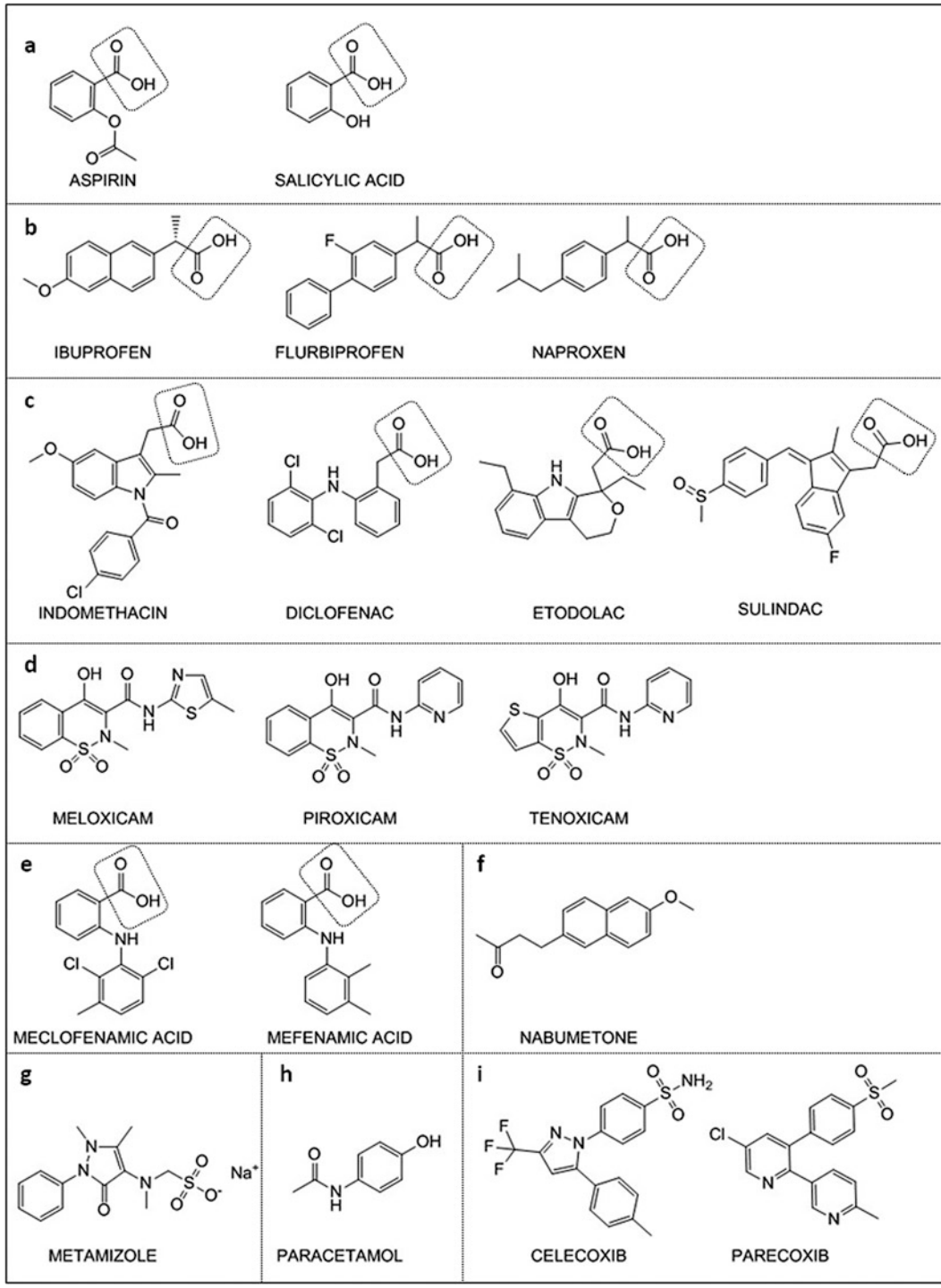


Fig. 1.1 Chemical structures of some NSAIDs including representative salicylates (**a**), propionates (**b**), acetic acid derivatives (**c**), enolic acid derivatives (oxicams) (**d**), fenamates (**e**), nabumetone (**f**), metamizole (or dipyrone) (**g**), paracetamol (or acetaminophen) (**h**), and diaryl heterocyclic compounds (coxibs) (I). Note the general presence of a carboxylic acid moiety in groups (**a–c**, **e**; *dashed rectangle*). It is also present in the nabumetone active metabolite (not represented)

Table 1.1 Classification of NSAIDs according to their parental chemical structure

Derivatives of	
Salicylic acid	Aspirin
	Diflunisal
Propionic acid	Ibuprofen
	Flurbiprofen
	Ketoprofen
	Naproxen
Acetic acid	Indomethacin
	Diclofenac
	Aceclofenac
	Etodolac
	Ketorolac
Enolic acid	Piroxicam
	Tenoxicam
	Meloxicam
	Lornoxicam
	Phenylbutazone
Fenamic acid	Mefenamic acid
	Meclofenamic acid
Alkanones	Nabumetone
Para-aminophenol	Acetaminophen or paracetamol
Pyrazole	Metamizole or dipyrone
	Propyphenazone
Diaryl heterocyclic compounds	Celecoxib
	Valdecoxib
	Rofecoxib
	Etoricoxib

Table 1.2 Values of p*K*a and logP of several NSAIDs

Drug	p*K*a	logP
Aspirin	3.49	1.19
Meloxicam	4.08	3.43
Diclofenac	4.15	4.51
Naproxen	4.15	3.18
Mefenamic acid	4.20	5.12
Parecoxib	4.24	3.51
Flurbiprofen	4.42	4.16
Indomethacin	4.50	4.27
Ketoprofen	4.45	3.12
Etodolac	4.65	2.50
Sulindac	4.70	3.42
Ibuprofen	4.91	3.97
Piroxicam	6.30	3.06
Paracetamol	9.38	0.46
Valdecoxib	10.06	2.82
Celecoxib	10.70	4.01

p*K*a: acid dissociation constant (values $\underline{1/\alpha}$ acidity); logP: octanol–water partition coefficient (values $\underline{\alpha}$ hydrophobicity) (Data obtained from PubChem and DrugBank)

cause peripheral sensitization by reducing the threshold of peripheral nociceptors, while PGE2 and other prostaglandins induce central nociceptive sensitization at the spinal dorsal horn neurons [4, 5]. Finally, PGE2 acts at the hypothalamus to increase body temperature by increasing heat production and reducing heat loss [1, 6]. Likewise, inhibition of prostanoid synthesis by NSAIDs is responsible for undesired side effects such as gastrointestinal and renal toxicities, since prostanoids are physiological regulators of gastrointestinal mucosal defense and renal homeostasis.

A key event in the evolution of this pharmacological group was the discovery and characterization of COX-2 [7–9] (Fig. 1.3). Unlike COX-1, which is constitutively expressed in most cells and responsible for many of the housekeeping functions mediated by prostanoids, COX-2 is expressed constitutively in a small number of tissues. However, it is induced in response to an extremely wide range of agonists that include cytokines and tumor promoters, which endows this isoenzyme with a significant role in inflammation and perhaps also in cancer. This paradigm has motivated an avid search for drugs capable of inhibiting "pathological" COX-2 activity while preserving the "physiological" COX-1 function [10]. However, later evidence has argued in favor of a mixed pattern in which both isoenzymes are involved in homeostasis and also in inflammatory processes. It seems that COX-1 activity contributes to inflammation in the early phases, until COX-2 is upregulated and takes up its role as a motor of the synthesis of pro-inflammatory prostanoids [1, 2, 11]. Both isoenzymes are also expressed in the spinal cord and mediate nociceptive stimuli [1, 12], while hyperthermia seems to depend mainly on COX-2 activity [1, 6]. On the other hand, besides the clear role of COX-1-derived prostanoids in the digestive mucosal barrier, renal homeostasis, and platelet aggregation, accumulating evidence indicates that

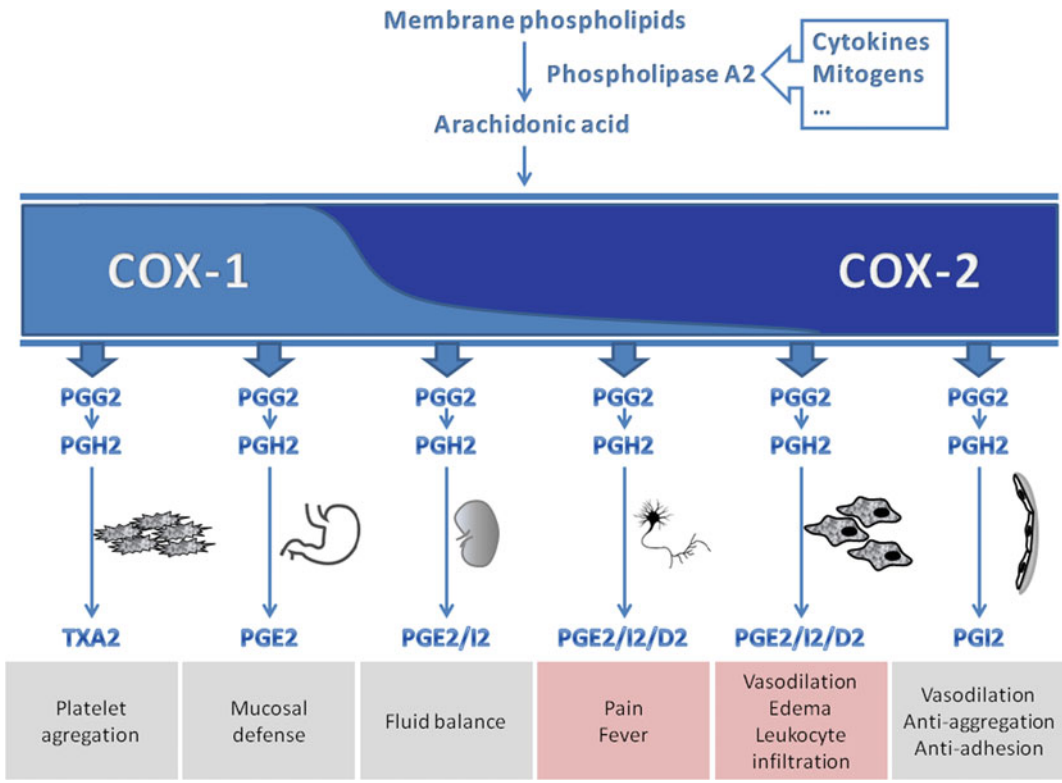


Fig. 1.2 Overview of the prostaglandin synthetic pathways with the relative contribution of COX-1 and COX-2 to different physiological and pathological functions

COX-2-dependent prostanoids formed in endothelial cells and the kidney counteract the effects of prothrombotic and atherogenic stimuli and contribute to arterial pressure homeostasis [13]. In addition to specific new COX-2 drugs, the selectivity for COX-1/COX-2 of the older NSAIDs has been reevaluated in order to understand differences in pharmacodynamic profiles and side effects.

Nonselective vs. Isoform-Specific COX Inhibitors

As previously explained, NSAIDs are usually subdivided into two classes:

- Classic or "nonselective" NSAIDs: inhibit both COX-1 and COX-2, with varied potencies on each isoenzyme
- COX-2-selective or "isoform-specific" NSAID inhibitors: designed to be more selective against COX-2

However, this classification is questionable, since COX-2 selectivity is a continuous variable rather than an absolute category, and all NSAIDs can inhibit both isoenzymes to some extent.

COX-1/COX-2 selectivity in vivo is predicted according to ex vivo assays performed in human whole blood (platelet COX-1 and macrophage COX-2) or in a combination of human whole blood and human lung cancer cells (as a consistent source of COX-2). These assays provide an estimate of potency and selectivity of inhibition of the two COX enzymes in a setting that takes into account the binding of NSAIDs to plasma proteins. Table 1.3 lists some IC80 values for several NSAIDs obtained in a broad comparative analysis using the aforementioned assay

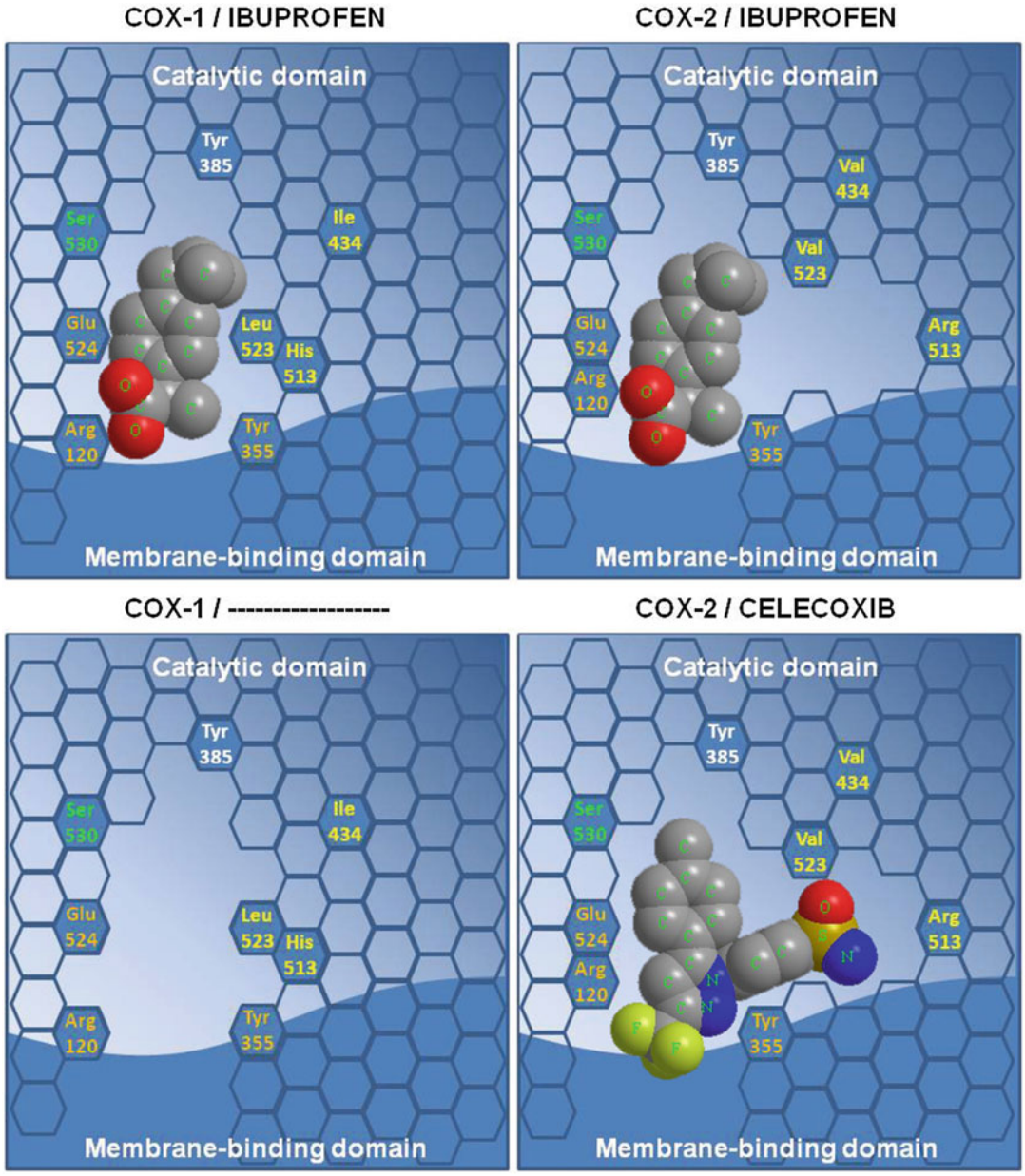


Fig. 1.3 Schematic diagram of the active site in COX-1 and COX-2 isoenzymes and the interaction with a nonselective (ibuprofen, *upper panel*) and a COX-2-selective (celecoxib, *bottom panel*) NSAID. Some key residues are shown: Arg120 stabilizes the carboxylate group that is present in most NSAIDs; Tyr385 is a highly conserved residue situated in the upper part of the largely hydrophobic binding channel that accommodates the aromatic ring structures; Leu523 in COX-1 is changed to Val523 in COX2, which allows the opening of a side pocket and accommodation of the bulkier diaryl heterocyclic compounds; His513 is changed to Arg513 in COX-2, which stabilizes the sulfonamide or methyl sulfone group of these COX-2 inhibitors by hydrogen bonding; Ile434 in COX-1 is changed to Val434 in COX-2, which allows a more stable binding of COX-2-selective inhibitors; and Ser530 is the residue that is acetylated by aspirin

Table 1.3 Ratio between the concentrations of drug inhibiting 80 % of COX-2 and COX-1 activities (IC80) determined in vitro for several NSAIDs (values >1 indicate a higher selectivity for COX-1; values <1 indicate a higher selectivity for COX-2)

Compound	IC80 ratio COX-2/COX-1
Ketorolac	294.00
Flurbiprofen	51.00
Ketoprofen	6.00
Indomethacin	4.30
Aspirin	3.80
Naproxen	3.00
Ibuprofen	2.60
Fenoprofen	1.00
Sodium salicylate	0.92
Diflunisal	0.75
Piroxicam	0.47
Meclofenamate	0.30
Sulindac sulfide	0.29
Diclofenac	0.23
Celecoxib	0.11
Meloxicam	0.09
Rofecoxib	0.05
Etodolac	0.04

Data from Warner et al. (1999) [14]

combining whole blood and cancer cells. IC80 was considered more appropriate than IC50 for comparisons, because normal plasma levels of NSAIDs are in the range of concentrations that produce 80 % inhibition of COX enzymes in this assay [14]. These numbers illustrate that some of the so-called traditional NSAIDs exert a COX-2-selective inhibition similar to that of coxibs, which are the prototypical COX-2-selective drugs. Although in vitro data varies from one study to another, a global analysis combined with information regarding therapeutic plasma levels and half-lives of the individual agents provides an estimable predictor of the pharmacodynamic and toxicodynamic effects of specific compounds.

A special case is that of paracetamol. This drug induces a redox-sensitive blockade of COX activities that is inhibited by the high extracellular concentrations of arachidonic acid and peroxide at the sites of inflammation [15, 16]. This pharmacodynamic particularity, together with the low concentrations of paracetamol observed in inflamed tissues (see below), justify its lack of anti-inflammatory activity.

Combination of structural, functional, and kinetic investigations outlines different patterns of interaction and expands the concept of COX-2 selectivity as a direct consequence of particular structural features (just a matter of size). By considering other influencing factors, such as kinetics and allosterism, the mechanisms of binding to COX-1 and COX-2 by different NSAIDs have been shown to be as impressively diverse as is explained in the following sections.

Structural Factors

COX-1 and COX-2 are dimers of 70 kDa subunits. The homodimer is membrane-bound and localized in the lumen of the endoplasmic reticulum and in the nuclear envelope. Although the global identity between COX-1 and COX-2 in a particular species involves around 60 % of the protein, cyclooxygenase active sites present a higher homology (>85 %), which limits the options available for obtaining selective interactions.

Each monomer of COX consists of three structural domains:

- A short N-terminal epidermal growth factor domain
- A membrane-binding domain
- A large, globular C-terminal catalytic domain that constitutes the major part of the COX monomer and is the site of arachidonic acid binding and transformation

As explained previously, arachidonic acid is transformed in two sequential reactions: double dioxygenation, to generate PGG2 in the cyclooxygenase active site, and the reduction of PGG2 to PGH2 at the peroxidase active site. For this reaction, the entrance to the COX active site occurs at the base of the membrane-binding domain and leads to a long hydrophobic channel that extends deep into the interior of the catalytic domain. This hydrophobic channel can be divided into the lobby and the binding site by a constriction of three residues (Arg120, Glu524, and Tyr355)

at the interface between the membrane-binding domain and the catalytic domain [17]. Most inhibitors bind in the COX active site above the constriction residues between the highly conserved residues Arg120 and Tyr385. Arg120 provides a positive charge that binds the negative charges of carboxylic acid substrates and inhibitors, serving in both isoenzymes as an anchor for acidic NSAIDs [10, 18, 19] (Fig. 1.3).

Some differences between COX-1 and COX-2 have been shown to be responsible for the selectivity of some NSAIDs for COX-2 (reviewed in [9, 10, 17]):

- The membrane-binding domain changes the last helix position and the location of Arg120 at the constriction site in COX-2. This change increases the space at the interface between the membrane-binding and catalytic domains, reduces steric and ionic crowding by Arg120, and, as a consequence, enhances the binding of nonacidic NSAIDs to COX-2.
- Above the constriction, the COX-2 active site presents spatial changes that result from changes in some amino acids (Ile523, His513, and Ile434 in COX-1 become Val523, Arg513, and Val434 in COX-2, respectively):
  - Val523 in COX-2 vs. Ile523 in COX-1 → this substitution makes accessible a small side pocket in the catalytic center of COX-2 that accommodates the sulfonamide or sulfone group of the diarylheterocycles celecoxib and rofecoxib. Mutating Val523 to an isoleucine restricts access to this side pocket, and COX-2 is no longer differentially sensitive to these inhibitors. Conversely, when Ile523 is substituted by Val523 in COX-1, this isoenzyme increases its affinity for COX-2-selective inhibitors.
  - Arg513 in COX-2 vs. His513 in COX-1 → alters the chemical environment of the side pocket by endowing it with a stable positive charge at its center. This arginine apparently interacts with polar moieties entering the pocket and contributes somewhat to the COX-2 selectivity of diarylheterocycles. In combination with the change of Ile523 to Val523, mutating His513 to an arginine in COX-1 makes this isoenzyme much more sensitive to COX-2 inhibitors.
  - Val434 in COX-2 vs. Ile434 in COX-1 → this modification in the surroundings of the COX active site allows a more stable binding of selective inhibitors to the COX-2 isoform.

Thus, despite the considerable homology between the two enzymes, this side pocket off the main active site channel, which is more accessible in COX-2 than in COX-1, makes the COX-2 active site approximately 27 % larger and allows the accommodation of bulkier NSAIDs such as diarylheterocycle derivatives. The fundamental structural factors responsible for the potent and selective inhibition of COX-2 of these drugs include (1) two adjacent aromatic rings as a central scaffold and (2) the presence of a sulfonamide or methyl sulfone group on one of the phenyl rings [10, 18].

Kinetic Factors

While all NSAIDs compete with arachidonate for the COX active site, each NSAID can inhibit COX in a time-dependent or time-independent manner, and this may be relevant for drug potency and COX-1/COX-2 selectivity. Time dependency is deduced from experiments which show that (1) the degree of COX inhibition induced by a drug depends on the period of time elapsed between addition of the inhibitor and addition of the substrate and (2) after reducing the concentration of the inhibitor, the recovery of COX activity occurs at a slow, sometimes almost undetectable, rate [20, 21]. According to this, different kinetic patterns have been observed [17, 18]:

i. Rapid and reversible binding followed by covalent modification (time-dependent)
ii. Rapid and reversible competitive inhibition (time-independent)

iii. Rapid and low-affinity competitive inhibition followed by a time-dependent transition to a high-affinity, slowly reversible, inhibitory mode
iv. A mixed pattern involving (ii) and (iii)

Among traditional NSAIDs, we can find prototypes of all kinetic patterns. The only example of the first type of inhibition is **aspirin**. This unique NSAID covalently modifies both COX-1 and COX-2 through acetylation of Ser-530 to inhibit these enzymes in a time-dependent fashion (i) [22]. Aspirin is significantly more potent against COX-1 than against COX-2, but the reason for this difference is unclear. **Ibuprofen and mefenamic acid** are examples of the second pattern (ii), causing a competitive and rapidly reversible COX inhibition through a single-step kinetic mechanism [22, 23].

Several different NSAIDs follow the third kinetic pattern (iii), although their interaction with the active site presents particularities in each case. **Indomethacin** exerts a time-dependent and functionally irreversible inhibition of COX through a two-step binding to COX enzymes. Indomethacin is recovered intact after prolonged incubation with either enzyme, suggesting that the time-dependent inhibition of COX is not caused by a covalent interaction [20, 23]. **Diclofenac's** kinetics are similar to those of indomethacin; however, it binds to the active site of COX-2 in a unique inverted binding mode different to that described for all the other NSAIDs analyzed [24]. A similar slow, tightly binding, time-dependent, functionally irreversible COX inhibition through a two-step mode is observed with **flurbiprofen** and **meclofenamic acid**, despite being structural analogues of ibuprofen and mefenamic acid, respectively [21]. With regard to COX-2-selective diarylheterocycle inhibitors, such as **rofecoxib** and **celecoxib**, a competitive, reversible kinetic with COX-1 is observed. However, with COX-2, these drugs exert an initial two-step competitive and reversible interaction that is followed by a third pseudoirreversible step leading to a tightly bound complex. This process causes a time-dependent inhibition that depends on the penetration of the sulfonamide or methylsulfonyl groups into the abovementioned side pocket. The significant differences between their kinetics on COX-1 and COX-2 seem critical to the selectivity for this isoenzyme [23, 25].

Finally, **naproxen** and some **oxicams** exhibit a "mixed" pattern of inhibition of COX (iv) that combines a quick and reversible blockade with a slow and functionally irreversible inhibition of the enzyme. This "mixed" pattern is observed with COX-2, while COX-1 blockade only presents the time-independent component [23].

A global analysis of these data suggests that tight binding/time dependency usually confers higher potency (with the exception of aspirin, which causes a covalent modification). When kinetics were analyzed in both isoenzymes, drugs exhibiting similar (e.g., ibuprofen and indomethacin) and different (naproxen, oxicams, coxibs) patterns of inactivation of COX-1 and COX-2 were identified, and in some cases this difference has an impact on their selectivity. Finally, the structural basis for time-dependent inhibition is not yet well defined and may vary for different drugs.

Allosteric Factors

Different studies suggest that cyclooxygenases exhibit enzymatic activity at a single COX active site at a given time [20, 26] and function as conformational heterodimers with an allosteric and a catalytic monomer [19]. Thus, the allosteric monomer regulates its catalytic partner by establishing a ligand-dependent cross talk between both monomers.

It was subsequently found that different fatty acids, which may or may not be COX substrates, are allosteric regulators of PGHSs and that a given fatty acid can elicit a stimulatory or inhibitory effect on arachidonic acid oxygenation depending on the COX isoform. Since nonsubstrate fatty acids such as palmitic acid bind preferentially to the allosteric subunit, they are used to determine the monomer targeted by different NSAIDs. These fatty acids interfere

with inhibitors that bind to the allosteric subunit, but have no effect on or even potentiate the actions of inhibitors that bind to the catalytic partner. By means of this kind of analysis, different patterns of NSAID binding to COX-2 heterodimers have again been observed (reviewed in Smith et al. [19]):

- Inhibitors that bind to the catalytic monomer (e.g., celecoxib, rofecoxib, indomethacin, diclofenac, or aspirin). These drugs can cause a complete inhibition by competing with the substrate for the active site in the enzyme (aspirin causes a covalent modification of this subunit).
- Inhibitors that bind to the allosteric monomer (e.g., flurbiprofen, naproxen). They induce an incomplete inhibition of COX.
- Inhibitors that bind to both monomers (e.g., ibuprofen and mefanamate). They can induce a mixed inhibition depending on the dose.

Most of these studies have been performed on COX-2; therefore, whether or not the pattern of interaction described for a particular inhibitor also applies to COX-1 is largely unknown at present.

The significance of COX allosterism remains unresolved, but, apart from its relevance in the mechanism of action of particular NSAIDs, the allosteric regulation of COX activity by common endogenous and dietary fatty acids raises the possibility that both the pathological role of COX and the pharmacological/therapeutic effects of NSAIDs are affected by circumstances that modulate the levels of these lipids (e.g., pathology, diet, etc.).

Thus, NSAIDs inhibit PG synthesis by binding to COX enzymes in many different ways, which is comprehensible considering the structural heterogeneity of this pharmacological group. However, there are still many unanswered questions regarding the mechanism of action of these drugs, even though some of them have been used for several decades (almost 120 years in the case of aspirin). This knowledge is important, as a better understanding of how both, explained and currently unknown factors, affect NSAID activity on COX isoenzymes may help in the future to develop drugs with more specific anti-inflammatory actions with less alteration of the housekeeping roles of prostanoids.

COX-Independent Actions

The significant varieties of molecular structures and pharmacological profiles observed in the NSAID group have driven the search for alternative mechanisms of action that could complement their common inhibitory activity on COX (Table 1.4). One field of research has analyzed the ability of NSAIDs to insert themselves inside the lipid bilayers of biological membranes, describing a range of actions mediated through this mechanism, including putative anti-inflammatory effects such as antioxidant activity or inhibition of phospholipase A2, or side effects like disruption of gastric mucosal barrier or mitochondrial toxicity [27]. Other investigations have focused on the ability of some NSAIDs to modulate transcription factors that control the inducible expression of many genes involved in inflammation, such as nuclear factor-kappa B (NF-kappa B) or AP-1, while other studies describe the ability of some NSAIDs to modulate signaling pathways involved in inflammatory responses, such as the MAPK or PI3k/Akt pathways, or to interact with nuclear receptors that regulate inflammation [28, 29]. Additionally, a variety of NSAIDs seem to inhibit the function of the adhesion molecules responsible for the leukocyte–endothelial cell interactions that initiate the inflammatory focus [30]. Although these alternative mechanisms have been implicated in many particular effects induced by different NSAIDs and observed in varying experimental conditions, there is no consensus with regard to their relative contribution to the anti-inflammatory activity of NSAIDs observed at the concentrations attained with clinically used doses.

Table 1.4 COX-independent direct cellular targets of different NSAIDs

Target	Effect	NSAIDs
Transcription factors	Inhibition of NF-κB	Aspirin
		Salicylate
		Ibuprofen
		Flurbiprofen
		Sulindac sulfide
		Indomethacin
	Inhibition of AP-1	Aspirin
Kinases	Inhibition of MAPK cascade	Aspirin
		Salicylate
		Sulindac sulfide
		Ibuprofen
		Celecoxib
	Inhibition of PI3k/Akt	Naproxen
		Oxaprozin
Nuclear receptors	Stimulation of PPAR-γ	Ibuprofen
		Indomethacin
	Inhibition of PPAR-δ	Indomethacin
		Sulindac sulfide
Leukocyte-adhesion molecules	Inhibition of L-selectin shedding	Aspirin
		Salicylate
		Ketoprofen
		Diclofenac
		Indomethacin
		Aceclofenac
		Meclofenamic acid
		Mefenamic acid
	Inhibition of ß2-integrin activation	Piroxicam
		Meloxicam
	Inhibition of VLA-4 activation	Diclofenac
		Indomethacin
		Aceclofenac

Pharmacokinetics

Absorption

NSAIDs are generally well absorbed following oral ingestion and present a high bioavailability (80–100 %), although there are some exceptions (e.g., diclofenac, celecoxib). Their absorption is generally quick, and peak plasma concentrations are usually observed within 2–3 h, except for some derivatives of the enolic acid (piroxicam, meloxicam, nabumetone) and certain diaryl heterocyclic compounds (e.g., celecoxib, rofecoxib). Food intake may delay absorption, but rarely decreases systemic availability. Some compounds, such as diclofenac or aspirin, undergo a significant first-pass effect that significantly reduces their bioavailability, while a first-pass metabolism generates the active drug in the case of others, like dipyrone, nabumetone, sulindac or etoricoxib (Table 1.5). When applied topically, NSAIDs' penetration of inflamed tissues and joints appears to be minimal, and detectable concentrations in synovial fluid observed after some topical treatments (i.e., with diclofenac) seem to depend on dermal absorption and systemic circulation.

Distribution

Most NSAIDs are extensively bound to plasma proteins (95–99 %), and this binding may be saturable with a potential for interaction with drugs that compete for the same binding sites. The distribution pattern has a significant impact on the pharmacological actions and side effects of NSAIDs. Most compounds achieve sufficient concentrations in the central nervous system to exert a central analgesic effect, while their kinetics in inflammatory foci seem to be affected by particular physicochemical characteristics, like acidity. Acidic drugs (p*K*a 4–5), including diclofenac, ibuprofen, ketoprofen, or lumiracoxib, seem to accumulate and persist in inflamed tissue, such as in the synovial fluid of inflamed joints (reviewed in Brune and Patrignani [31]). This accumulation may be the consequence of several factors:

- The local acidic microenvironment caused by inflammation facilitates nonionic diffusion of these drugs into the cell interior; once there, the higher intracellular pH causes drug ionization. This process, termed ion trapping, increases the intracellular concentration of the drug.

Table 1.5 Pharmacokinetic data of common NSAIDs

Drug	Bioavailability (oral, %)	First-pass	Peak C_p (*h*)	Protein binding	$t_{1/2}$ (*h*)	Active metabolites	Urinary excretion (%)
Salicylates							
Aspirin	>80	+++	0.4	80–90	0.25–0.3	Salicylic acid	
Salicylic acid	100	–	1–2	95	2–3[a]		2–30
Diflunisal	90		2–3	99	8–12		
Para-aminophenol derivative							
Paracetamol	75–90	+	0.5–1	20–50	2[b]		
Pyrazolones							
Dipyrone	>90[c]	+++	0.5–1	15	2–4[c]	4-Methyl-amino-antipyrine	5
				58[c]		Amino-antipyrine	
Propyphenazone	>90		1–3	10–20	1–1.5		0.6
Acetic acid derivatives							
Indomethacin	90–100	±	1–2	99	1–6		20
Diclofenac	54	+++	2–3	99	1–2		<1
Aceclofenac	100	–	1.25–3	99	4–5		
Etodolac			1	99	7		
Ketorolac	80–100	±	0.5–1	99	4–6[d]		5–10
Sulindac	90		1–2	90	7	Sulindac sulfide	
			8[e]		18[e]		
Propionic acid derivatives							
Ibuprofen	>80	–	0.25–0.5	99	2–4		<1
Naproxen	99	±	1	99[f]	14		10
Fenoprofen	85		2	99	2		
Flurbiprofen	92	–	1–2	99	6		2
Ketoprofen	100	±	1–2	98	2		
Fenamates							
Mefenamic acid	>90	–	2–4	high	3–4		<6
Meclofenamate			0.5–2	99	2–3		
Enolic acid derivatives							
Meloxicam	89	11	5–10	90	15–20		<1
Piroxicam	100	–	3–5	99	45–50		<5
Alcanones							
Nabumetone	35[g]		2–8[g]	99[g]	24[g]	6-Methoxy-2-naphthylacetic acid (6MNA)[h]	
Diaryl heterocyclic derivatives							
Celecoxib	22–40		2–3	97	8–12[i]		<1
Etoricoxib	100		1	92	22		<2
Parecoxib				98[h]	0.3	Valdecoxib	<5[h]
					8–11[h]		

Peak C_p: time to peak plasma drug concentration; urinary excretion: as unaltered drug
[a]Dose-dependent half-life
[b]Half-life increases in liver disease, elderly, and children
[c]As 4-methyl-amino-antipyrine
[d]Half-life increases in elderly, liver, and renal disease
[e]As sulindac sulfide
[f]Increased free fraction and half-life in elderly
[g]As 6-methoxy-2-naphthylacetic acid
[h]Reduced in liver disease
[i]Increased in cases of P450-2C9 genetic polymorphisms, co-treatment with P450-2C9 inhibitors or liver disease
[h]As valdecoxib

- Changes in the hemodynamics of tissue during inflammation, including increased localized blood flow and edema, allows protein-bound and protein-unbound drugs to escape into the tissue.
- The high concentration of albumin in inflamed tissues and synovial fluid retains drugs that present a high affinity for this protein.
- The mildly acidic extracellular pH may reduce their binding to plasma proteins and increase the free fraction of the drug.

These factors would modulate the kinetics in the inflammatory focus, thereby prolonging the therapeutic action of the drug beyond that expected based on analysis of plasma pharmacokinetics. However, ion trapping also results in acidic compounds achieving high concentrations in the stomach wall and kidney, in which blockade of prostanoid synthesis causes the typical organ toxicity elicited by these compounds. Due to their lack of acidic structure, other COX inhibitors, such as dipyrone and paracetamol, are distributed homogenously throughout the body at therapeutic doses and induce analgesia, but induce no or very slight anti-inflammatory effects. This is partly due to their low concentration in inflamed tissues [31].

Elimination

The majority of NSAIDs are cleared from plasma by hepatic biotransformation followed by renal excretion of their metabolites. Renal excretion of active drugs is negligible in most cases (except indomethacin or salicylic acid) (Table 1.5). Some have active metabolites (e.g., nabumetone, sulindac) and nearly all undergo varying degrees of biliary excretion and reabsorption (enterohepatic circulation), a process that seems to contribute to NSAID enteropathy. Some NSAIDs are metabolized by phase I (oxidation, hydroxylation, demethylation) followed by phase II (glucuronidation, other conjugations) mechanisms, while others suffer only phase II reactions. The family of NSAIDs includes drugs with very different half-lives, from 1 to 4 h in the

Table 1.6 Particularities of some NSAIDs

Specific characteristics of individual NSAIDs
Aspirin
• Most widely consumed NSAID
• Used as antiplatelet agent
• Only NSAID that causes irreversible inhibition of COX
• After absorption, quickly hydrolyzed to salicylic acid
• Excretion of salicylates extremely variable: dose- and pH-dependent elimination; reduced in renal disease
Celecoxib
• COX-2 selectivity
• Fewer gastrointestinal side effects
• Increased risk of cardiovascular events
• Metabolism reduced in hepatic impairment
• Inhibitor of CYP2D6
Diclofenac
• Potent NSAID
• Selectivity for COX-2 similar to that of celecoxib
• Short half-life, substantial first-pass effect, accumulation in synovial fluid
Dipyrone
• Prodrug
• Analgesic and antipyretic activity
• Weak anti-inflammatory action
• Some antispasmodic activity
Etodolac
• Some degree of COX-2 selectivity
Etoricoxib
• Long half-life
• Metabolism reduced in hepatic impairment
Ibuprofen
• Most commonly used NSAID (besides aspirin)
• Interferes with the antiplatelet effects of aspirin
Indomethacin
• Potent nonselective COX inhibitor
• Limited use due to its toxicity
• Undergoes enterohepatic cycling
Nabumetone
• Prodrug of 6-methoxy-2-naphthylacetic acid (6MNA)
Oxicams
• Long half-life, allowing once daily dosing
Paracetamol
• Analgesic and antipyretic
• Very weak anti-inflammatory action: probably related to the inhibitory effect of peroxides on its blocking effect on COX and to low levels of paracetamol in the inflammatory focus due to its lack of acidity

(continued)

Table 1.6 (continued)

Specific characteristics of individual NSAIDs
• Low incidence of gastrointestinal side effects
• Severe hepatic damage when overdose
Parecoxib
• Prodrug of valdecoxib
• Inhibitor of CYP2C9 and CYP2C19
• Metabolism reduced in elderly and hepatic impairment
Sulindac
• Prodrug of sulindac sulfide
• Complex pharmacokinetics: metabolized to active drug; extensive enterohepatic circulation

case of ibuprofen, diclofenac, or acetaminophen to 20–60 h in the case of oxicams. COX-2-selective drugs present an intermediate half-life (Table 1.5).

Some authors have hypothesized that the short half-life of acidic compounds confers an advantage in that their rapid disappearance from the central compartment allows the recovery of COX-2 activity in endothelial cells at the end of each dosing interval, while analgesia resulting from COX blockade in the inflamed tissue is continuously inhibited by the accumulated drug. This rationale could also be applied to other tissues and organs in which prostaglandin synthesis exerts a homeostatic action [31, 32].

The clearance of many NSAIDs is reduced in the elderly due to changes in hepatic metabolism. Additionally, older patients may present lower levels of plasma albumin and, consequently, higher concentrations of unbound NSAIDs. These elevated NSAID concentrations, in addition to impaired gastric mucosal defenses, explain the higher susceptibility to gastrointestinal complications observed in older patients.

Some clinically relevant characteristics of specific NSAIDs are summarized in Table 1.6.

References

1. Smyth EM, Grosser T, Wang M, et al. Prostanoids in health and disease. J Lipid Res. 2009;50(Suppl):S423–8.
2. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol. 2011;31:986–1000.
3. Aoki T, Narumiya S. Prostaglandins and chronic inflammation. Trends Pharmacol Sci. 2012;33:304–11.
4. Ito S, Okuda-Ashitaka E, Minami T. Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. Neurosci Res. 2001;41:299–332.
5. Samad TA, Sapirstein A, Woolf CJ. Prostanoids and pain: unraveling mechanisms and revealing therapeutic targets. Trends Mol Med. 2002;8:390–6.
6. Phillis JW, Horrocks LA, Farooqui AA. Cyclooxygenases, lipoxygenases, and epoxygenases in CNS: their role and involvement in neurological disorders. Brain Res Rev. 2006;52:201–43.
7. Xie WL, Chipman JG, Robertson DL, et al. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci U S A. 1991;88:2692–6.
8. Kujubu DA, Fletcher BS, Varnum BC, et al. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J Biol Chem. 1991;266:12866–72.
9. Flower RJ. The development of COX2 inhibitors. Nat Rev Drug Discov. 2003;2:179–91.
10. Marnett LJ. The COXIB experience: a look in the rearview mirror. Annu Rev Pharmacol Toxicol. 2009;49:265–90.
11. McAdam BF, Mardini IA, Habib A, et al. Effect of regulated expression of human cyclooxygenase isoforms on eicosanoid and isoeicosanoid production in inflammation. J Clin Invest. 2000;105:1473–82.
12. Zhang FY, Wan Y, Zhang ZK, et al. Peripheral formalin injection induces long-lasting increases in cyclooxygenase 1 expression by microglia in the spinal cord. J Pain. 2007;8:110–7.
13. Patrono C, Baigent C. Nonsteroidal anti-inflammatory drugs and the heart. Circulation. 2014;129:907–16.
14. Warner TD, Giuliano F, Vojnovic I, et al. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. Proc Natl Acad Sci U S A. 1999;96:7563–8.
15. Ouellet M, Percival MD. Mechanism of acetaminophen inhibition of cyclooxygenase isoforms. Arch Biochem Biophys. 2001;387:273–80.
16. Boutaud O, Aronoff DM, Richardson JH, et al. Determinants of the cellular specificity of acetaminophen as an inhibitor of prostaglandin H (2) synthases. Proc Natl Acad Sci U S A. 2002;99:7130–5.
17. Garavito RM, Mulichak AM. The structure of mammalian cyclooxygenases. Annu Rev Biophys Biomol Struct. 2003;32:183–206.
18. Blobaum AL, Marnett LJ. Structural and functional basis of cyclooxygenase inhibition. J Med Chem. 2007;50:1425–41.
19. Smith WL, Urade Y, Jakobsson PJ. Enzymes of the cyclooxygenase pathways of prostanoid biosynthesis. Chem Rev. 2011;111:5821–65.

20. Kulmacz RJ, Lands WE. Stoichiometry and kinetics of the interaction of prostaglandin H synthase with - anti-inflammatory agents. J Biol Chem. 1985;260:12572–8.
21. Rome LH, Lands WE. Structural requirements for time-dependent inhibition of prostaglandin biosynthesis by anti-inflammatory drugs. Proc Natl Acad Sci U S A. 1975;72:4863–5.
22. Roth GJ, Stanford N, Majerus PW. Acetylation of prostaglandin synthase by aspirin. Proc Natl Acad Sci U S A. 1975;72:3073–6.
23. Gierse JK, Koboldt CM, Walker MC, et al. Kinetic basis for selective inhibition of cyclo-oxygenases. Biochem J. 1999;339(Pt 3):607–14.
24. Rowlinson SW, Kiefer JR, Prusakiewicz JJ, et al. A novel mechanism of cyclooxygenase-2 inhibition involving interactions with Ser-530 and Tyr-385. J Biol Chem. 2003;278:45763–9.
25. Walker MC, Kurumbail RG, Kiefer JR, et al. A three-step kinetic mechanism for selective inhibition of cyclo-oxygenase-2 by diarylheterocyclic inhibitors. Biochem J. 2001;357:709–18.
26. Yuan C, Rieke CJ, Rimon G, et al. Partnering between monomers of cyclooxygenase-2 homodimers. Proc Natl Acad Sci U S A. 2006;103:6142–7.
27. Pereira-Leite C, Nunes C, Reis S. Interaction of non-steroidal anti-inflammatory drugs with membranes: in vitro assessment and relevance for their biological actions. Prog Lipid Res. 2013;52:571–84.
28. Tegeder I, Pfeilschifter J, Geisslinger G. Cyclooxygenase-independent actions of cyclooxygenase inhibitors. FASEB J. 2001;15:2057–72.
29. Gurpinar E, Grizzle WE, Piazza GA. COX-independent mechanisms of cancer chemoprevention by anti-inflammatory drugs. Front Oncol. 2013;3:181.
30. Diaz-Gonzalez F, Sanchez-Madrid F. NSAIDs: learning new tricks from old drugs. Eur J Immunol. 2015;45:679–86.
31. Brune K, Patrignani P. New insights into the use of currently available non-steroidal anti-inflammatory drugs. J Pain Res. 2015;8:105–18.
32. Brune K, Renner B, Hinz B. Using pharmacokinetic principles to optimize pain therapy. Nat Rev Rheumatol. 2010;6:589–98.

Principles, Mechanisms of Action, and Future Prospects of Anti-inflammatory Drugs

2

Bibiana Rius and Joan Clària

Introduction

Bioactive lipid mediators are major regulators of inflammatory response. Seminal discoveries in the field of lipid mediators generated from the omega-6 polyunsaturated fatty acid, arachidonic acid, have identified prostaglandins (PGs) as essential constituents of inflammation in response to injury. Later on, the identification of cyclooxygenase (COX) as the enzyme responsible for the formation of PGs offered a novel paradigm in the therapeutic modulation of inflammation. Indeed, this enzyme evolved to become the primary target for nonsteroidal anti-inflammatory drugs (NSAIDs), which block the formation of PGs by inhibiting COX activity. Subsequent investigations defined other enzymes belonging to the COX pathway that participate in the formation of PGs, the identification of which offered novel opportunities for blocking/inhibiting this inflammatory pathway. Among these, the discovery of COX-2 in the early 1990s, the enzyme involved in the production of PGs during the inflammatory process, revolutionized the field and provided novel avenues for the design and development of safer anti-inflammatory drugs collectively known as COXIBs. The main advantage of these anti-inflammatory compounds was that they were able to reduce inflammation while sparing the formation of PGs from COX-1, which is the main COX isoform involved in the production of PGs responsible for housekeeping functions such as the maintenance of gastric mucosa and the integrity of renal function. Unfortunately, COXIBs fell into disgrace when their use was associated with a higher incidence of thrombotic events. Luckily, alternative scenarios including the design of compounds selectively inhibiting microsomal PGE synthase 1 (mPGES-1) have emerged as a means to reduce PGE_2 formation without affecting other COX products. Other promising strategies are the use of PG agonists and antagonists acting on specific prostanoid receptors. The latter approach offers more advantages in terms of safety and specificity as compared to the traditional upstream COX inhibitors, although the ten types and subtypes of membrane prostanoid receptors are hamper-ing progress in this area. Other therapeutic opportunities that have been considered are dualCOX-lipoxygenase (LOX) inhibitors, cyclo-pentenone PGs, NSAIDs coupled to nitric oxide (NO) donors, and NSAIDs coupled to H_2S-releasing compounds. Finally, the so-called aspirin-triggered lipoxins, a new genus of lipid mediators that promote the resolution of inflammation, have attracted special interest.

B. Rius, Ph.D. • J. Clària, Ph.D. (✉)
Biochemistry and Molecular Genetics Service, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), CIBERehd, and Department of Biomedical Sciences, Barcelona University School of Medicine, Villarroel 170, Barcelona 08036, Spain
e-mail: jclaria@clinic.ub.es

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_2

Eicosanoids

Eicosanoids comprise a large family of biologically active lipid mediators originating from arachidonic acid, an essential long-chain omega-6 polyunsaturated (4 double bonds) fatty acid with a backbone of 20-carbon atoms. The term eicosanoids derives from the Greek term *eicosa* (20) which refers to the peculiarity that all these arachidonic acid derivatives retain the parent 20-carbon structure. In resting cells, arachidonic acid is stored within the cell membrane and esterified to glycerol in the phospholipids, which are the most abundant structural lipid components in mammalian cells [1, 2]. Phospholipids are amphipathic molecules composed of a glycerol backbone with two fatty acids esterified to the sn (stereospecific numbering) 1 and 2 positions (sn1 and sn2) and a phosphate group bound to the third hydroxyl group. This phosphate group is esterified to another hydroxyl group on another hydrophilic compound, such as choline, ethanolamine, serine, or inositol, forming different phospholipids with unique properties [1, 2]. Upon stimulation, the enzyme phospholipase A_2 (PLA_2) catalyzes the hydrolysis of phospholipids at the sn2 position in a single-step reaction, releasing arachidonic acid into the intracellular space [1, 2]. Other phospholipases such as phospholipase C (PLC) or phospholipase D (PLD) do not release free arachidonic acid directly. Rather, they generate arachidonate-containing diacylglycerol and phosphatidic acid, from which arachidonic acid is subsequently released by diacylglycerol and monoacylglycerol lipases, respectively [1, 2].

Once released to the cytoplasm, free arachidonic acid is highly toxic to the cell and therefore is either rapidly converted into biologically active lipid mediators (i.e., eicosanoids), reincorporated into phospholipids, or diffused outside the cell. There are two major routes of eicosanoid biosynthesis in mammalian cells: the COX and LOX pathways, which are complemented by a third distinct enzymatic pathway, the cytochrome P450 epoxygenase or CYP pathway [3]. The COX pathway results in the formation of prostaglandins (PGs) and thromboxane (TXA_2), which are known for their powerful physiological properties and their critical role in inflammation [4–6]. On the other hand, the LOX pathway comprises three major LOXs, designated 5-LOX, 12-LOX, and 15-LOX. 5-LOX converts arachidonic acid into 5(*S*)-hydroxyeicosatetraenoic acid (5-HETE) and leukotrienes (LTs), which also represent another consolidated pharmacological target in inflammation, whereas 12-LOX and 15-LOX generate the corresponding 12- and 15-HETEs, respectively [4–7]. Alternatively, arachidonic acid can be converted into epoxyeicosatrienoic acids (EETs) through the CYP pathway [8]. These CYP metabolites are subsequently converted by the enzyme soluble epoxide hydrolase into inactive compounds designated diHETEs [9]. Since to date no cognate receptors or second messengers have been identified for these eicosanoids, they will not be discussed in this review.

In recent years, new families of eicosanoids generated by sequential interaction between individual LOX or between COX and LOX interactions have been described. The first mediators of this novel class ever described were the lipoxins (LXs), which are conjugated trihydroxytetraene-containing eicosanoids generated from arachidonic acid [10]. These mediators are the result of transcellular biosynthesis initiated by 15-LOX/5-LOX, 5-LOX/12-LOX, or aspirin-acetylated COX-2/5-LOX interactions [10]. In contrast to the majority of eicosanoids, which have consolidated proinflammatory properties, LXs are anti-inflammatory and not only act as "stop signals" for inflammation but also promote its active resolution [11]. More information on these mediators is given later on of this chapter.

COX Pathway

COX is the key enzyme in the biosynthesis of PGs and TXB_2 from arachidonic acid [5, 6, 12]. There are two distinct COX isoforms,

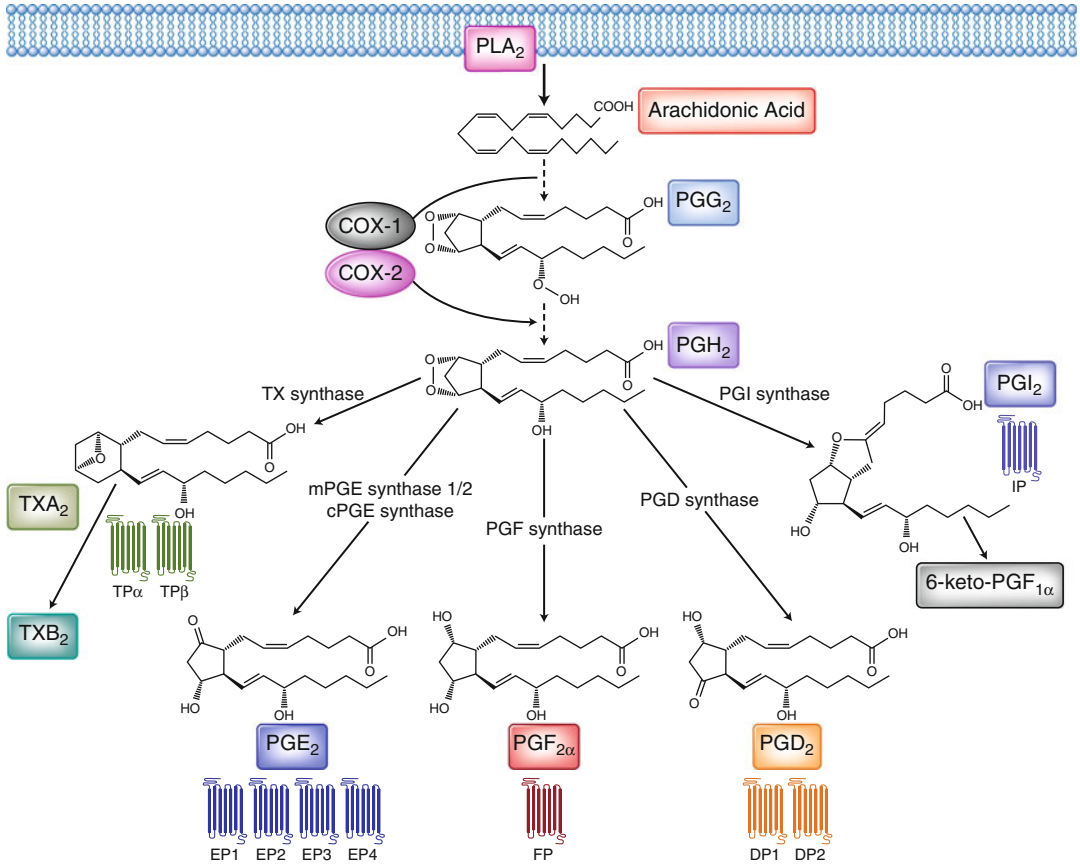


Fig. 2.1 Schematic diagram of the cyclooxygenase (COX) pathway. Once released from membrane phospholipids by phospholipase A_2, arachidonic acid is transformed by COX isoforms (COX-1 and COX-2) into prostaglandin (PG) G_2, which is subsequently reduced to PGH_2. PGH_2 is a highly unstable endoperoxide that is rapidly converted by specific synthases into PGs of the E, D, F, and I series as well as into thromboxane (TX) A_2. Both PGI_2 (prostacyclin) and TXA_2 have a very short half-life and are rapidly hydrolyzed to the inactive compounds 6-keto-$PGF_{1\alpha}$ and TXB_2, respectively. Each COX product interacts with its specific receptor(s) on target cells and tissues. Ten different receptors have been described: four for PGE_2, two for PGD_2, two for TXA_2, and one each for $PGF_{2\alpha}$ and PGI_2

designated COX-1 and COX-2, that generate the same structural products (i.e., PGs). However, COX-1 is a constitutive enzyme expressed in virtually all cells, whereas COX-2 has limited expression in most tissues but is induced by inflammatory mediators. Induction of COX-2 is seen in response to interleukin (IL)-1β, tumor necrosis factor (TNF)-α, interferon (IFN) γ, and lipopolysaccharide (LPS), and therefore, it is generally accepted as the COX isoform involved in inflammatory response [13, 14]. In any event, both COX isoforms sequentially transform arachidonic acid into PGG_2 and, subsequently, into PGH_2, which is finally converted into PGs of the D, E, F, and I series as well as into TXA_2 by specific terminal synthases (Fig. 2.1). The biosynthesis of each of these products is cell specific and depends on which synthase is predominant in a particular cell type. Consequently, any given cell type tends to specialize in the formation of one of these eicosanoids as its major product. For example, endothelial cells mainly produce PGI_2 (prostacyclin) from PGH_2 by means of PGI synthase, and platelets release TXA_2 from PGH_2 through the action of TX synthase. Both PGI_2 and TXA_2 have a very short half-life and

are rapidly hydrolyzed to the inactive compounds 6-keto-$PGF_{1\alpha}$ and TXB_2, respectively [12]. PGH_2 can be alternatively converted into $PGF_{2\alpha}$ by PGF synthase, which is mainly expressed in the uterus. PGH_2 is also converted into PGD_2 by the action of PGD synthase, of which two distinct types have been identified: lipocalin-type PGD synthase and hematopoietic-type PGD synthase [5]. PGD_2 is readily dehydrated to the cyclopentenone PGs of the J_2 series (PGJ_2 and 15-deoxy-Δ (delta)^{12,14}-PGJ_2 (15d-PGJ_2)) (see below). PGE_2 is formed by the enzyme PGE synthase (PGES) present in virtually every cell type. There are three different PGES isoforms (mPGES-1, cPGES-1, and mPGES-2), of which mPGES-1 was the first to be identified and characterized [15]. Owing to their instability, PGs and TXA_2 exert their functions mainly in the proximity of their sites of synthesis. Thus, they typically act as autocrine or paracrine hormones, maintaining homeostasis within their cells of origin or in neighboring cells in the tissue. Ten different types and subtypes of receptors, which belong to the G protein-coupled rhodopsin-type receptor superfamily of seven transmembrane domains, mediate the biological effects of PGs [16] (Fig. 2.1). Four of the receptor subtypes bind PGE_2 (EP1, EP2, EP3, and EP4), two bind PGD_2 (DP1 and DP2), two bind TXA_2 (TPα and TPβ), and the rest are single receptors for $PGF_{2\alpha}$ and PGI_2 (FP and IP, respectively) [16]. In addition to these classical membrane receptors, PGs and especially cyclopentenone PGs such as the PGD_2 final metabolite 15d-PGJ_2 can also transduce signals upon direct ligand binding to nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) [17]. These receptors are found in three different isoforms (i.e., PPARα, PPARδ, and PPARγ) and act as ligand-activated transcription factors with a DNA-binding domain that recognizes response elements in the promoter region of specific target genes linked to inflammation, cell proliferation, apoptosis, and differentiation [18].

The formation of PGs has been reported in almost every tissue and body fluid. With the exception of seminal fluid, PGs are not stored in tissues or cells. Instead, once synthesized, they are released and/or exported to the extracellular space. Owing to instability, PGs and TXA_2 exert their functions mainly in the proximity of their sites of synthesis. Thus, they typically act as autocrine or paracrine hormones, maintaining homeostasis within their cells of origin or in neighboring cells in the tissue. In general terms, COX products play a major role in inflammation and participate in the regulation of smooth muscle tone, hemostasis, thrombosis, parturition, and protection of gastrointestinal and renal integrity as well as in the progression of cancer.

Among the different PGs, PGE_2 plays a crucial role in the development of the five cardinal signs of inflammation: *edema*, *erythema*, *pain*, *fever*, and *loss of function*. In this regard, PGE_2 increases vascular permeability contributing to fluid extravasation and the appearance of *edema* (*swelling*), in a synergistic fashion with other soluble factors such as complement, bradykinin, histamine, and LTs [5]. In addition, PGE_2 is a potent vasodilator that increases tissue blood flow, contributing to the appearance of the characteristic *erythema* (*redness*) [19]. PGE_2 also sensitizes peripheral sensory nerve endings located at the site of inflammation and acts in the spinal cord to evoke hyperalgesia *pain* [20, 21]. Finally, PGE_2 is crucial in the appearance of *fever* [22]. *Pyresis* is the consequence of increased levels of PGE_2 in the central nervous system secondary to the actions of the proinflammatory cytokines IL-1β and TNF-α produced by activated immune cells in the systemic circulation [23]. It is of note that PGs are able to potentiate and prolong the action of other mediators of inflammation such as bradykinin, histamine, neurokinins, and complement [5].

PGI_2 (prostacyclin) is the chief COX product of the vascular endothelium [5]. It is mostly produced by endothelial cells and has vasodilatory properties and works as an inhibitor of platelet aggregation [5]. In contrast, TXA_2 is produced by platelets and is a potent vasoconstrictor and pro-thrombotic agent [5]. There is a fine balance between TXA_2 and PGI_2 in the regulation of systemic blood pressure and thrombogenesis. $PGF_{2\alpha}$ is also a prostanoid

with vasoconstrictor properties mainly produced by vascular and uterine smooth muscle [5]. $PGF_{2\alpha}$ induces the contraction of the uterus during labor and reproduction and induces bronchoconstriction in the lungs [5]. Finally, PGD_2 is a major product of mast cells and is actively involved in allergy and asthma [5].

COX-2

COX-2 was identified as a second COX isoform, which, unlike the constitutive isoform COX-1, is inducible and belongs to the category of immediate-early genes [24–26]. The COX-2 gene is localized on chromosome 1, is about 8 kb long, has 10 exons, and is transcribed as 4.6, 4.0, and 2.8 kb mRNA variants [27, 28]. The cDNA for COX-2 encodes a polypeptide, which, before cleavage of the signal sequence, contains 604 amino acids with an apparent molecular mass of 70 kDa [27, 29]. Sequence analysis of the COX-2 5′-flanking region has revealed several potential transcription regulatory elements including a TATA box, a NF-IL-6 motif, two AP-2 sites, three Sp1 sites, two NF-kB sites, a *Cre* motif, and an E-box [28]. COX-2 was originally identified as a unique, inducible gene product in studies addressing cell growth signaling pathways as well as in investigations on COX activity in response to cytokines and other inflammatory factors (reviewed in references [4, 13, 14, 30]). In fact, COX-2 is markedly induced by IL-1α, IL-1β, TNF−α, IFNγ, LPS, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and oncogenes such as v-src and v-ras [13, 14, 30]. Induction of COX-2 has been reported in many cell types including fibroblasts, monocytes and macrophages, epithelial, endothelial, smooth muscle, mesangial and mast cells, synoviocytes, osteoblasts, and central nervous system neurons [13, 14, 30].

The amino acid sequences of COX-1 and COX-2 from a single species are about 60 % identical and catalyze identical reactions and exhibit the same kinetic constants for the conversion of arachidonic acid to PGs [24, 25, 29]. However, the two COX isoforms have distinct tissue distribution and regulation. COX-1 is a constitutive isoform widely distributed throughout the gastrointestinal system, the kidneys, the vascular smooth muscle, and platelets and is presumably involved in the *housekeeping* functions of PGs such as cytoprotection of the gastric mucosa and the integrity of platelet and renal functions [31]. On the contrary, COX-2, which is not commonly found in differentiated cells in the absence of stimulation, has been referred to as the *inducible* isoform because, like other immediate-early genes, it can be rapidly upregulated in response to growth factors and cytokines [31]. This led to the dogma that the inducible COX-2 isoform was responsible for the synthesis of PGs involved in inflammatory response, whereas COX-1-derived PGs were involved in preserving the physiological functions of these prostanoids. This dogma is not entirely accurate, since COX-1 can be induced or upregulated under certain conditions, whereas COX-2 can be constitutively expressed in organs such as the brain and the kidneys [30, 31]

The primary role of COX-2 in gastrointestinal cancer deserves specific mention. Normal gastric mucosa scarcely expresses COX-2, but COX-2 expression and PGE_2 levels are upregulated through the multistep process of gastric carcinogenesis [32]. Since Ristimäki et al. described an elevated expression of COX-2 in gastric cancer for the first time [33], a number of studies has evaluated the relationship between COX-2 and cancer. The increased production of PGs observed in tumors likely reflects enhanced COX-2 activity since nearly 85 % of adenocarcinomas show between a two- and a fifty-fold increase in COX-2 expression at both mRNA and protein levels compared with matched, macroscopically normal, colonic mucosa from the same patient [34, 35]. Thus, COX-2 likely plays a role in early gastric carcinogenesis, although the precise mechanisms leading to the elevated expression of COX-2 are still not fully elucidated. Nevertheless, evidence suggests that proinflammatory cytokines, gastrin, mitogen, and growth factors could be involved in this process [36]. On the other hand, COX-2-overexpressing cells produce large amounts of vascular endothelial growth factor (VEGF), a

key pro-angiogenic factor that stimulates endothelial cell migration, proliferation of cancer cells, and angiogenesis [37]. Moreover, several mechanisms may concur to enhance COX-2 gene expression in cancer: in particular, mutations of APC and *ras*, activation of EGF receptor and IGF-I receptor pathways and the heregulin/HER-2 receptor pathway, and direct COX-2 induction by the Epstein-Barr virus oncoprotein and latent membrane protein 1 [38–40].

COX Inhibitors

The COX pathway offers unprecedented therapeutic opportunities in the arena of anti-inflammation. Seminal discoveries by Vane, Ferreira et al. and Smith et al. [41–43] were published in 1971 linking the ability of NSAIDs to suppress inflammation to the inhibition of COX and PG biosynthesis. At present, NSAIDs are among the most widely prescribed class of over-the-counter medications showing proven clinical utility in treating pain, fever, and inflammation [5]. A list of currently marketed NSAIDs is provided in Table 2.1. Among these NSAIDs, aspirin (acetylsalicylic acid) plays an undisputed central role in inflammation therapy. In fact, aspirin is the most widely consumed NSAID worldwide and the standard against which all new anti-inflammatory agents are compared. Aspirin has a long history of use and availability without prescription, and because of its low cost and safety, aspirin is the drug of choice for relieving inflammation and mild to moderate pain and fever. In addition to the well-known anti-inflammatory, analgesic, and antipyretic properties, aspirin also inhibits platelet aggregation and therefore is useful in preventing myocardial infarction and stroke [44]. Moreover, numerous epidemiological studies have also shown that the long-term use of low doses of aspirin represents a potentially viable option in the prevention of sporadic colon cancer [45] (see below).

The pharmacological properties of aspirin are related to its ability to acetylate COX, leading to the irreversible inhibition of the biosynthesis of

Table 2.1 List of the most common drugs marketed (brand names given in brackets) targeting the COX pathway

NSAIDS
Acetylsalicylic acid (Aspirin)
Choline salicylate (Arthropan)
Diclofenac potassium (Cataflam)
Diclofenac sodium (Voltaren)
Diclofenac sodium with misoprostol (Arthrotec)
Diflunisal (Dolobid)
Etodolac (Lodine)
Fenoprofen calcium (Nalfon)
Flurbiprofen (Ansaid)
Ibuprofen (Advil, Motrin)
Indomethacin (Indocin)
Ketoprofen (Actron, Orudis, Orudis KT, Oruvail)
Magnesium salicylate (Arthritab, Bayer Select, Doan's Pills, Magan, and others)
Meclofenamate sodium (Meclomen)
Mefenamic acid (Ponstel)
Meloxicam (Mobic)
Nabumetone (Relafen)
Choline and Magnesium salicylates (CMT, Tricosal, Trilisate)
Naproxen (Aleve, Naprosyn)
Oxaprozin (Daypro)
Piroxicam (Feldene)
Salsalate (Amigesic, Anaflex 750, Disalcid, Marthritic, Mono-Gesic, and others)
Sodium salicylate (various generics)
Sulindac (Clinoril)
Tolmetin sodium (Tolectin)
COXIBS
Celecoxib (Celebrex)
Etoricoxib (Arcoxia)
Lumiracoxib (Prexige)
NS-389 (Piroxicam)
Parecoxib (Dynastat)
Valdecoxib (Bextra, Dynastat)
RECEPTOR AGONISTS AND ANTAGONISTS
AA-2114 (Seratrodast)
Alprostadil (Edex)
BAY-U-3405 (Baynas)
Bimatoprost (Allergan, Lumigan)
Carboprost tromethamine (Hemabate)
Dinoprostone (Prepidil)
Iloprost (Ventavis)
Latanoprost (Xalatan)
Misoprostol (Cytotec)
Travoprost (Travatan)
Treprostinil (Remodulin, Tyvaso, Orenitram)
Tromethamine (Hebamate)

the eicosanoids (i.e., PGs and TXA_2). However, there are properties of aspirin that are independent of COX and PG inhibition. For example, aspirin-like drugs are able to either activate the heat shock transcriptional factor and the p38 mitogen-activated protein kinase or to inhibit the mitogen-activated protein kinases p44Erk1 and p42Erk2 and the activity of transcriptional factors such as nuclear factor-kB and activator protein 1 [46–48]. Therefore, complete knowledge of the mechanisms of action underlying the pleiotropic effects of aspirin is still a subject of interest and debate.

Unfortunately, apart from the beneficial anti-inflammatory, antipyretic, and analgesic effects, NSAIDs also exert unwanted side effects, particularly in the gastrointestinal tract [49]. This is due to the fact that traditional or conventional NSAIDs nonspecifically inhibit both COX-1 and COX-2 isoforms. In other words, COX-1-derived PGs are mainly involved in housekeeping functions including gastrointestinal cytoprotection, whereas COX-2-derived PGs are mostly responsible for inflammation, and consequently inhibition of both COX-1 and COX-2 by traditional NSAIDs (i.e., aspirin, indomethacin, ibuprofen, and meclofenamate) produces gastrotoxicity. That is, at concentrations required to inhibit PG biosynthesis at sites of inflammation (COX-2 activity), they also elicit a marked suppression of PG production in the gastrointestinal and renal systems (COX-1 activity).

Selective COX-2 Inhibitors (COXIBs)

The discovery of COX-2 and the characterization of its role in inflammation were crucial for understanding why some existing NSAIDs including etodolac (*Lodine*®), meloxicam (*Mobic*®), and nimesulide (*Mesulid*® and others, currently withdrawn from the market) were associated with a lower range of deleterious effects. The most plausible explanation for this phenomenon was that these NSAIDs have a higher selectivity for COX-2 in comparison with COX-1. In any event, the most important advance in the field of inflammation occurred when drug companies took up the search for a new class of compounds specifically designed to selectively inhibit COX-2 without affecting COX-1-dependent PG biosynthesis. These new series of compounds were generically designated as COXIBs. The first generation of selective COX-2 inhibitors displayed high selectivity for blocking COX-2 activity in vitro and proved to be as efficacious as standard NSAIDs in a number of in vivo models of inflammation (rat carrageenan-induced foot-pad edema and rat adjuvant-induced arthritis) and hyperalgesia (rat carrageenan-induced hyperalgesia) [50–52]. These preclinical results led to the rational design of the first clinical trials for selective COX-2 inhibitors, which were sufficient to prove that these compounds were useful for relieving the signs and symptoms of osteoarthritis and rheumatoid arthritis and for alleviating pain following dental extraction, while reducing the incidence of gastrointestinal ulcers and erosions seen with standard NSAID therapy [53–57]. This novel class of compounds aroused particular interest for combating inflammation in diseases such as liver cirrhosis, in which renal function is critically dependent on COX-1-derived PGs [58–61]. The two first selective COX-2 inhibitors approved and marketed were celecoxib (*Celebrex*®) and rofecoxib (*Vioxx*®). A second generation of selective COX-2 inhibitors including valdecoxib (*Bextra*®), etoricoxib (*Arcoxia*®), parecoxib, an injectable prodrug of valdecoxib (*Dynastat*®), and lumiracoxib (*Prexige*®) (Table 2.1) was also approved for the treatment of osteoarthritis, rheumatoid arthritis, primary dysmenorrhea, and postoperative pain. Since their introduction into the market in 1999, selective COX-2 inhibitors have become hugely popular and one of the world's best selling drug class. Unfortunately, rofecoxib (*Vioxx*) was withdrawn from the market in 2004 based on the findings from the prospective, randomized, placebo-controlled clinical trial, adenomatous polyp prevention on *Vioxx* (APPROVe), which demonstrated an increased relative risk for confirmed cardiovascular events, such as heart attacks and strokes, in patients taking *Vioxx*

compared to those taking placebo [62]. It has been postulated that the increased cardiovascular risk associated with COX-2 inhibitors may be secondary to prostacyclin/TXB_2 imbalance, since prostacyclin inhibits platelet aggregation and causes vasodilatation and is derived mainly from COX-2, whereas TXB_2 causes platelet aggregation and vasoconstriction and is mainly a COX-1 product.

An interesting aspect of COX-2 is that this isoform plays a crucial role in cell growth, angiogenesis, and cancer progression [37–39]. Consequently, COXIBs were also envisioned from the very first moment as promising anticancer agents. The use of these compounds in clinical and experimental studies has provided clear proof that COX-2 is indeed involved in the cancer preventive actions of NSAIDs. In a randomized clinical trial, the COX-2 inhibitor celecoxib effectively inhibited the growth of adenomatous polyps and caused regression of existing polyps in patients with hereditary familial adenomatous polyposis [63]. Studies in rodents have also demonstrated that pharmacological inhibition of COX-2 activity prevents chemically induced carcinogenesis and intestinal polyp formation in an experimental model of FAP [40]. Interestingly, animal studies have shown that celecoxib is able to potentiate the antitumor activity of conventional chemotherapy and radiation [64, 65], an effect that could be related to the recently uncovered COX-2 capability of blocking p53- or genotoxic stress-induced apoptosis [66]. Cell growth and angiogenesis can be blocked in vitro by selective COX-2 inhibitors, highlighting the role of COX-2 in cancer progression [67, 68]. Nevertheless, a significant antiproliferative effect following selective COX-2 inhibition has been observed in colon cancer cells that do not express COX-2 [69]. It has been suggested that the therapeutic activity of COX-2 inhibitors might also be related to their ability to inhibit IkB kinase (IKK) activity [70]. This finding together with the observation that sulindac sulfone, a sulindac metabolite devoid of COX inhibitory activity, is able to reduce colon cancer cell growth [71] suggests that COX-2-independent pathways and/or pathways unrelated to PGs are also involved in the antineoplastic effects of NSAIDs and selective COX-2 inhibitors.

mPGES-1 Inhibitors

Given the controversy surrounding the COXIBS, increased interest emerged regarding the pharmacological modulation of PG production through inhibition of specific PG synthases. Among the different PG synthases, PGE synthase was of particular interest because this enzyme is responsible for PGE_2 biosynthesis. In theory, pharmacologic inhibition of PGE synthase activity could decrease the formation of the proinflammatory prostanoid PGE_2 while sparing the production of other prostanoids with vascular protective effects such as prostacyclin. In 1999, Jakobsson and coworkers [15] reported the cloning and characterization of human PGE synthase, now designated mPGES-1, which is a member of the membrane-associated proteins involved in the eicosanoid and glutathione metabolism superfamily with the ability to catalyze the conversion of PGH_2 into PGE_2. Following this discovery, a cytosolic form of PGE synthase, termed cPGES-1, which also isomerizes PGH_2 to PGE_2 rather specifically in the presence of glutathione, was also cloned [72]. cPGES is ubiquitously expressed and identical to p23 [73]. In addition, a second isoform of membrane-associated PGE synthase, designated mPGES-2, was identified in 2002 [74]. Among the three different PGE synthases, mPGES-1 has received much attention because it is an inducible enzyme functionally coupled with COX-2 [15, 72–75]. Indeed, protein expression for mPGES-1 and COX-2 is concomitantly induced by IL-1β [15, 76]. Moreover, in a series of elegant experiments, Murakami et al. demonstrated that cotransfection of human mPGES-1 and COX-2 into HEK 293 cells results in a higher PGE_2 production when cells are subsequently stimulated with ionophore or IL-1β than cotransfection of mPGES-1 with COX-1, thus providing evidence that mPGES-1 preferentially couples with COX-2 activity [77]. The fact that

mice lacking the mPGES-1 gene have impaired inflammatory, pain, and fever responses clearly highlights the role of this enzyme in inflammation [78, 79]. At the moment, a number of compounds specifically targeting mPGES-1 are under development, although they are not yet available for clinical use.

Agonists and Antagonists of Prostanoid Receptors

The modulation of the COX pathway by compounds acting on specific prostanoid receptors provides advantages over upstream COX, COXIBs, and mPGES-1 inhibitors, because they can offer more specificity to their actions. Unfortunately, progress in this field has been slow and difficult, mainly because of the existence of such a large number of prostanoid receptors and their function similarity. Nevertheless, the cloning and characterization of specific prostanoid receptors have facilitated the development of synthetic agonists and antagonists for some of these receptors. Most of these compounds have proven to be very useful in the identification of the biological role of a given prostanoid receptor, and some have shown therapeutic potential (Table 2.1). Some examples are misoprostol (*Cytotec*®), an EP3/EP2 agonist used as an adjunct to COX inhibitor therapy to reduce gastric irritation and bleeding [80]; alprostadil (*Edex*®), an EP4/EP2 agonist used for erectile dysfunction [81]; travoprost (*Travatan*®), latanoprost (*Xalatan*®), and bimatroprost (*Allergan*® *or Lumigan*®), which are FP agonists marketed for the treatment of glaucoma and ocular hypertension [82]; carbaprost tromethamine (*Hebamate*®), a 15-methyl analogue of naturally occurring prostaglandin F2α prescribed for termination of pregnancy and also used for postpartum hemorrhage [83]; iloprost (*Ventavis*®), an IP agonist used in pulmonary hypertension; treprostinil (*Remodulin*®, *Tyvaso*®, and *Orenitram*® among others), a PGI_2 analogue used to treat pulmonary arterial hypertension [84]; beraprost sodium, the first chemically stable orally active prostacyclin analogue currently only approved in Japan [85]; dinoprostone (*Prepidil*®), natural occurring PGE_2 which is a pharmacologic agent administered intravaginally or intracervically for ripening the cervix [86]; and AA-2114 (*Seratrodast*®) and BAY-U-3405 (*Baynas*®), which are orally active TX receptor antagonists available for the treatment of asthma [87, 88].

Cyclopentenone PGs

Cyclopentenone PGs (cyPGs) are products of the nonenzymatic dehydration of PGs. CyPGs are structurally defined by the presence of a highly reactive α,β-unsaturated carbonyl moiety in the cyclopentenone ring [89]. From a biological point of view, the most relevant cyPGs are those derived from the dehydration of PGD_2, including the PGs of the J_2 series: PGJ_2, Δ^{12}-PGJ_2, and 15d-PGJ_2. Unlike other PGs, no specific transmembrane receptors for cyPGs have been identified to date. Instead, 15d-PGJ_2 is a natural ligand of PPARγ and appears to exert its effects through binding and activation of this member of the nuclear receptor superfamily of ligand-activated transcription factors [90]. Other actions independent of PPARγ have been reported for cyPGs, including downregulation of NF-kB transcriptional activity [91], inhibition of cytokine production by monocytes [92], and direct inhibition of key enzymes of the eicosanoid cascade, namely, cytosolic phospholipase A_2, COX-2, and mPGES-1 [93, 94].

CyPGs have a broad spectrum of biological effects and, unlike conventional PGs, display powerful immunomodulatory and anti-inflammatory properties [95]. CyPGs have been shown to suppress chronic inflammation and pannus formation in rats with adjuvant-induced arthritis [96] and to have a protective role in models of renal ischemia-reperfusion injury [97] and inflammatory bowel disease [98]. Interestingly, in rats with carrageenan-induced pleurisy, in which the generation of 15d-PGJ_2 takes place during the resolution phase, administration of cyPGs brings about acute inflammatory

resolution, whereas inhibition of 15d-PGJ_2 synthesis is associated with an exacerbation of inflammation [99, 100]. In addition, cyPGs suppress viral replication, stimulate osteogenesis, exhibit antiproliferative effects on cancer cells, and attenuate the tumorigenic potential of cancer cells in nude mice [89, 95, 101]. Unfortunately, these compounds have not progressed toward clinical development.

Other Approaches

Drugs Acting on the 5-LOX Pathway

Arachidonate 5-LOX is the key enzyme in the biosynthesis of LTs. It initially transforms free arachidonic acid to 5-HPETE through the stereospecific abstraction of the pro-*S* hydrogen at carbon-7, followed by insertion of molecular O_2 at carbon-5 [102]. 5-HPETE is either reduced to 5-HETE or subjected to the stereospecific removal of the pro-*R* hydrogen at carbon-10 to generate the highly unstable allylic epoxide LTA_4 [103]. Once formed, LTA_4 is rapidly transformed to either LTB_4 via stereoselective hydration by LTA_4 hydrolase [104] or to LTC_4 through glutathione conjugation catalyzed by LTC_4 synthase [105]. Sequential metabolic reactions catalyzed by γ-glutamyl transferase and a specific membrane-bound dipeptidase convert LTC_4 into LTD_4 and LTE_4, respectively. Together LTC_4, D_4, and E_4 are termed cysteinyl-leukotrienes (Cys-LTs) and in the past were referred to as the slow-reacting substances of anaphylaxis.

Over the past 25 years, a number of pharmacological agents that modify the 5-LOX pathway and the biosynthesis of LTs have been developed to treat inflammatory diseases such as asthma, ulcerative colitis, arthritis, and psoriasis. These agents, which are generically known as LT-modifying drugs, include 5-LOX and FLAP inhibitors and Cys-LT receptor antagonists. Drugs that directly block 5-LOX activity were the first pharmacological compounds considered as LT-modifying drugs. Many of the molecules originally developed were discarded because of severe side effects and never entered the market, although some are currently used for in vitro research [106]. Caffeic acid, AA-861 and BW-775C, fall within this category. Nordihydroguaretic acid (NDGA) also known as masoprocol (*Actinex*®) was a potent 5-LO inhibitor used to treat actinic keratoses, although it was withdrawn from the USA and Canada in 1996. Other molecules designed to chelate the active iron, such as the N-hydroxyurea derivative Zileuton, have been developed. Zileuton (*Zyflo*®) has been marketed as therapy for the prevention and chronic treatment of asthma in adults and children 12 years of age or older. A different approach to inhibit 5-LOX activity is by means of FLAP inhibitors. The indole-based compound AM803 underwent clinical investigation and passed phase II trials with asthma patients [107]. A similar compound named AM103 underwent phase II clinical trials for treatment of respiratory disorders [108]. A very potent and selective FLAP inhibitor BAYX1005 was developed by Bayer and passed a phase II clinical trial for myocardial infarction as the compound DG-031 (*Veliflapon*®) from the company deCODE genetics [109]. However, while entering phase III for the prevention of heart attacks and stroke, participant recruitment was suspended.

LT receptor antagonists are another important class of LT-modifying drugs. Orally active receptor antagonists directed against the Cys-LT1 receptor have been marketed [110–112]. The Cys-LT1 receptor antagonists montelukast (*Singulair*®), pranlukast (*Ultair*®), and zafirlukast (*Accolate*®) were tested in a number of clinical trials which demonstrated improvement of pulmonary function and reduction of asthma exacerbations, especially in exercise-induced asthma [113]. On the other hand, LTB_4 receptor antagonists such as SC-41,930 are CP-105,696 were shown to be efficacious in reducing the arthritis index and ankle bone destruction in IL-1-accelerated collagen-induced arthritis and to reduce atherosclerosis lesion progression in mice [114, 115].

Dual COX-2/5-LO Inhibitors

Considering the proinflammatory properties of COX-2- and 5-LO-derived eicosanoids, dual COX-2/5-LO inhibitors should, in theory, have a superior anti-inflammatory profile than individual selective COX-2 and 5-LO inhibitors. Although no human data are available analyzing the superiority of the anti-inflammatory efficacy of inhibiting two pathways versus inhibition of a single pathway, experimental and cellular studies indicate that dual inhibitors may have some disease-modifying activity and may stop disease progression by reducing the expression of matrix metalloproteinase-13 and IL-1β as well as chondrocyte death [116, 117].

While in theory it is quite easy to design drugs acting on one enzyme, it is more daunting to design a drug that selectively inhibits two different enzymes, especially if these are not structurally related. One of the first compounds with dual COX/5-LO inhibitory activity was tepoxalin, a pyrazole-containing hydroxamic acid able to chelate the nonheme iron atom of 5-LO [118]. Tepoxalin underwent clinical evaluation for psoriasis and rheumatoid arthritis but unfortunately was discontinued in phase II [119]. This drug received animal healthcare approval later on for reduction of inflammation and relief of pain caused by acute and chronic musculoskeletal disorders such as arthritis. A COX/5-LO inhibitor also evaluated in clinical trials for arthritis was S-2474, which displayed excellent anti-inflammatory and analgesic activities associated with remarkable gastric safety [120, 121]. RWJ-63556, a compound structurally related to the selective COX-2 inhibitor nimesulide, was another potent orally active COX-2/5-LO inhibitor with remarkable anti-inflammatory activity in experimental carrageenan-induced inflammation [122]. An interesting activity profile was also noted for ER-34122, which suppressed progression of PMN infiltration, subsynovial soft tissue edema, and multiplication of synovial lining cells in the early stages of arthritis in a mouse model of systemic lupus erythematosus [123, 124].

Licofelone, also known as ML-3000, deserves special mention. Licofelone is a pyrrolizine derivative and an arachidonic acid substrate analogue that inhibits both COX and 5-LO. Unlike most of the previously described dual inhibitors, licofelone is neither an antioxidant nor an iron chelator [125, 126]. Licofelone was shown to inhibit COX in bovine and human platelets and 5-LO in bovine and human granulocytes [126]. Moreover, licofelone exhibits not only anti-inflammatory but also potent analgesic, antipyretic, and antithrombotic activities with little or no gastrointestinal damage in experimental animals [125–127]. In addition, in guinea pigs challenged with arachidonic acid or antigen and in sheep challenged with antigen, licofelone displayed potent antiasthmatic activity [128]. Licofelone showed an excellent gastrointestinal profile, much better than conventional NSAIDs and equivalent to selective COX-2 inhibitors in phase III trials [129, 130]. Furthermore, in healthy subjects, licofelone is well tolerated with no hepatotoxicity and has a good pattern of tissue distribution, with the highest levels being reached in the lung, liver, kidneys, heart, and large and small intestine [127, 129, 130].

NSAIDs Releasing Nitric Oxide (NO) or Hydrogen Sulfide (H_2S)

A new class of NSAIDs that offers new perspectives is the COX-inhibiting NO donators (CINODs) which are generated by adding a NO-generating moiety to a parent NSAID via an ester linkage [131]. CINODs are designed to reduce the potential toxicity of the parent drug, while maintaining its analgesic and anti-inflammatory effects. In this regard, NO cooperates with endogenous PGs in the maintenance of gastric integrity and microcirculation by potentiating gastric alkaline mucus secretion and inhibiting gastric acid secretion [132, 133]. NO also modulates leukocyte-endothelial interactions as demonstrated in in vivo microscopy experiments in single venules [134]. All these findings raised the possibility that NO could be

GI protective in NSAID-induced gastric damage, which is characterized by increased leukocyte adherence, reduced gastric blood flow, and impaired mucosal repair [135]. Naproxcinod®, Nicox's lead drug, was the first CINOD ever evaluated in preclinical and clinical studies. It is metabolized to naproxen and has been shown to donate NO in vitro and in vivo [135]. Phase III clinical trials of Naproxcinod® are currently underway, with the aim of reducing potential toxicity while maintaining its analgesic and anti-inflammatory effects.

More recently, H_2S-releasing derivatives of NSAIDs have been developed. H_2S is a normal component of our bodies where it is present in very low concentrations. This gas is produced through a number of pathways, the most common being related to the metabolism of L-cysteine, cystine, and homocysteine [136]. As with NO, H_2S seems to play an important role in a variety of physiologic processes and diseases. Among others, H_2S plays an important role in neuromodulation, hypertension, inflammation, gastric mucosal integrity, and vascular tone [137–140]. H_2S, which is also produced by the gastric mucosa like NO, contributes to the ability of this tissue to counteract the damage induced by several luminal substances. The production of H_2S was found to be reduced following NSAID administration, supposedly through the inhibition of the expression of a key enzyme for conversion of L-cysteine into H_2S, the enzyme cystathionine γ-lyase [141]. The provision of H_2S donors could avoid the decrease in gastric blood flow induced by current NSAIDs as well as prevent NSAID-induced leukocyte adherence. Thus, as with the CINODs and dual LOX/COX inhibitors, the existing preclinical data appear to indicate a potential for H_2S-releasing NSAIDs to provide similar anti-inflammatory efficacy as traditional NSAIDS without the burden of gastric toxicity.

Aspirin-Triggered 15-Epi-lipoxins

Aspirin-triggered lipoxins (ATL) have received the most attention as a novel anti-inflammatory approach [10, 142, 143]. The acetylation capacity of aspirin is a critical aspect in the ATL biosynthetic pathway, and this property is not shared but any other NSAID. Indeed, this biosynthetic pathway triggered by aspirin is initiated by acetylation of COX-2, which switches the enzyme catalytic activity from a PG synthase to 15-LOX [142]. Thus, PG biosynthesis by aspirin-acetylated COX-2 is inhibited, and arachidonic acid is transformed to 15R-HETE. The further conversion of 15R-HETE to 15-epi-LXA_4 (ATL) by a 5-LOX present in immune cells is the result of a process called transcellular biosynthesis. This process involves cell-cell interaction and processing of a metabolic intermediate generated by one cell (donor cell) by a vicinal cell (acceptor cell) for the production of an active eicosanoid that neither cell can generate alone [144]. ATLs are 15-epimers of LXs, which have a unique spectrum of bioactions indicative of anti-inflammatory and pro-resolution properties. The most relevant biological action of these aspirin-triggered eicosanoids (i.e., ATLs) is that they work as putative endogenous "breaking signals" for leukocyte recruitment and therefore play a key role in the resolution of inflammation [10]. For example, these eicosanoids inhibit chemotaxis, selectin- and integrin-mediated adhesion to and transmigration across endothelial monolayers in response to LTB_4 and formylmethionyl-leucyl-phenylalanine, TNF-α-stimulated superoxide generation, and degranulation and interleukin-1 release by neutrophils [10]. In vivo, LX stable analogues inhibit LTB_4-induced leukocyte rolling and adherence and neutrophil margination and extravasation [10]. LX analogues inhibit TNFα-stimulated leukocyte trafficking and chemokine secretion in murine air pouches and when applied topically to mouse ears dramatically inhibit leukocyte infiltration and vascular permeability [10]. In addition, ATL analogues protect mice from renal ischemia-reperfusion injury and glomerulonephritis [10]. In an animal model of periodontal disease, LX and ATL analogues attenuate gingivitis and leukocyte recruitment [10]. Intravenous delivery of LXs and ATL inhibits acute dermal inflammation and neutrophil infiltration

of skin microabcesses and lungs in LTB_4 receptor transgenic mice [10]. In a murine model of asthma, stable LX and ATL analogues attenuate airway hyperreactivity and inflammation and accelerate resolution of pulmonary edema [10]. Administration of a metabolically stable LXA_4 analogue in a mouse model of chronic airway inflammation and infection associated with cystic fibrosis suppresses neutrophilic inflammation, decreases pulmonary bacterial burden, and attenuates disease severity [10]. Finally, a randomized clinical trial in healthy subjects demonstrated that low-dose aspirin (81 mg daily), used for long-term antithrombotic prophylaxis, initiates the production of anti-inflammatory ATL contrary to the inhibition of the pro-thrombotic TXA_2 [145]. Overall, LXs and ATL are anti-inflammatory and pro-resolution eicosanoids that work efficiently in reducing the signs and symptoms of inflammation in a wide range of disease models. Consequently, this property may effectively mediate, at least in part, the beneficial actions of aspirin.

More recently, aspirin was shown to trigger the conversion of omega-3-PUFA (i.e., eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to another group of anti-inflammatory and pro-resolution lipid mediators termed ASA-triggered resolvins and ASA-triggered-protectins [146, 147]. Similar to what has been described for the biosynthesis of ATL, endothelial cells expressing COX-2 acetylated by aspirin transform DHA into 17R-HDHA which is further converted by 5-LOX into the corresponding 17R-RvD1, 17R-RvD2, and other 17R-D resolvins, which are collectively known as aspirin-triggered (AT) resolvins [11, 148]. ASA-triggered protectin D1 (AT-PD1) is biosynthesized in a similar process. Finally, biosynthesis of resolvins of the E-series from EPA is initiated with the formation of 18R-hydroperoxy-EPE (18R-HEPE) by endothelial cells expressing aspirin-acetylated COX-2 [147]. 18R-HEPE is transformed by transcellular biosynthesis in neighboring 5-LOX-containing leukocytes into RvE1 (5S,12R,18R-trihydroxy-EPA) via a 5S,6-epoxide intermediate [147]. Collectively, these omega-3-derived lipid mediators also exert anti-inflammatory and pro-resolution actions both in vitro and in vivo and contribute to the understanding of the preventive actions observed with both aspirin and dietary omega-3PUFA.

Conclusions

For the last 40 years, COX-derived PGs have evolved as the best consolidated inflammatory mediators among the plethora of bioactive lipid mediators generated from arachidonic acid. A large number of over-the-counter medications based on the inhibition of these lipid mediators (i.e., NSAIDs) are still the most currently available class of drugs to fight inflammation, pain, and fever. Despite numerous efforts to improve the safety, the use of drugs targeting PG biosynthesis is still the front line of inflammation therapy. COX-2 inhibitors, for example, were safer than NSAIDs and exhibited a better gastric tolerance, but fail because of unexpected thrombotic events. At present much hope has been raised over the use of compounds that specifically target PG receptors hat inhibit the activity of specific terminal synthases, and the outcome of this effort will be the subject of discussion in the coming years. Finally, the use of drugs that modulate the PG cascade in combination with the modulation of other pathways of lipid mediator biosynthesis is a subject that will receive much attention in the next years. For example, the interaction of NSAIDs, which target the omega-6 arachidonic acid-derived products, with the omega-3 family of polyunsaturated fatty acids, which also function as substrates for the same COX enzymes, is a matter of interest in the search for novel strategies to harness unremitting inflammation.

Acknowledgments Our lab is supported by Spanish Ministerio de Economía y Competitividad (MEC) (SAF15/63674-R and PIE14/00045 to J.C.) under European Regional Development Funds (ERDF). CIBERehd is funded by the Instituto de Salud Carlos III. B.R. has a fellowship from MEC (BES-2013-063705).

References

1. Balsinde J, Dennis EA. Function and inhibition of intracellular calcium-independent phospholipase A2. J Biol Chem. 1997;272(26):16069–72.
2. Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. J Lipid Res. 2009;50(Suppl):S237–42.
3. Dennis EA, Norris PC. Eicosanoid storm in infection and inflammation. Nat Rev Immunol. 2015;15(8):511–23.
4. Romano M, Claria J. Cyclooxygenase-2 and 5-lipoxygenase converging functions on cell proliferation and tumor angiogenesis: implications for cancer therapy. FASEB J. 2003;17(14):1986–95.
5. Dudzinski DM, Serhan CN. Pharmacology of eicosanoids. In: Golan DE, Armstrong EJ, Galanter JM, Armstrong AW, Arnaout RA, Rose HS, editors. Principles of pharmacology: The pathophysiologic basis of drug therapy. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 627–46.
6. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001;294(5548):1871–5.
7. Haeggstrom JZ, Funk CD. Lipoxygenase and leukotriene pathways: biochemistry, biology, and roles in disease. Chem Rev. 2011;111(10):5866–98.
8. Zeldin DC. Epoxygenase pathways of arachidonic acid metabolism. J Biol Chem. 2001;276(39):36059–62.
9. Lopez-Vicario C, et al. Inhibition of soluble epoxide hydrolase modulates inflammation and autophagy in obese adipose tissue and liver: role for omega-3 epoxides. Proc Natl Acad Sci U S A. 2015;112(2):536–41.
10. Serhan CN. Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. Prostaglandins Leukot Essent Fatty Acids. 2005;73(3-4):141–62.
11. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature. 2014;510(7503):92–101.
12. Smith WL, Song I. The enzymology of prostaglandin endoperoxide H synthases-1 and -2. Prostaglandins Other Lipid Mediat. 2002;68–69:115–28.
13. Clària J. Cyclooxygenase-2 biology. Curr Pharm Des. 2003;9(27):2177–90.
14. Morita I. Distinct functions of COX-1 and COX-2. Prostaglandins Other Lipid Mediat. 2002;68–69:165–75.
15. Jakobsson PJ, et al. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci U S A. 1999;96(13):7220–5.
16. Breyer RM, et al. Prostanoid receptors: subtypes and signaling. Annu Rev Pharmacol Toxicol. 2001;41:661–90.
17. Fukushima M. Biological activities and mechanisms of action of PGJ2 and related compounds: an update. Prostaglandins Leukot Essent Fatty Acids. 1992;47(1):1–12.
18. Evans RM, Barish GD, Wang YX. PPARs and the complex journey to obesity. Nat Med. 2004;10(4):355–61.
19. Williams TJ, Peck MJ. Role of prostaglandin-mediated vasodilatation in inflammation. Nature. 1977;270(5637):530–2.
20. Bley KR, et al. The role of IP prostanoid receptors in inflammatory pain. Trends Pharmacol Sci. 1998;19(4):141–7.
21. Yamamoto T, Nozaki-Taguchi N. Analysis of the effects of cyclooxygenase (COX)-1 and COX-2 in spinal nociceptive transmission using indomethacin, a non-selective COX inhibitor, and NS-398, a COX-2 selective inhibitor. Brain Res. 1996;739(1-2):104–10.
22. Milton AS. Thermoregulatory actions of eicosanoids in the central nervous system with particular regard to the pathogenesis of fever. Ann N Y Acad Sci. 1989;559:392–410.
23. Ushikubi F, et al. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. Nature. 1998;395(6699):281–4.
24. Kujubu DA, et al. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J Biol Chem. 1991;266(20):12866–72.
25. Xie WL, et al. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci U S A. 1991;88(7):2692–6.
26. O'Banion MK, et al. A serum- and glucocorticoid-regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein. J Biol Chem. 1991;266(34):23261–7.
27. Jones DA, et al. Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. J Biol Chem. 1993;268(12):9049–54.
28. Tanabe T, Tohnai N. Cyclooxygenase isozymes and their gene structures and expression. Prostaglandins Other Lipid Mediat. 2002;68–69:95–114.
29. Hla T, Neilson K. Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci U S A. 1992;89(16):7384–8.
30. Herschman HR. Prostaglandin synthase 2. Biochim Biophys Acta. 1996;1299(1):125–40.
31. Otto JC, Smith WL. Prostaglandin endoperoxide synthases-1 and -2. J Lipid Mediat Cell Signal. 1995;12(2-3):139–56.
32. Nardone G, et al. Expression of COX-2, mPGE-synthase1, MDR-1 (P-gp), and Bcl-xL: a molecular pathway of H pylori-related gastric carcinogenesis. J Pathol. 2004;202(3):305–12.
33. Ristimaki A, et al. Expression of cyclooxygenase-2 in human gastric carcinoma. Cancer Res. 1997;57(7):1276–80.

34. Eberhart CE, et al. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994;107 (4):1183–8.
35. Kutchera W, et al. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: evidence for a transcriptional effect. Proc Natl Acad Sci U S A. 1996;93(10):4816–20.
36. Konturek PC, et al. Influence of gastrin on the expression of cyclooxygenase-2, hepatocyte growth factor and apoptosis-related proteins in gastric epithelial cells. J Physiol Pharmacol. 2003;54(1):17–32.
37. Tsujii M, et al. Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell. 1998;93 (5):705–16.
38. Vadlamudi R, et al. Regulation of cyclooxygenase-2 pathway by HER2 receptor. Oncogene. 1999;18 (2):305–14.
39. Murono S, et al. Induction of cyclooxygenase-2 by Epstein-Barr virus latent membrane protein 1 is involved in vascular endothelial growth factor production in nasopharyngeal carcinoma cells. Proc Natl Acad Sci U S A. 2001;98(12):6905–10.
40. Oshima M, et al. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell. 1996;87(5):803–9.
41. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971;231(25):232–5.
42. Ferreira SH, Moncada S, Vane JR. Indomethacin and aspirin abolish prostaglandin release from the spleen. Nat New Biol. 1971;231(25):237–9.
43. Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. Nat New Biol. 1971;231(25):235–7.
44. Patrono C. Aspirin as an antiplatelet drug. N Engl J Med. 1994;330(18):1287–94.
45. Thun MJ, Namboodiri MM, Heath Jr CW. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med. 1991;325(23):1593–6.
46. Jurivich DA, et al. Effect of sodium salicylate on the human heat shock response. Science. 1992;255 (5049):1243–5.
47. Pillinger MH, et al. Modes of action of aspirin-like drugs: salicylates inhibit erk activation and integrin-dependent neutrophil adhesion. Proc Natl Acad Sci U S A. 1998;95(24):14540–5.
48. Kopp E, Ghosh S. Inhibition of NF-kappa B by sodium salicylate and aspirin. Science. 1994;265 (5174):956–9.
49. Gargallo CJ, Sostres C, Lanas A. Prevention and treatment of NSAID gastropathy. Curr Treat Options Gastroenterol. 2014;12(4):398–413.
50. Seibert K, et al. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc Natl Acad Sci U S A. 1994;91(25):12013–7.
51. Masferrer JL, et al. Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic. Proc Natl Acad Sci U S A. 1994;91 (8):3228–32.
52. Anderson GD, et al. Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. J Clin Invest. 1996;97(11):2672–9.
53. Simon LS, et al. Preliminary study of the safety and efficacy of SC-58635, a novel cyclooxygenase 2 inhibitor: efficacy and safety in two placebo-controlled trials in osteoarthritis and rheumatoid arthritis, and studies of gastrointestinal and platelet effects. Arthritis Rheum. 1998;41(9):1591–602.
54. Simon LS, et al. Anti-inflammatory and upper gastrointestinal effects of celecoxib in rheumatoid arthritis: a randomized controlled trial. JAMA. 1999;282(20):1921–8.
55. Emery P, et al. Celecoxib versus diclofenac in long-term management of rheumatoid arthritis: randomised double-blind comparison. Lancet. 1999;354(9196):2106–11.
56. Malmstrom K, et al. Comparison of rofecoxib and celecoxib, two cyclooxygenase-2 inhibitors, in postoperative dental pain: a randomized, placebo- and active-comparator-controlled clinical trial. Clin Ther. 1999;21(10):1653–63.
57. Lanas A. Clinical experience with cyclooxygenase-2 inhibitors. Rheumatology (Oxford). 2002;41(Supp 1):16–22. discussion 35-42.
58. Claria J, Arroyo V. Prostaglandins and other cyclooxygenase-dependent arachidonic acid metabolites and the kidney in liver disease. Prostaglandins Other Lipid Mediat. 2003;72 (1-2):19–33.
59. Bosch-Marce M, et al. Selective inhibition of cyclooxygenase 2 spares renal function and prostaglandin synthesis in cirrhotic rats with ascites. Gastroenterology. 1999;116(5):1167–75.
60. Lopez-Parra M, et al. Cyclooxygenase-1 derived prostaglandins are involved in the maintenance of renal function in rats with cirrhosis and ascites. Br J Pharmacol. 2002;135(4):891–900.
61. Claria J, et al. Effects of celecoxib and naproxen on renal function in nonazotemic patients with cirrhosis and ascites. Hepatology. 2005;41 (3):579–87.
62. Bresalier RS, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med. 2005;352 (11):1092–102.
63. Steinbach G, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med. 2000;342(26):1946–52.
64. Milas L, et al. Enhancement of tumor response to -gamma-radiation by an inhibitor of cyclooxygenase-2 enzyme. J Natl Cancer Inst. 1999;91(17):1501–4.
65. Trifan OC, et al. Cyclooxygenase-2 inhibition with celecoxib enhances antitumor efficacy and reduces diarrhea side effect of CPT-11. Cancer Res. 2002;62 (20):5778–84.

66. Han JA, et al. P53-mediated induction of Cox-2 counteracts p53- or genotoxic stress-induced apoptosis. EMBO J. 2002;21(21):5635–44.
67. Sheng H, et al. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. J Clin Invest. 1997;99(9):2254–9.
68. Kawamori T, et al. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. Cancer Res. 1998;58 (3):409–12.
69. Elder DJ, et al. Induction of apoptotic cell death in human colorectal carcinoma cell lines by a cyclooxygenase-2 (COX-2)-selective nonsteroidal anti-inflammatory drug: independence from COX-2 protein expression. Clin Cancer Res. 1997;3 (10):1679–83.
70. Shishodia S, Koul D, Aggarwal BB. Cyclooxygenase (COX)-2 inhibitor celecoxib abrogates TNF-induced NF-kappa B activation through inhibition of activation of I kappa B alpha kinase and Akt in human non-small cell lung carcinoma: correlation with suppression of COX-2 synthesis. J Immunol. 2004;173 (3):2011–22.
71. Piazza GA, et al. Apoptosis primarily accounts for the growth-inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction. Cancer Res. 1997;57 (12):2452–9.
72. Tanioka T, et al. Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. J Biol Chem. 2000;275 (42):32775–82.
73. Murakami M, Kudo I. Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. Prog Lipid Res. 2004;43 (1):3–35.
74. Tanikawa N, et al. Identification and characterization of a novel type of membrane-associated prostaglandin E synthase. Biochem Biophys Res Commun. 2002;291(4):884–9.
75. Mancini JA, et al. Cloning, expression, and up-regulation of inducible rat prostaglandin e synthase during lipopolysaccharide-induced pyresis and adjuvant-induced arthritis. J Biol Chem. 2001;276(6):4469–75.
76. Thoren S, Jakobsson PJ. Coordinate up- and down-regulation of glutathione-dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells. Inhibition by NS-398 and leukotriene C4. Eur J Biochem. 2000;267(21):6428–34.
77. Murakami M, et al. Cellular prostaglandin E2 production by membrane-bound prostaglandin E synthase-2 via both cyclooxygenases-1 and -2. J Biol Chem. 2003;278(39):37937–47.
78. Trebino CE, et al. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. Proc Natl Acad Sci U S A. 2003;100 (15):9044–9.
79. Engblom D, et al. Microsomal prostaglandin E synthase-1 is the central switch during immune-induced pyresis. Nat Neurosci. 2003;6(11):1137–8.
80. Watkinson G, Akbar FA. Misoprostol in peptic ulcer disease. Prostaglandins. 1987;33(Suppl):78–92.
81. McMahon CG. Erectile dysfunction. Intern Med J. 2014;44(1):18–26.
82. Covert D, Robin AL. Adjunctive glaucoma therapy use associated with travoprost, bimatoprost, and latanoprost. Curr Med Res Opin. 2006;22 (5):971–6.
83. Bai J, Sun Q, Zhai H. A comparison of oxytocin and carboprost tromethamine in the prevention of postpartum hemorrhage in high-risk patients undergoing cesarean delivery. Exp Ther Med. 2014;7(1):46–50.
84. Whittle BJ, et al. Binding and activity of the prostacyclin receptor (IP) agonists, treprostinil and iloprost, at human prostanoid receptors: treprostinil is a potent DP1 and EP2 agonist. Biochem Pharmacol. 2012;84(1):68–75.
85. Galie N, et al. Effects of beraprost sodium, an oral prostacyclin analogue, in patients with pulmonary arterial hypertension: a randomized, double-blind, placebo-controlled trial. J Am Coll Cardiol. 2002;39(9):1496–502.
86. Sawai SK, O'Brien WF. Outpatient cervical ripening. Clin Obstet Gynecol. 1995;38(2):301–9.
87. Narumiya S, FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. J Clin Invest. 2001;108(1):25–30.
88. Dogne JM, et al. Therapeutic potential of thromboxane inhibitors in asthma. Expert Opin Investig Drugs. 2002;11(2):275–81.
89. Straus DS, Glass CK. Cyclopentenone prostaglandins: new insights on biological activities and cellular targets. Med Res Rev. 2001;21 (3):185–210.
90. Forman BM, et al. 15-Deoxy-delta 12, -14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell. 1995;83 (5):803–12.
91. Cernuda-Morollon E, et al. 15-Deoxy-Delta 12,14-prostaglandin J2 inhibition of NF-kappaB-DNA binding through covalent modification of the p50 subunit. J Biol Chem. 2001;276(38):35530–6.
92. Thieringer R, et al. Activation of peroxisome proliferator-activated receptor gamma does not inhibit IL-6 or TNF-alpha responses of macrophages to lipopolysaccharide in vitro or in vivo. J Immunol. 2000;164(2):1046–54.
93. Tsubouchi Y, et al. Feedback control of the arachidonate cascade in rheumatoid synoviocytes by 15-deoxy-Delta(12,14)-prostaglandin J2. Biochem Biophys Res Commun. 2001;283 (4):750–5.
94. Quraishi O, Mancini JA, Riendeau D. Inhibition of inducible prostaglandin E-2 synthase by 15-deoxy-Delta(12,14)-prostaglandin J(2) and polyunsaturated fatty acids. Biochem Pharmacol. 2002;63 (6):1183–9.

95. Gilroy DW, et al. Inflammatory resolution: new opportunities for drug discovery. Nat Rev Drug Discov. 2004;3(5):401–16.
96. Kawahito Y, et al. 15-deoxy-delta(12,14)-PGJ (2) induces synoviocyte apoptosis and suppresses adjuvant-induced arthritis in rats. J Clin Invest. 2000;106(2):189–97.
97. Chatterjee PK, et al. The cyclopentenone prostaglandin 15-deoxy-Delta(12,14)-prostaglandin J2 ameliorates ischemic acute renal failure. Cardiovasc Res. 2004;61(3):630–43.
98. Cuzzocrea S, et al. The cyclopentenone prostaglandin 15-deoxy-delta(12,14)- PGJ2 attenuates the development of colon injury caused by dinitrobenzene sulphonic acid in the rat. Br J Pharmacol. 2003;138(4):678–88.
99. Gilroy DW, et al. Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med. 1999;5(6):698–701.
100. Gilroy DW, et al. Inducible cyclooxygenase-derived 15-deoxy(Delta)12-14PGJ2 brings about acute inflammatory resolution in rat pleurisy by inducing neutrophil and macrophage apoptosis. FASEB J. 2003;17(15):2269–71.
101. Clay CE, et al. Influence of J series prostaglandins on apoptosis and tumorigenesis of breast cancer cells. Carcinogenesis. 1999;20(10):1905–11.
102. Borgeat P, Hamberg M, Samuelsson B. Transformation of arachidonic-acid and homo-gamma-linolenic acid by rabbit polymorphonuclear leukocytes—monohydroxy acids from novel lipoxygenases. J Biol Chem. 1976;251(24):7816–20.
103. Rouzer CA, Matsumoto T, Samuelsson B. Single protein from human-leukocytes possesses 5-lipoxygenase and leukotriene-a4 synthase activities. Proc Natl Acad Sci U S A. 1986;83 (4):857–61.
104. Radmark O, et al. Leukotriene A4 hydrolase in human leukocytes. Purification and properties. J Biol Chem. 1984;259(20):12339–45.
105. Lam BK, Frank Austen K. Leukotriene C4 synthase. A pivotal enzyme in the biosynthesis of the cysteinyl leukotrienes. Am J Respir Crit Care Med. 2000;161 (2 Pt 2):S16–9.
106. Ford-Hutchinson AW, Gresser M, Young RN. 5--Lipoxygenase. Annu Rev Biochem. 1994;63:383–417.
107. Stock NS, et al. 5-Lipoxygenase-activating protein (FLAP) inhibitors. Part 4: development of 3-[3-tert-butylsulfanyl-1-[4-(6-ethoxypyridin-3-yl)benzyl]-5-(5-methylpyridin-2-y lmethoxy)-1H-indol-2-yl]-2,2-dimethylpropionic acid (AM803), a potent, oral, once daily FLAP inhibitor. J Med Chem. 2011;54 (23):8013–29.
108. Bain G, et al. Pharmacodynamics and pharmacokinetics of AM103, a novel inhibitor of 5-lipoxygenase-activating protein (FLAP). Clin Pharmacol Ther. 2010;87(4):437–44.
109. Hakonarson H, et al. Effects of a 5-lipoxygenase-activating protein inhibitor on biomarkers associated with risk of myocardial infarction: a randomized trial. JAMA. 2005;293(18):2245–56.
110. Devillier P, Baccard N, Advenier C. Leukotrienes, leukotriene receptor antagonists and leukotriene synthesis inhibitors in asthma: an update. Part I: synthesis, receptors and role of leukotrienes in asthma. Pharmacol Res. 1999;40(1):3–13.
111. Creticos PS, et al. Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. N Engl J Med. 1984;310(25):1626–30.
112. Werz O. 5-Lipoxygenase: cellular biology and molecular pharmacology. Curr Drug Targets Inflamm Allergy. 2002;1(1):23–44.
113. Nathan RA, Kemp JP, Antileukotriene Working G. Efficacy of antileukotriene agents in asthma management. Ann Allergy Asthma Immunol. 2001;86 (6 Suppl 1):9–17.
114. Kuwabara K, et al. Effects of the second-generation leukotriene B(4) receptor antagonist, LY293111Na, on leukocyte infiltration and collagen-induced arthritis in mice. Eur J Pharmacol. 2000;402(3):275–85.
115. Aiello RJ, et al. Leukotriene B4 receptor antagonism reduces monocytic foam cells in mice. Arterioscler Thromb Vasc Biol. 2002;22(3):443–9.
116. Boileau C, et al. The regulation of human MMP-13 by licofelone, an inhibitor of cyclo-oxygenases and 5-lipoxygenase, in human osteoarthritic chondrocytes is mediated by the inhibition of the p38 MAP kinase signalling pathway. Ann Rheum Dis. 2005;64(6):891–8.
117. Jovanovic DV, et al. In vivo dual inhibition of cyclooxygenase and lipoxygenase by ML-3000 reduces the progression of experimental osteoarthritis: suppression of collagenase 1 and interleukin-1beta synthesis. Arthritis Rheum. 2001;44(10):2320–30.
118. Argentieri DC, et al. Tepoxalin: a dual cyclooxygenase/5-lipoxygenase inhibitor of arachidonic acid metabolism with potent anti-inflammatory activity and a favorable gastrointestinal profile. J Pharmacol Exp Ther. 1994;271 (3):1399–408.
119. Fiorucci S, et al. Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy? Biochem Pharmacol. 2001;62(11):1433–8.
120. Janusz JM, et al. New cyclooxygenase-2/5-lipoxygenase inhibitors. 1. 7-tert-buty1-2,3-dihydro-3,3-dimethylbenzofuran derivatives as gastrointestinal safe antiinflammatory and analgesic agents: discovery and variation of the 5-keto substituent. J Med Chem. 1998;41(7):1112–23.
121. Inagaki M, et al. Novel antiarthritic agents with 1,2-isothiazolidine-1,1-dioxide (gamma-sultam) skeleton: cytokine suppressive dual inhibitors of cyclooxygenase-2 and 5-lipoxygenase. J Med Chem. 2000;43(10):2040–8.

122. Kirchner T, et al. Evaluation of the antiinflammatory activity of a dual cyclooxygenase-2 selective/5-lipoxygenase inhibitor, RWJ 63556, in a canine model of inflammation. J Pharmacol Exp Ther. 1997;282(2):1094–101.
123. Horizoe T, et al. ER-34122, a novel dual 5-lipoxygenase/cyclooxygenase inhibitor with potent anti-inflammatory activity in an arachidonic acid-induced ear inflammation model. Inflamm Res. 1998;47(10):375–83.
124. Horizoe T, et al. Effects of ER-34122, a novel dual 5-lipoxygenase/cyclooxygenase inhibitor, on indices of early articular lesion in MRL/MpJ-lpr/lpr mice. Inflamm Res. 1999;48(8):432–6.
125. Tries S, Neupert W, Laufer S. The mechanism of action of the new antiinflammatory compound ML3000: inhibition of 5-LOX and COX-1/2. Inflamm Res. 2002;51(3):135–43.
126. Laufer SA, et al. (6,7-Diaryldihydropyrrolizin-5-yl) acetic acids, a novel class of potent dual inhibitors of both cyclooxygenase and 5-lipoxygenase. J Med Chem. 1994;37(12):1894–7.
127. Wallace JL, et al. ML 3000 reduces gastric prostaglandin synthesis without causing mucosal injury. Eur J Pharmacol. 1994;271(2-3):525–31.
128. Bertolini A, Ottani A, Sandrini M. Dual acting anti-inflammatory drugs: a reappraisal. Pharmacol Res. 2001;44(6):437–50.
129. Celotti F, Durand T. The metabolic effects of inhibitors of 5-lipoxygenase and of cyclooxygenase 1 and 2 are an advancement in the efficacy and safety of anti-inflammatory therapy. Prostaglandins Other Lipid Mediat. 2003;71(3-4):147–62.
130. Charlier C, Michaux C. Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. Eur J Med Chem. 2003;38(7-8):645–59.
131. Wallace JL, Viappiani S, Bolla M. Cyclooxygenase-inhibiting nitric oxide donators for osteoarthritis. Trends Pharmacol Sci. 2009;30(3):112–7.
132. Brzozowski T, et al. Physiological mediators in non-steroidal anti-inflammatory drugs (NSAIDs)-induced impairment of gastric mucosal defense and adaptation. Focus on nitric oxide and lipoxins. J Physiol Pharmacol. 2008;59 Suppl 2:89–102.
133. Fiorucci S, et al. Nitric oxide (NO)-releasing naproxen (HCT-3012 [(S)-6-methoxy-alpha-methyl-2-naphthaleneacetic Acid 4-(nitrooxy) butyl ester]) interactions with aspirin in gastric mucosa of arthritic rats reveal a role for aspirin-triggered lipoxin, prostaglandins, and NO in gastric protection. J Pharmacol Exp Ther. 2004;311 (3):1264–71.
134. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. Proc Natl Acad Sci U S A. 1991;88(11):4651–5.
135. Wallace JL. Building a better aspirin: gaseous solutions to a century-old problem. Br J Pharmacol. 2007;152(4):421–8.
136. Wang R. Two's company, three's a crowd: can H_2S be the third endogenous gaseous transmitter? FASEB J. 2002;16(13):1792–8.
137. Abe K, Kimura H. The possible role of hydrogen sulfide as an endogenous neuromodulator. J Neurosci. 1996;16(3):1066–71.
138. Zhong G, et al. The role of hydrogen sulfide generation in the pathogenesis of hypertension in rats induced by inhibition of nitric oxide synthase. J Hypertens. 2003;21(10):1879–85.
139. Li L, et al. Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse. FASEB J. 2005;19(9):1196–8.
140. Fiorucci S, et al. The emerging roles of hydrogen sulfide in the gastrointestinal tract and liver. Gastroenterology. 2006;131(1):259–71.
141. Fiorucci S, et al. Inhibition of hydrogen sulfide generation contributes to gastric injury caused by anti-inflammatory nonsteroidal drugs. Gastroenterology. 2005;129(4):1210–24.
142. Claria J, Serhan CN. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. Proc Natl Acad Sci U S A. 1995;92(21):9475–9.
143. Claria J, Lee MH, Serhan CN. Aspirin-triggered lipoxins (15-epi-LX) are generated by the human lung adenocarcinoma cell line (A549)-neutrophil interactions and are potent inhibitors of cell proliferation. Mol Med. 1996;2(5):583–96.
144. Marcus AJ. Transcellular metabolism of eicosanoids. Prog Hemost Thromb. 1986;8:127–42.
145. Chiang N, et al. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. Proc Natl Acad Sci U S A. 2004;101(42):15178–83.
146. Serhan CN, Chiang N. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. Br J Pharmacol. 2008;153 Suppl 1: S200–15.
147. Serhan CN, et al. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med. 2000;192 (8):1197–204.
148. Serhan CN, Petasis NA. Resolvins and protectins in inflammation resolution. Chem Rev. 2011;111 (10):5922–43.

Part II

Clinical Effects and Drug Safety

Efficacy of NSAIDs in the Treatment of Rheumatic Diseases

3

Marc C. Hochberg and Lee S. Simon

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are a chemically diverse group of compounds that share three cardinal characteristics; they are anti-inflammatory, analgesic, and antipyretic [1, 2]. They are approved by regulatory authorities for the treatment of patients with osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and some forms of juvenile idiopathic arthritis. They are also used for the treatment of acute gout, acute pain, dysmenorrhea, and chronic low back pain. There are at least 20 chemically different NSAIDs currently available in Europe and the United States that can be subdivided into two groups, although there is some overlap. These groups include not only the "traditional" nonselective cyclooxygenase (COX) inhibitors that inhibit both the COX-1 and COX-2 enzymes but also the COX-2 selective inhibitors (coxibs, e.g., celecoxib and etoricoxib). In general, traditional nonselective NSAIDs have similar efficacy to one another, although there is variability in an individual patient's response to different NSAIDs. The coxibs have similar efficacy to traditional nonselective NSAIDs in the treatment of patients with rheumatic diseases but a significantly decreased incidence of serious upper gastrointestinal adverse effects [3, 4].

NSAIDs are one of the most commonly used classes of drugs in developed countries. It has been reported that more than 17 million Americans use these agents on a daily basis for the relief of pain and, at times, swelling related to inflammation [5]. With the aging of the US population, the Centers for Disease Control predicted a significant increase in the prevalence of painful arthritis and rheumatic conditions and thus an increased use of NSAIDs. Approximately 60 million NSAID prescriptions are written each year in the United States; the number for elderly patients exceeds those for younger patients by approximately 3.6-fold. Based on calendar year 2013 data for the US, the five most commonly prescribed NSAIDs were ibuprofen followed by meloxicam, naproxen, diclofenac, and celecoxib [6]; more recent unpublished data through May 2015 show that celecoxib and diclofenac have changed positions in the top five list.

All NSAIDs approved by the US Food and Drug Administration carry the same boxed warning for cardiovascular and gastrointestinal risk. These state "NSAIDs may cause an increased risk of serious cardiovascular thrombotic events, myocardial infarction, and stroke, which can be fatal. This risk may increase with duration of use.

M.C. Hochberg, M.D., M.P.H., M.A.C.P., M.A.C.R. (✉)
Departments of Medicine and Epidemiology and Public Health, University of Maryland School of Medicine, 10 S. Pine St., MSTF 8-34, Baltimore, MD 21201, USA
e-mail: mhochber@medicine.umaryland.edu

L.S. Simon, M.D., F.A.C.P., F.A.C.R.
SDG, LLC, Cambridge, MA, USA

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_3

Patients with cardiovascular disease or risk factors for cardiovascular disease may be at greater risk" and "NSAIDs cause an increased risk of serious gastrointestinal adverse events including bleeding, ulceration, and perforation of the stomach or intestines, which can be fatal. These events can occur at any time during use and without warning symptoms. Elderly patients are at greater risk for serious gastrointestinal events," respectively.

The authors recently have reviewed the mechanism of action, clinical pharmacology, and adverse effects of NSAIDs in the treatment of patients with osteoarthritis [7]; the remainder of this chapter will cover the efficacy of this class of agents in the treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and acute gout. We focus on orally administered NSAIDs, as topical NSAIDs are only recommended for patients with mild to moderate pain of osteoarthritis of the hand and knee.

Osteoarthritis

Several professional societies, including the American College of Rheumatology, European League of Associations of Rheumatology, European Society for Clinical and Economic Outcomes in Osteoarthritis (ESCEO), and the Osteoarthritis Research Society International, have published evidence-based recommendations for the management of patients with osteoarthritis [8–13]. All of these professional societies recommend the use of oral NSAIDs in patients with persistent pain and stiffness that have not responded adequately to acetaminophen (paracetamol) with or without concomitant use of topical NSAIDs or, in the ESCEO recommendations [13], the use of the slow-acting symptomatic drugs glucosamine sulfate and chondroitin sulfate which are approved as biologic products in some countries in Europe.

Several recent systematic reviews and meta-analyses have documented the modest efficacy of oral NSAIDs in the treatment of patients with osteoarthritis [14–17]. Myers and colleagues performed a systematic review and network meta-analysis to examine the efficacy of oral NSAIDs as well as duloxetine and opioids in osteoarthritis [14]. They identified randomized controlled trials (RCTs) in patients with osteoarthritis of 12 or more weeks in duration that used the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) as an outcome measure; 32 articles with 47 active treatment arms were included. All four oral NSAIDs studied, ibuprofen, naproxen, celecoxib and etoricoxib, were significantly more efficacious than placebo and were noted to have similar efficacy to duloxetine; note that this study was funded by Eli Lilly and Co., the manufacturer of duloxetine. Kongtharvonskul and colleagues performed a systematic review and network meta-analysis to examine the efficacy of oral NSAIDs as well as glucosamine and diacerein in osteoarthritis [15]. They identified RCTs and quasi-experimental observational studies in patients with osteoarthritis of the knee or hip that used pain score, function, patient and physician global assessments, and joint space width difference as an outcome measure; 31 articles with 40 active treatment arms were included. All oral NSAIDs studied were significantly more efficacious than placebo for pain and were noted to have similar efficacy to both glucosamine and diacerein. van Walsem and colleagues performed a systematic review and network meta-analysis to compare the efficacy of oral NSAIDs for pain in patients with osteoarthritis [16]. They searched MEDLINE, EMBASE, and the Cochrane Library in June 2013 to identify randomized controlled trials (RCTs) in patients with osteoarthritis of 2 or more weeks in duration; 138 studies including patients with osteoarthritis were included in the analysis. All five oral NSAIDs studied, diclofenac, ibuprofen, naproxen, celecoxib and etoricoxib, were significantly more efficacious than placebo for pain. Diclofenac, at a dose of 150 mg per day, had greater than 90 % probability of being more efficacious than acetaminophen and celecoxib at both 6 and 12 weeks; note that Novartis Pharma AG, the manufacturer of diclofenac, funded both the study and the writing of the manuscript.

Bannuru and colleagues performed a systematic review and network meta-analysis to examine

the efficacy of acetaminophen, oral NSAIDs including ibuprofen, naproxen, diclofenac, and celecoxib, as well as intra-articular corticosteroids and hyaluronates in osteoarthritis of the knee; note that the results of this study may be more reliable as it was funded by the Agency for Healthcare Research and Quality and the National Institutes of Health [17]. The investigators searched MEDLINE, EMBASE, Web of Science, Google Scholar, and the Cochrane Central Register of Controlled Trials from inception to 15 August 2014 and performed hand searches of conference proceedings from several professional societies from January 1990 to August 2014. They identified 137 RCTs in patients with osteoarthritis of the knee published between 1980 and 2014 that involved a total of 33,243 randomly assigned participants; 129 RCTs with over 32,000 participants contributed to the analysis of pain-related outcomes and 76 RCTs with over 24,000 participants to physical function outcomes. All oral NSAIDs studied were significantly more efficacious than both placebo and acetaminophen for both pain and function; there were no significant differences in efficacy for pain and function between the individual NSAIDs in pair-wise comparisons.

The choice of the individual NSAID by the individual practitioner is usually based on a combination of relative safety, frequency of administration, and cost, as there is no convincing evidence of superior efficacy for one drug versus another within the class [18, 19]. Since use of all oral NSAIDs, even COX-2-selective inhibitors, is associated with an increased risk for serious upper GI side effects [3, 4], it is recommended that concomitant therapy be given with a proton pump inhibitor especially in patients who are at a moderate-to-high risk for these events. In patients at high risk for serious upper GI side effects who need to receive an oral NSAID, the best option is the combination of a COX-2-selective inhibitor with a proton pump inhibitor. For patients at low risk of a cardiovascular thrombotic event, the decision regarding choice of an NSAID depends on the GI risk. For patients at moderate risk of a cardiovascular thrombotic event, one would consider using naproxen with a proton pump inhibitor as naproxen does not appear to be associated with an increased risk of cardiovascular thrombotic events compared with placebo based on indirect comparisons [4]. For patients at high risk of a cardiovascular thrombotic event, especially those taking low-dose aspirin and with a prior history of a myocardial infarction, cerebrovascular accident, or known stable coronary heart disease, oral NSAIDs should be avoided, and alternative analgesic agents should be used to manage the patient's pain. In addition, oral NSAIDs should be avoided in patients with established moderate-to-severe chronic kidney disease (estimated glomerular filtration rate <45 mL/min) and moderate-to-severe congestive heart failure (New York Heart Association Class III and IV).

Rheumatoid Arthritis

Kvien reviewed the use of NSAIDs in the treatment of rheumatoid arthritis [20]. He noted that several meta-analyses have demonstrated efficacy of both traditional and COX-2-selective NSAIDs in the treatment of rheumatoid arthritis (RA) without evidence of a difference in efficacy between these types of NSAIDs (see also References [21–23]). NSAID-induced efficacy can be demonstrated even when such therapy is superimposed on disease-modifying therapies [24].

Chen and colleagues performed a systematic review of randomized controlled trials to determine the clinical effectiveness and cost-effectiveness of COX-2-selective NSAIDs for rheumatoid arthritis [25]. They searched electronic databases to November 2003 and industry submissions to NICE in 2003. They found that COX-2-selective NSAIDs were more efficacious than placebo and had similar efficacy not only to each other but also to traditional nonselective NSAIDs for the symptomatic relief of rheumatoid arthritis. Economic analyses suggested that the use of COX-2-selective NSAIDs was unattractive from a cost-effectiveness standpoint, although this may change with the availability of generic celecoxib in the US market.

Gotzsche searched the following databases to identify studies: Medline 1966 to September 2009, EMBASE 1980 to September 2009, and the Cochrane Database of Systematic Reviews 2009, Issue 3 (1966 to date of issue); an additional search within the Cochrane Library was carried out for the Database of Abstracts of Reviews of Effects (DARE) and Health Technology Assessment (HTA) [26]. He concluded that there was high-quality evidence that COX-2-selective inhibitors were as effective at reducing signs and symptoms of RA as traditional NSAIDs and that different traditional NSAIDs seemed as effective as each other. An earlier systematic review that included four crossover studies noted that NSAIDs were preferred more often than acetaminophen by both patients and providers [27].

In general, NSAIDs should be used as first-line therapy for the relief of signs and symptoms of RA. They should be administered at their full anti-inflammatory dose (e.g., ibuprofen 800 mg three times daily, naproxen 500 mg twice daily, diclofenac 50 mg three times daily or 75 mg twice daily) in conjunction with a proton pump inhibitor (e.g., omeprazole 20 mg once daily) to reduce the risk of upper gastrointestinal (GI) adverse events. Ibuprofen should be avoided in patients taking low-dose aspirin for cardioprophylaxis, and diclofenac should be avoided in patients taking concomitant methotrexate. If the provider chooses to use a COX-2-selective inhibitor (e.g., celecoxib 200 mg twice daily or etoricoxib 60 mg once daily but not available in the United States), then a proton pump inhibitor is generally not indicated unless the patient has had a prior history of peptic ulcer disease or upper GI bleeding or is particularly elderly or frail. Improvement in pain, stiffness, and physical function should occur within 2–4 weeks; if there is no clinically important improvement after 4 weeks, then the provider should consider switching to another agent. NSAIDs usually do not reduce acute-phase reactants nor have they been demonstrated to modify radiographic progression in patients with RA [28].

Ankylosing Spondylitis

Prior to the availability of the newer biologic therapies, the NSAIDs were the mainstay of therapy for the various forms of axial and peripheral spondyloarthritides. Kroon and colleagues performed a systematic review and meta-analysis to determine the benefits and harms of NSAIDs in axial spondyloarthritis (formerly known as ankylosing spondylitis [AS]) [29]. The authors searched CENTRAL, Medline, and EMBASE through 18 June 2014 to identify randomized controlled trials (RCTs) or quasi-RCTs of NSAIDs versus placebo or any comparator in adults with axial spondyloarthritis and observational cohort studies studying the long-term effect ($\geq$6 months) of NSAIDs on radiographic progression or adverse events. The main comparisons were traditional or COX-2-selective NSAIDs versus placebo. The major outcomes were pain, Bath ankylosing spondylitis disease activity index (BASDAI), Bath ankylosing spondylitis functional index (BASFI), radiographic progression, number of withdrawals due to adverse events (AEs), and number of serious AEs. Two authors independently selected trials for inclusion, assessed the risk of bias, extracted data, and assessed the quality of evidence for major outcomes. They included 39 studies, 35 of which were RCTs, with almost 5000 patients. Traditional NSAIDs were more efficacious than placebo at 6 weeks for pain relief, improving disease activity as measured by BASDAI and physical function as measured by BASFI. Similarly, COX-2-selective inhibitors also were more efficacious than placebo at 6 weeks for these same outcomes. There were no significant differences in benefits between traditional and COX-2-selective NSAID classes for the efficacy endpoints.

In general, NSAIDs should be used as first-line therapy for the relief of signs and symptoms of axial spondyloarthritis [30]. They should be administered at their full anti-inflammatory dose (e.g., indomethacin 50 mg three times daily or 75 mg sustained release twice daily, naproxen 500 mg twice daily, diclofenac 50 mg three

times daily or 75 mg twice daily) in conjunction with a proton pump inhibitor (e.g., omeprazole 20 mg once daily) to reduce the risk of serious upper gastrointestinal adverse events. If the provider chooses to use a COX-2-selective inhibitor (e.g., celecoxib 200 mg twice daily or etoricoxib 90 mg once daily which is not available in the United States), then a proton pump inhibitor is generally not indicated unless the patient has had a prior history of peptic ulcer disease or upper gastrointestinal bleeding. Improvement in pain, stiffness, and physical function should occur within 2–4 weeks; if there is no clinically important improvement after 4 weeks, then the provider should consider switching to another agent or adding a tumor necrosis factor inhibitor. NSAIDs may inhibit radiographic progression in the spine in patients with elevated levels of C-reactive protein [31]. Importantly, the results of a recent RCT suggest that continuous treatment with diclofenac at a dose of 150 mg per day did not reduce radiographic progression compared with on-demand dosing [32].

Acute Gout

Khanna and Fitzgerald recently reviewed recommendations for the treatment of acute gout [33]. The American College of Rheumatology published evidence-based recommendations for the treatment of acute gout in 2012 [34]. They searched PubMed, EMBASE, and the Cochrane Central Register of Controlled Trials through 31 March 2011 and included a total of 26 manuscripts and two meeting abstracts that reported results of randomized controlled trials of treatment of acute gout. They recommended initiating monotherapy with full-dose oral NSAIDs, systemic glucocorticoids or colchicine. The task force did not preferentially recommend any one specific NSAID as first-line treatment, although the strongest evidence for efficacy was present for indomethacin, which had been shown to be similarly efficacious to glucocorticoids and was used as the positive control in non-inferiority trials of etoricoxib and lumiracoxib. The task force recommended that full-dose oral NSAID should be continued until the gout attack has completely resolved. These recommendations are consistent with those from the British Society of Rheumatology, EULAR, and the 3e initiative [35–37]. There appears to be no clinically important difference in efficacy between traditional nonselective NSAIDs, COX-2-selective inhibitors, and glucocorticoids for the treatment of acute gout [38–41].

As with osteoarthritis, full-dose NSAIDs should not be used for treating acute attacks of gout in patients with a known history of cardiovascular thrombotic disease, poorly controlled hypertension, moderate-to-severe chronic kidney disease, or moderate-to-severe congestive heart failure; these comorbidities are particularly common in this patient population [42].

Conclusion

NSAIDs are known to be analgesic, anti-inflammatory, and antipyretic and are efficacious for the management of pain and other symptoms in patients with rheumatic diseases. Their role in management of patients continues to be controversial, however, largely because of common and potentially severe adverse effects as well as the observation that they are not disease modifying in patients with rheumatoid arthritis. Numerous clinical practice guidelines and recommendations provide help to the practicing clinician in her decision about their use in the treatment of the individual patient with rheumatic disease; in addition, quality indicators related to the safe use of NSAIDs in clinical practice have been published [43, 44].

References

1. Day RO, Graham GG. Non-steroidal antiinflammatory drugs (NSAIDs). BMJ. 2013;346:f3195.
2. Patrono C. Non-steroidal anti-inflammatory drugs. Chapter 53 in Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. Rheumatology. 6th ed. Philadelphia: Mosby/Elsevier; 2015. p. 415–22.

3. Castellsague J, Riera-Guardia N, Calingaert B, Varas-Lorenzo C, Fourrier-reglat A, Nicotra F, et al. Individual NSAIDs and upper gastrointestinal complications: a systematic review and meta-analysis of observational studies (the SOS project). Drug Saf. 2012;35:1127–46.
4. Coxib and traditional NSAID Trialists' (CNT) Collaboration. Vascular and upper gastrointestinal effects of nonsteroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomized trials. Lancet. 2013;382:769–79.
5. Baum C, Kennedy DL, Forbes MB. Utilization of nonsteroidal anti-inflammatory drugs. Arthritis Rheum. 1985;28:686–91.
6. Aitken M, Kleinrock M, Lyle J, Caskey L. Medicine use and shifting costs of healthcare: a review of the use of medicines in the United States in 2013. Parsinnapany, NJ: IMS Institute for Healthcare Informatics; 2014.
7. Simon LS, Hochberg MC. Nonsteroidal anti-inflammatory drugs. In: Biljsma JWJ, Doherty M, Lohmander S, editors: Oxford textbook of osteoarthritis and crystal arthropathy. 3rd ed. Oxford: Oxford University Press; 2016, in press.
8. Jordan KM, Arden NK, Doherty M, Bannwarth B, Bijlsma JW, Dieppe P, et al. EULAR recommendations 2003: an evidence based approach to the management of knee osteoarthritis. Ann Rheum Dis. 2003;62:1145–55.
9. Zhang W, Doherty M, Arden N, Bannwarth B, Bijlsma J, Gunther KP, et al. EULAR evidence based recommendations for the management of hip osteoarthritis. Ann Rheum Dis. 2005;64:669–81.
10. Hochberg MC, Altman RD, April KT, Benkhalti M, Guyatt G, McGowan J, et al. American College of Rheumatology 2012 recommendations for the use of nonpharmacologic and pharmacologic therapies in osteoarthritis of the hand, hip, and knee. Arthritis Care Res (Hoboken). 2012;64:465–74.
11. McAlindon TE, Bannuru RR, Sullivan MC, Arden NK, Berenbaum F, Bierma-Zeinstra SM, et al. OARSI guidelines for the non-surgical management of knee osteoarthritis. Osteoarthritis Cart. 2014;22:363–88.
12. National Clinical Guideline Centre (UK). Osteoarthritis: care and management in adults. London: National Institute for Health and Care Excellence. 2014 Feb.
13. Bruyere O, Cooper C, Pelletier JP, Branco J, Luisa Brandi M, Guillemin F, et al. An algorithm recommendation for the management of knee osteoarthritis in Europe and internationally: a report from a task force of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). Semin Arthritis Rheum. 2014;44 (3):253–63.
14. Myeers J, Wielage RC, Han B, Price K, Gahn J, Paget M-A, et al. The efficacy of duloxetine, non-steroidal anti-inflammatory drugs, and opioids in osteoarthritis: a systematic literature review and meta-analysis. BMC Musculoskelet Disord. 2014;15:76.
15. Kongtharvonskul J, Anothaisintawee T, McEvoy M, Attia J, Woratanarat P, Thankkinstrian A. Efficacy and safety of glucosamine, diacerein, and NSAIDs in osteoarthritis knee: a systematic review and network meta-analysis. Eur J Med Res. 2015;20:24.
16. van Walsen A, Pandhi S, Nixon RM, Guyot P, Karabis A, Moore RA. Relative benefit-risk comparing diclofenac to other traditional non-steroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors in patients with osteoarthritis or rheumatoid arthritis: a network meta-analysis. Arth Res Ther. 2015;17:66.
17. Bannuru RR, Schmid CH, Kent DM, Vaysbrot EE, Wong JB, McAlindon TE. Comparative effectiveness of pharmacologic interventions for knee osteoarthritis: a systematic review and network meta-analysis. Ann Intern Med. 2015;162:46–54.
18. Burmester G, Lanas A, Biasucci L, Hermann M, Lohmander S, Olivieri I, et al. The appropriate use of non-steroidal anti-inflammatory drugs in rheumatic disease: opinions of a multidisciplinary European expert panel. Ann Rheum Dis. 2011;70(5):818–22.
19. Scarpignato C, Lanas A, Blandizzi C, Lems WF, Hermann M, Hunt RH, for the International NSAID Consensus Group. Safe prescribing of non-steroidal anti-inflammatory drugs in patients with osteoarthritis: an expert consensus addressing benefits as well as gastrointestinal and cardiovascular risks. BMC Med. 2015;13:55.
20. Kvien TK. Nonsteroidal anti-inflammatory drugs and coxibs. In: Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH, editors. Rheumatoid arthritis. Philadelphia: Mosby/Elsevier; 2009. p. 295–9.
21. Gotzsche PC. Non-steroidal anti-inflammatory drugs. BMJ. 2000;320(7241):1058–61.
22. Garner S, Pidan D, Frankish R, et al. Celecoxib for rheumatoid arthritis. Cochrane Database Syst Rev. 2002;4, CD003831.
23. Garner S, Pidan D, Frankish R, et al. Rofecoxib for rheumatoid arthritis. Cochrane Database Syst Rev. 2005;1, CD003685.
24. Simon LS, Weaver AL, Graham DY, Kivitz AJ, Lipsky PE, Hubbard RC, Isakson PC, Verburg KM, Shawn SY, Zhao WW, Geis GS. The antiinflammatory and upper gastrointestinal effects of celecoxib in rheumatoid arthritis: a randomized, controlled trial. JAMA. 1999;282:1921–8.
25. Chen Y-F, Jobanputra P, Barton P, Bryan S, Fry-Smith A, Harris G, et al: Cyclooxygenase-2-selective non-steroidal anti-inflammatory drugs (etodolac, meloxicam, celecoxib, rofecoxib, etoricoxib, valdecoxib and lumiracoxib) for osteoarthritis and rheumatoid arthritis: a systematic review and economic evaluation. Health Technol Assess 2008;12(11):1–278.
26. Gotzsche PC. NSAIDs. BMJ Clin Evid. 2010 Jun 28; pii 1108.

27. Wienecke T, Gotzsche PC. Paracetamol versus nonsteroidal anti-inflammatory drugs for rheumatoid arthritis. Cochrane Database Syst Rev. 2004;1, CD003789.
28. Crofford L. Use of NSAIDs in treating patients with arthritis. Arthritis Res Ther. 2013;15 Suppl 3:S2 [Epub 2013 Jul 24].
29. Kroon FPB, van der Burg LRA, Ramiro S, Landewe RBM, Buchbinder R, Falzon L, van der Heijde D. - Non-steroidal anti-inflammatory drugs (NSAIDs) for axial spondyloarthritis (ankylosing spondylitis and non-radiographic axial spondyloarthritis). Cochrane Database Syst Rev. 2015;7, CD010952.
30. Poddubnyy D, van der Heijde D. Therapeutic controversies in spondyloarthritis: nonsteroidal anti-inflammatory drugs. Rheum Dis Clin North Am. 2012;38(3):601–11.
31. Poddubnyy D, Rudwaleit M, Haibel H, Listing J, Marker-Hermann E, Zeidler H, Bruan J, Sieper J. Effect of non-steroidal anti-inflammatory drugs on radiographic spinal progression in patients with axial spondyloarthritis: results from the German Spondyloarthritis Inception Cohort. Ann Rheum Dis. 2012;71(10):1616–22.
32. Sieper J, Listing J, Poddubnyy D, Song IH, Hermann KG, Callhoff J, Syrbe U, Braun J, Rudwaleit M. Effect of continuous versus on-demand treatment of ankylosing spondylitis with diclofenac over 2 years on radiographic progression of the spine: results from a randomised multicenter trial (ENRADAS). Ann Rheum Dis. 2016;75(8):1438–43.
33. Khanna PP, FitzGerald J. Evolution of management of gout: a comparison of recent guidelines. Curr Opin Rheumatol. 2015;27:139–46.
34. Khanna D, Khanna PP, FitzGerald JD, Singh MK, Bae S, Neogi T, et al. 2012 American College of Rheumatology guidelines for management of gout. Part II: therapy and anti-inflammatory prophylaxis of acute gouty arthritis. Arthritis Care Res. 2012;64:1447–61.
35. Jordan KM, Cameron JS, Snaith M, et al. British Society for Rheumatology and British Health professionals in Rheumatology guideline for the management of gout. Rheumatology (Oxford). 2007;46:1372–4.
36. Sivera F, Andres M, Carmona L, et al. Multinational evidence-based recommendations for the diagnosis and management of gout: integrating systematic literature review and expert opinion of a broad panel of rheumatologists in the 3e initiative. Ann Rheum Dis. 2014;73:328–35.
37. Zhang W, Doherty M, Bardin T, et al. EULAR evidence based recommendations for gout. Part II: management. Report of a task force of the EULAR Standing Committee for International Clinical Studies including Therapeutics (ESCISIT). Ann Rheum Dis. 2006;65:1312–24.
38. Khanna PP, Gladue HS, Singh MK, et al. Treatment of acute gout: a systematic review. Semin Arthritis Rheum. 2014;44:31–8.
39. Wechalekar MD, Vinik O, Moi JH, et al. The efficacy and safety of treatments for acute gout: results from a series of systematic literature reviews including Cochrane reviews on intraarticular glucocorticoids, colchicine, nonsteroidal anti-inflammatory drugs, and interleukin-1 inhibitors. J Rheumatol. 2014;41 Suppl 92:15–25.
40. van Durme CM, Wechalekar MD, Buchbinder R, Schlesinger N, van der Heijde D, Landewe RB. - Non-steroidal anti-inflammatory drugs for acute gout. Cochrane Database Syst Rev. 2014;9, CD010120.
41. van Durme CM, Wechalekar MD, Landewe RB. Nonsteroidal anti-inflammatory drugs for treatment of acute gout. JAMA. 2015;313:2276–7.
42. Keenan RT, O'Brien WR, Lee KH, Crittenden DB, Fisher MC, Goldgarb DS, Krasnokutsky S, Oh C, Pillinger MH. Prevalence of contraindications and prescription of pharmacologic therapies for gout. Am J Med. 2011;124:155–63.
43. Saag KG, Olivieri JJ, Patino F, Mikuls TR, Allison JJ, MacLean CH. Measuring quality in arthritis care: the Arthritis Foundations's quality indicator set for analgesics. Arthritis Rheum. 2004;51:337–49.
44. Risser A, Donovan D, Heintzman J, Page T. NSAID prescribing precautions. Am Fam Phys. 2009;80:1371–8.

Adverse Effects of NSAIDs in the Gastrointestinal Tract: Risk Factors of Gastrointestinal Toxicity with NSAIDs

4

Sunny H. Wong and Francis K.L. Chan

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) belong to a class of drugs that has potent analgesic and anti-inflammatory effects. They act mainly by inhibiting cyclooxygenase (COX) enzymes including COX-1 and COX-2, which are involved in prostaglandin synthesis. This class of medications includes the traditional, non-selective NSAIDs that inhibit both COX-1 and COX-2, and the newer selective COX-2 inhibitors. They are highly effective in treating various painful conditions such as osteoarthritis and dysmenorrhoea [1]. Unlike other NSAIDs, aspirin is used primarily for its anti-platelet effect to reduce cardiothrombotic risks.

Though being commonly prescribed, these drugs carry a substantial risk of gastrointestinal toxicity. They can have serious adverse gastrointestinal side effects, including peptic ulcers, bleeding or perforation. Ulcers are found on upper gastrointestinal endoscopy in up to one-third of regular NSAID users, and every year about 1–2 % of these patients develop symptoms or complications [2]. In addition to peptic ulcers, NSAIDs can also cause injury to the lower gastrointestinal tract, and more than half of chronic NSAID users have endoscopic evidence of small bowel mucosal injury [3].

With the declining incidence of *Helicobacter pylori* in many countries, aspirin and other NSAIDs have emerged as an important cause of peptic ulcer and its complications. The magnitude of the problem is further contributed by a changing disease epidemiology, with an ageing population and an increased usage of aspirin and other NSAIDs for many various medical conditions. These have resulted in a different disease pattern among patients seen in gastroenterology units. This chapter focuses on the gastrointestinal toxicity and risk factors of NSAIDs.

Epidemiology

NSAIDs are among the most commonly prescribed medications worldwide. It is estimated that NSAIDs are used by more than 30 million people every day [4]. More than 111 million prescriptions were written for NSAIDs annually in the United States [5], whereas in Europe, NSAIDs represented more than 7.7 % of all prescriptions [6].

Many studies have reported the secular trends of gastrointestinal complications associated with NSAIDs. In a population-based study in

S.H. Wong, M.B.Ch.B. (Hons.), D.Phil.
F.K.L. Chan, M.D., D.Sc. (✉)
Institute of Digestive Disease, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Science, and Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, 30-32 Ngan Shing Street, Shatin, Hong Kong, China
e-mail: wonghei@cuhk.edu.hk; fklchan@cuhk.edu.hk

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_4

Denmark, the proportion of NSAID-related peptic ulcers increased from 39 % in 1993 to 53 % in 2002 [7]. In Scotland, the rise in the incidence of upper gastrointestinal bleeding was related to the increasing use of aspirin and other NSAIDs to a lesser extent [8]. Studies have shown that NSAIDs and aspirin have accounted for most cases of serious complicated gastroduodenal ulcers [9, 10].

Apart from upper gastrointestinal adverse events, there is evidence that NSAID-related toxicity can also occur in the lower gastrointestinal tract [11]. A population-based study in Spain showed a decreasing trend in upper gastrointestinal events; but conversely, there has been an increasing trend in lower gastrointestinal events leading to hospitalization between 1996 and 2005 [12]. Though more common among patients with upper gastrointestinal events, NSAID use was also recorded in 11.1 % of patients with lower gastrointestinal events [12]. Two randomized clinical trials have found that the rates of upper and lower gastrointestinal complications were no different among NSAID users [2, 13].

Pathogenesis

The gastrointestinal toxicity of NSAIDs is mediated largely through the inhibition of cyclooxygenase (COX) enzyme activity, resulting in a suppression of prostaglandin synthesis [14]. The precursor of prostaglandins, arachidonic acid, is catalysed by two isoenzymes COX-1 and COX-2. The gene for COX-1 is expressed constitutively, and it helps to maintain the integrity of the gastric epithelium and the mucous barrier, whereas the gene for COX-2 contains a corticosteroid-responsive repressor element and is inducible by inflammatory stimuli. As such, the analgesic and anti-inflammatory properties are mediated through the inhibition of COX-2, whereas the gastrointestinal toxicities are mediated through the inhibition of COX-1 [15]. Conventional NSAIDs inhibit both isoenzymes, including the irreversible blockage by aspirin, whereas newer NSAIDs target COX-2 selectively to reduce the gastrointestinal side effects mediated by inhibition of COX-1.

Apart from the COX inhibition, other pathogenic mechanisms have been suggested for NSAID-induced gastropathy. Aspirin and other NSAIDs can diffuse through the gastric mucus into surface epithelial cells, where they dissociate into ionized forms and trap hydrogen ions to cause mucosal damage [16]. These drugs can also directly alter cell morphology and membrane permeability to mediate the cytotoxicity without involving the COX pathway [17, 18]. Furthermore, mast cells have been shown to have a critical role in the repair of NSAID-induced gastric injury. Mast-cell-deficient mice have been found to develop more gastric ulcers after exposure to piroxicam, a non-selective NSAID, compared to wild-type and tumour necrosis factor (TNF)-deficient mice [19]. These mechanisms may provide insight for the development of novel treatments for NSAID-induced gastropathy.

NSAIDs can also cause significant injury in the small and large intestines. Similar to the stomach, the decrease in prostaglandin production after NSAIDs can disrupt the intestinal homeostasis including mucosal blood flow and permeability [20]. In addition, NSAIDs can cause direct damage to the enterocytes through uncoupling of mitochondrial oxidative phosphorylation [21], and the enterohepatic recirculation of NSAIDs further enhances the cytotoxicity [22]. The gut microbiota has also been implicated in the pathogenesis, as germ-free mice do not develop intestinal ulcers but would become susceptible when colonized with commensal microbiota [23, 24].

Gastrointestinal Toxicity of NSAIDS

Upper Gastrointestinal Tract

Multiple studies have shown that traditional NSAIDs increase the risk of upper gastrointestinal ulcers and complications by about fourfold [25–28]. Gastrointestinal injuries such as

sub-epithelial haemorrhage, erosions or ulcerations can occur in 30–50 % of patients taking NSAIDs, although most lesions are asymptomatic [2, 29]. Symptomatic peptic ulcers occur in one of every 20 NSAID users and in one of seven elderly adults using NSAIDs [30]. The annual incidence of an NSAID-related upper gastrointestinal event is 2.0–4.5 %, and the risk of severe complications is 0.2–1.9 % [30–32]. The risk appears to be highest among new users in the first two months, but remains elevated until two months after cessation of NSAID treatment [26]. NSAIDs that have a long half-life and profound inhibition of both COX isozymes, such as piroxicam and naproxen, are associated with a greater risk of upper gastrointestinal events [33]. A clear dose-dependent increase in risk was observed, with a meta-analysis reporting a risk ratio of 3.0 for low-dose and 6.9 for high-dose use of NSAIDs, respectively [26].

Aspirin, like the other traditional NSAIDs, inhibits the COX enzymes and is associated with gastrointestinal risks. The use of low-dose aspirin is associated with a 1.5- to 3.2-fold greater risk of an upper gastrointestinal event [34]. The absolute risk is increased by 0.12 % per year with a number needed to harm of 833 patients [34]. The gastric antrum and prepyloric area are the most frequent areas of mucosal injury [35].

Lower Gastrointestinal Tract

Apart from the upper gastrointestinal tract, aspirin and other NSAIDs can also damage the lower gastrointestinal tract distal to the ligament of Treitz, to a similar or even greater extent [2, 36]. NSAIDs induce small intestinal and colonic injury with a wide spectrum of manifestations, from clinically silent mucosal injury to significant ulcerations with bleeding, intestinal obstruction or perforation [2, 37]. The mucosal injuries, collectively known as NSAID enteropathy, may result in increased intestinal permeability, inflammation and chronic blood loss.

The availability of video capsule endoscopy has allowed studies on the incidence of NSAID-induced small intestinal injuries. Capsule endoscopy showed that NSAIDs could induce mucosal injury in 75 % and macroscopic ulcers in 40 % of patients [3, 38]. The mucosal injury appeared to be milder in patients taking low-dose aspirin [39, 40]. Colonic erosions, ulcers or even diaphragmatic strictures have also been observed in NSAID users during colonoscopy studies [41–44].

To capture clinically significant events in the gastrointestinal tract, a composite endpoint combining gastroduodenal, small bowel or large bowel haemorrhage or perforation, gastric outlet obstruction or clinically significant anaemia of defined or presumed gastrointestinal origin was developed [45, 46]. In the 6-month study period with 2,246 patients receiving diclofenac and omeprazole, 81 (3.8 %) patients met the composite endpoint with the majority developing anaemia of defined or presumed gastrointestinal origin. This may have explained the increasing number of hospitalizations due to complications of the lower gastrointestinal tract, whereas the corresponding numbers for upper gastrointestinal complications are decreasing [12].

Other Gastrointestinal Symptoms

Apart from these complications, NSAIDs can commonly cause gastrointestinal symptoms like dyspepsia, nausea, vomiting and abdominal pain in up to 40 % of patients [47]. In another study involving 986 patients taking low-dose aspirin, about 15 % reported upper gastrointestinal symptoms [48]. These symptoms are not predictive of an ulcer or complication, and about 50–60 % of patients with complications do not have a warning symptom [49] (Table 4.1).

Table 4.1 Risk factors for NSAID ulcers

Concomitant use of multiple NSAIDs (including aspirin)
Concomitant use with anticoagulants
Concomitant use with corticosteroids
Helicobacter pylori infection
Past history of complicated ulcers Advanced age

Risk Factors for Gastrointestinal Toxicity

The risk of ulcer complications increases by approximately fourfold in patients taking NSAIDs [25, 50–54], particularly in patients with multiple risk factors. These risk factors include advanced age, concomitant use with aspirin, anticoagulants or corticosteroids, history of a complicated ulcer and *H. pylori* infection [55]. Advanced age has consistently found to be a primary risk factor for adverse gastrointestinal events, and the risk appears to increase linearly at about 4 % per year of age [50, 52, 56, 57].

Concomitant Use of Aspirin and Another NSAID

With the increasing use of aspirin for various cardiovascular indications, concomitant use of aspirin and another NSAID is common, and this predisposes to an elevated risk of ulcer complications. An early study showed that the odds ratio of ulcer bleeding in patients on concomitant aspirin and another NSAID was about 7.7, compared to 3.3 and 4.9 in patients who took aspirin or an NSAID alone, respectively [58]. A similar risk profile was observed in a recent population-based study involving 114,835 patients with upper gastrointestinal bleeding, with an approximately twofold increase in the incidence of bleeding among patients taking aspirin and another NSAID concomitantly [27].

Concomitant Use with Anticoagulants

Although there is no evidence that anticoagulant increases the risk of developing an NSAID-related ulcer, concomitant use of an NSAID and anticoagulant does increase the risk of ulcer bleeding. Compared to non-users of either drug, there was a nearly 13-fold increase in the risk of developing peptic ulcer bleeding in concurrent users of oral anticoagulants and NSAIDs [59]. In a recent database analysis of 114,835 patients with upper gastrointestinal bleeding in Europe, the combination of anticoagulants with aspirin and other traditional NSAIDs had an incidence rate ratio of 8.7 and 6.9, respectively [27].

Over the past decade, novel anticoagulants including dabigatran, rivaroxaban, apixaban and edoxaban have gained acceptance in clinical practice for a variety of cardiovascular indications. Although these novel anticoagulants can effectively reduce cardiovascular risk and do not require typical blood test monitoring, some of them are associated with an increased risk of gastrointestinal bleeding [60–62]. A systematic review and meta-analysis in 2013 showed a pooled odds ratio of 1.45 for gastrointestinal bleeding associated with these novel oral anticoagulants compared with standard therapy [63]. In particular, dabigatran was consistently associated with an increased risk of major gastrointestinal bleeding, especially in African Americans and chronic kidney disease patients [64]. While these novel anticoagulants may not directly cause mucosal injury or act synergistically with NSAIDs, these may increase the risk of gastrointestinal bleeding even more so than warfarin [65–67]. NSAIDs should be prescribed with extreme caution in patients undergoing anticoagulation therapy.

Concomitant Use with Corticosteroids

There is still a debate whether systemic corticosteroid alone can cause peptic ulcer directly [68–70]. In a recent database study, the use of corticosteroid alone was associated with upper gastrointestinal bleeding at a similar magnitude as that with non-selective NSAIDs [27]. Concomitant use of NSAIDs and corticosteroids greatly increases the risk of ulcers and complications. This might be due to the inhibition of ulcer healing [71], as direct drug interaction between the two drugs has not been consistently observed [72]. In a previous study, concomitant use of NSAIDs and corticosteroids increased the ulcer risk by about fourfold, compared with NSAIDs alone [73, 74]. There was also an increased risk of major upper gastrointestinal bleeding [27, 72] and perforation [75, 76].

Helicobacter pylori Infection

Helicobacter pylori infection is an independent risk factor for upper gastrointestinal injury. Their interaction is complex and has been subject to much controversy. *H. pylori* can induce inflammation on the gastric mucosa that has already been injured due to the inhibition of COX-1. Previous meta-analyses and systematic reviews have shown that the presence of *H. pylori* infection is associated with an increased risk of NSAID-related gastrointestinal complications [54, 77]. The presence of *H. pylori* and NSAIDs increased the risk of ulcer bleeding by more than sixfold, whereas *H. pylori* alone and NSAIDs increased the risk by about 1.8- and 4.8-fold, respectively [54]. The risk of ulcer is highest during the initiation of NSAID therapy, thereafter, the risk falls substantially but remains present with long-term use [25, 50, 52, 53]. Eradication of *H. pylori*, especially in NSAID-naïve users, is beneficial in preventing peptic ulcers and complications [78, 79].

H. pylori may play an even more significant role in low-dose aspirin users than in other traditional NSAID users [80]. In a randomized study of low-dose aspirin users receiving either eradication therapy or omeprazole for 6 months, eradication of *H. pylori* was as effective as the omeprazole eradication group in preventing recurrent bleeding [81]. The risk of recurrent bleeding with low-dose aspirin is low after *H. pylori* eradication, and these patients may not require antiulcer prophylaxis in the absence of other risk factors [82].

Past History of Complicated Ulcers

For reasons not completely understood, having a history of ulcer complications is the most important risk factor for NSAID-related ulcer complications. The odds of complications were estimated to be 13-fold compared to NSAID users with no prior ulcer complications [51]. There is some indirect evidence that ulcers tend to recur at their previous locations [83, 84]. This suggests that local factors, such as a weakened mucosal barrier at the site of previous ulceration, might be important.

Stratification by Gastrointestinal Risks

Given the vast number of people taking aspirin and NSAIDs for various cardiovascular and musculoskeletal conditions, having a proper risk assessment and risk reduction strategy would be important in managing these patients. Based on the MUCOSA trial [85], the gastrointestinal risk of ulcer complications can be grouped into three categories: low risk (no risk factors), moderate risk (one or two risk factors) and high risk (more than two risk factors, previous ulcer complications or concomitant use of aspirin, corticosteroids or anticoagulants). The annual incidence of NSAID-related ulcer complications was 0.8 % in patients with low risk but more than 7.6 % in patients with high risk. Furthermore, apart from the gastrointestinal risk, it is important to consider the cardiovascular risk as these medications can alter the cardiovascular risk.

Strategies to Reduce NSAID-Associated Ulcers

Two main strategies have been developed to prevent the development of gastrointestinal mucosal injury in NSAID users: co-therapy with a gastro-protective agent or substitution of a traditional NSAID with a COX-2 inhibitor. These gastro-protective agents may include a histamine-2 receptor antagonist (H2RA), misoprostol or a proton-pump inhibitor (PPI). Systematic reviews have shown that these strategies are variably effective to reduce the risk of NSAID-related ulcers and complications [86, 87].

Histamine-2 Receptor Antagonist

In a pooled analysis of five randomized controlled trials of H2RAs for the prevention of NSAID-induced peptic ulcers, standard doses of H2RA reduced the incidence of duodenal ulcers

but not gastric ulcers [86]. Other randomized trials also found a low rate of endoscopic ulcers in NSAID users taking a high-dose H2RA regimen [88–90]; nevertheless, whether high-dose H2RAs prevent ulcer complications is not clear. A meta-analysis showed that H2RAs might not significantly reduce the risk of symptomatic ulcers among patients receiving NSAIDs [91]. As for aspirin-related injury, a study showed that famotidine is effective in preventing gastric and duodenal ulcers in patients taking low-dose aspirin [92].

Misoprostol

The efficacy of misoprostol in preventing NSAID-induced ulcers has been evaluated in several randomized trials [32, 93, 94]. A meta-analysis indicated that all doses of misoprostol (400–800 μg per day) could reduce the risk of NSAID-induced ulcers on endoscopy [86, 95]. Misoprostol appears superior to H2RA in reducing gastric ulcers, with a lower rate of endoscopic ulcers in NSAID users receiving misoprostol than those receiving ranitidine [96].

Furthermore, full-dose misoprostol (800 μg per day) has been shown effectively to reduce ulcer complications [32]. In a randomized trial with rheumatoid arthritis patients receiving NSAIDs, misoprostol lowered the rate of ulcer complications by 40 % [32]. Despite its efficacy, up to 30 % of patients who received misoprostol experienced gastrointestinal upset, causing limitation to its clinical use. Even though endoscopic studies had suggested that lower doses of misoprostol (400–600 μg per day) could prevent NSAID-induced ulcers with fewer side effects [94], such low doses of misoprostol failed to prevent ulcer complications in high-risk patients [93].

Proton-Pump Inhibitors

The risk of peptic ulcer in long-term NSAID users can be significantly reduced by PPIs [86, 95]. In two previous randomized studies comparing omeprazole 20 mg daily with either standard-dose ranitidine (150 mg twice daily) and half-dose misoprostol (200 μg twice daily), omeprazole was more effective than standard-dose ranitidine and comparable with half-dose misoprostol in preventing endoscopic ulcers, especially with duodenal ulcers [97, 98]. A randomized trial compared two doses of esomeprazole (40 and 20 mg once daily) with standard-dose ranitidine for the healing of gastric ulcers in patients who continued to receive NSAIDs. Both doses of esomeprazole were superior to ranitidine [99]. Although full-dose misoprostol (200 μg 4 times daily) appeared to be more effective than lansoprazole in preventing gastric ulcers in long-term NSAID users without *H. pylori* infection, it offered no practical advantage over lansoprazole due to the high withdrawal rate in the misoprostol group [100].

In two multicentre randomized studies involving at-risk patients taking non-selective NSAIDs and COX-2 inhibitors, esomeprazole was highly effective in preventing ulcers. The overall 6-month ulcer rates were 17.0 %, 5.2 % and 4.6 % in patients receiving placebo, esomeprazole 20 mg and esomeprazole 40 mg, respectively [101]. Other PPIs appeared to be similarly effective, with ulcer-free rates of 91–95 % observed in long-term NSAID users receiving pantoprazole or omeprazole for ulcer prevention in another endoscopic study [102].

One randomized study has investigated the role of PPIs in reducing the risk of NSAID-related ulcer bleeding. In the study, long-term omeprazole therapy was compared to *H. pylori* eradication therapy for the prevention of recurrent ulcer bleeding in *H. pylori*-infected patients with a recent history of NSAID-related ulcer bleeding who continued to use naproxen [84]. Recurrent ulcer bleeding occurred in 18.8 % of patients undergoing eradication therapy, compared with only 4.4 % of patients receiving omeprazole. The efficacy of PPIs was further supported by an observational study, in which PPI therapy was found to be associated with a significant reduction in the risk of upper gastrointestinal bleeding among chronic NSAID users [103].

The fact that PPIs reduce, but do not eliminate, the risk of NSAID-induced ulcer complications suggests that acid-peptic injury may not be the only mechanism involved in ulcer pathogenesis. Though rarely discussed, NSAIDs can produce ulcers in achlorhydric patients [104]. It is uncertain whether PPIs remain effective in achlorhydric patients or patients with an altered anatomy due to previous gastric surgery. Furthermore, while PPIs can reduce peptic ulcers and complications in patients taking aspirin or other NSAIDs, their beneficial effect is not expected beyond the duodenum [105]. The use of omeprazole could not prevent NSAID-induced intestinal damage, either in healthy individuals [38, 106, 107] or patients [3] evaluated by video capsule or faecal calprotectin measurement. A previous study suggested that PPIs might even worsen the NSAID-induced intestinal damage, through disruption of the small bowel microbial ecology [108].

COX-2 Inhibitors

The discovery of the two COX isoforms and understanding of their functions have allowed development of highly selective COX-2 inhibitors to achieve their therapeutic effects while minimizing gastrointestinal toxicity. Previous randomized controlled trials [30, 109] and a systematic review [87] showed that when compared to non-selective NSAIDs, the COX-2 inhibitors led to significantly fewer gastroduodenal ulcers and ulcer complications, as well as fewer withdrawals caused by gastrointestinal symptoms. The relative risk was 0.26 for gastroduodenal ulcers and 0.39 for ulcer complications [87]. The COX-2 inhibitors appeared as effective as a combination of non-selective NSAIDs with a PPI in patients at risk for ulcers. In a randomized study comparing diclofenac plus omeprazole versus celecoxib for secondary prevention of ulcer bleeding, a similar proportion of patients had recurrent bleeding at 6 months [110]. Nevertheless, neither treatment can eliminate the risk of recurrent bleeding in high-risk patients. Concomitant use of low-dose aspirin can also increase the gastrointestinal risks of COX-2 inhibitors to a rate similar to that of NSAIDs alone [109, 111]. In another randomized trial comparing celecoxib alone versus celecoxib plus esomeprazole in patients with a history of NSAID-induced ulcer bleeding, 8.9 % of the celecoxib-alone group had recurrent ulcer bleeding compared with none of the combined therapy group [110]. The combination of a COX-2 inhibitor and a PPI or misoprostol probably offers the best gastrointestinal protection for very high-risk patients, although this approach remains to be proven in prospective trials.

As with non-selective NSAIDs, COX-2 inhibitors can damage the lower gastrointestinal tract, although the frequency appears to be lower. A systematic review suggested that COX-2 inhibitors might cause significantly less adverse effects than non-selective NSAIDs in the lower gastrointestinal tract [34], as well as several other controlled trials [2, 45]. Other studies suggested that COX-2 inhibitors might be beneficial, even when compared to a PPI added to a non-selective NSAID [106, 107].

Furthermore, the gastrointestinal benefits of COX-2 inhibitors over non-selective NSAIDs seem to remain for patients requiring aspirin. Fewer people taking COX-2 inhibitors and aspirin had ulcers and complications than those taking non-selective NSAIDs and aspirin [76, 112, 113]. These benefits are supported by large epidemiological studies, showing aspirin with either celecoxib or rofecoxib reduced adverse gastrointestinal events by about 50 % compared with non-selective NSAIDs [114].

Cardiovascular Risks of NSAIDS

Despite the improved gastrointestinal safety profile of COX-2 inhibitors, the cardiovascular risk of this medicinal class has been a subject of concern [112, 115, 116] (Table 4.2). In a previous randomized study evaluating the gastrointestinal outcomes of a COX-2 inhibitor, the incidence of acute myocardial events was four times higher among patients receiving rofecoxib

Table 4.2 Balancing gastrointestinal (GI) and cardiovascular (CV) risks in prescribing analgesics

CV risk\GI risk	Low	Moderate	High
Low	NSAID at lowest effective dose	NSAID plus either PPI or misoprostol	COX-2 inhibitor plus either PPI or misoprostol
High	Naproxen plus PPI or misoprostol	Naproxen plus PPI or misoprostol	Avoid NSAID or COX-2 inhibitor if possible

Low GI risk denotes no risk factors; moderate GI risk denotes 1–2 risk factors; high GI risk denotes ≥3 risk factors. High CV risk denotes the requirement for prophylactic aspirin for primary or secondary prevention of serious CV events

than patients receiving naproxen [30]. Further data regarding the cardiovascular safety of rofecoxib were derived from a colorectal polyp prevention study [117], in which patients receiving rofecoxib had nearly twice the risk of suffering a heart attack or stroke compared to patients receiving a placebo. This has led to the voluntary withdrawal of rofecoxib from the worldwide market in 2004. Other studies have also found higher risks of cardiovascular events for other COX-2 inhibitors, including parecoxib and valdecoxib [116]. The cardiovascular risk for celecoxib appears to be dose-dependent in two meta-analyses [28, 118], with the cardiovascular effects of celecoxib 200 mg daily being statistically insignificant. In contrast to non-selective NSAIDs, celecoxib does not impair the anti-platelet activity of low-dose aspirin [119, 120].

Emerging evidence suggests that not only COX-2 inhibitors but also non-selective NSAIDs can increase the cardiovascular risk [28, 121, 122]. A meta-analysis evaluating the cardiovascular and gastrointestinal effects of NSAIDs, including COX-2 inhibitors and non-selective NSAIDs, showed comparable cardiovascular risks with high-dose diclofenac, ibuprofen and COX-2 inhibitors, but a lower risk with naproxen [28]. The favourable cardiovascular profile of naproxen is consistent with experimental studies showing its capability to inhibit COX-1 to result in platelet inhibition [123]. Major cardiovascular events were increased by about a third by a COX-2 inhibitor or diclofenac, primarily due to an increase in coronary events, but also vascular events to a lesser extent. All NSAID regimens increased upper gastrointestinal complications, with a rate ratio of 1.81 for COX-2 inhibitors and up to 4.22 for naproxen [28].

Balancing Cardiovascular and Gastrointestinal Risks

NSAIDs are very effective drugs, but they carry significant cardiovascular and gastrointestinal risks along with toxicities to the liver and kidneys. The clinical challenge for the physicians is to maintain the therapeutic effects of NSAIDs while minimizing the adverse events. The choice of medications can be guided by taking individual patient's cardiovascular and gastrointestinal risks into account [55].

For patients with low cardiovascular risk, the management plan can be made primarily according to the gastrointestinal risk. For patients with a low gastrointestinal risk, rational use of a less toxic NSAID at a lowest effective dose is a reasonable approach. For patients at moderate gastrointestinal risk, co-therapy with a gastroprotective agent such as a PPI or misoprostol or substitution with a COX-2 inhibitor would be appropriate. For patients with high gastrointestinal risk, the combination of a COX-2 inhibitor and either a PPI or misoprostol offers the best gastrointestinal protection, although this approach remains to be examined in a prospective trial.

The cardiovascular risk associated with many NSAIDs mandates a careful clinical assessment especially when prescribing to patients with a high background cardiovascular risk. Patients known to have a high cardiovascular risk should receive low-dose aspirin irrespective of NSAID use [124]. Because of the potential

cardiovascular hazards of COX-2 inhibitors and most non-selective NSAIDs, patients with high cardiovascular risk should avoid using these drugs if possible. Ibuprofen can attenuate the cardio-protective effect of aspirin, possibly through competitive binding to platelet COX-1 and interfering the inhibition of TXA2 synthesis [125–127]. Concomitant use of ibuprofen and low-dose aspirin should therefore be avoided. If an NSAID is deemed necessary in patients at high cardiovascular risk, naproxen is a safer option as it does not appear to increase the cardiothrombotic risk. However, it remains uncertain whether the cardio-protective effect of naproxen will persist at lower doses or when naproxen is co-prescribed with low-dose aspirin. One major drawback of concomitant use of NSAIDs and low-dose aspirin is that the combination will markedly increase the risk of ulcer complications. Thus, co-therapy with a PPI or misoprostol is necessary even if patients do not have other gastrointestinal risk factors. Despite some controversies, celecoxib is another option for patients receiving low-dose aspirin for cardiovascular prophylaxis [128] as it does not seem to impair the anti-platelet activity of aspirin [119, 120]. Other forms of analgesics such as paracetamol, opioids or corticosteroids may be considered.

Conclusions

While the risk factors and strategies for the prevention of NSAID-induced upper gastrointestinal toxicity have been studied extensively, our knowledge about lower gastrointestinal toxicity of NSAIDs remains limited. To date, there is no effective preventive treatment for lower gastrointestinal complications associated with NSAID use. Emerging data suggest that profound acid suppression may not be beneficial in terms of NSAID-induced lower gastrointestinal damage and that we probably need a trade-off of different strategies to protect the entire gastrointestinal tract. Another dilemma is about the cardiothrombotic risks of NSAIDs. Physicians have to make individualized decisions to balance gastrointestinal and cardiothrombotic risks when prescribing NSAIDs.

References

1. Day RO, Graham GG. Non-steroidal anti-inflammatory drugs (NSAIDs). BMJ. 2013;346:f3195. Epub 2013/06/13.
2. Laine L, Connors LG, Reicin A, Hawkey CJ, Burgos-Vargas R, Schnitzer TJ, et al. Serious lower gastrointestinal clinical events with nonselective NSAID or coxib use. Gastroenterology. 2003;124(2):288–92. Epub 2003/01/31.
3. Graham DY, Opekun AR, Willingham FF, Qureshi WA. Visible small-intestinal mucosal injury in chronic NSAID users. Clin Gastroenterol Hepatol. 2005;3(1):55–9. Epub 2005/01/13.
4. Singh G. Gastrointestinal complications of prescription and over-the-counter nonsteroidal anti-inflammatory drugs: a view from the ARAMIS database. Arthritis, Rheumatism, and Aging Medical Information System. Am J Ther. 2000;7(2):115–21. Epub 2001/04/25.
5. Shaheen NJ, Hansen RA, Morgan DR, Gangarosa LM, Ringel Y, Thiny MT, et al. The burden of gastrointestinal and liver diseases, 2006. Am J Gastroenterol. 2006;101(9):2128–38. Epub 2006/07/20.
6. Jones R. Nonsteroidal anti-inflammatory drug prescribing: past, present, and future. Am J Med. 2001;110(1A):4S–7S. Epub 2001/02/13.
7. Lassen A, Hallas J. Schaffalitzky de Muckadell OB. Complicated and uncomplicated peptic ulcers in a Danish county 1993-2002: a population-based cohort study. Am J Gastroenterol. 2006;101(5):945–53. Epub 2006/04/01.
8. Taha AS, Angerson WJ, Prasad R, McCloskey C, Blatchford O. Upper gastrointestinal bleeding and the changing use of COX-2 non-steroidal anti-inflammatory drugs and low-dose aspirin. Aliment Pharmacol Ther. 2007;26(8):1171–8. Epub 2007/09/27.
9. Ishikawa S, Inaba T, Mizuno M, Miyake Y, Okada H, Ishikawa H, et al. Characteristics of serious complicated gastroduodenal ulcers in Japan. Hepatogastroenterology. 2012;59(113):147–54. Epub 2012/01/19.
10. Taha AS, Angerson WJ, Prasad R, McCloskey C, Gilmour D, Morran CG. Clinical trial: the incidence and early mortality after peptic ulcer perforation, and the use of low-dose aspirin and nonsteroidal anti-inflammatory drugs. Aliment Pharmacol Ther. 2008;28(7):878–85. Epub 2008/07/23.
11. Lanas A, Sopena F. Nonsteroidal anti-inflammatory drugs and lower gastrointestinal complications. Gastroenterol Clin North Am. 2009;38(2):333–52. Epub 2009/05/19.

12. Lanas A, Garcia-Rodriguez LA, Polo-Tomas M, Ponce M, Alonso-Abreu I, Perez-Aisa MA, et al. Time trends and impact of upper and lower gastrointestinal bleeding and perforation in clinical practice. Am J Gastroenterol. 2009;104(7):1633–41. Epub 2009/07/04.
13. Laine L, Takeuchi K, Tarnawski A. Gastric mucosal defense and cytoprotection: bench to bedside. Gastroenterology. 2008;135(1):41–60. Epub 2008/06/14.
14. Wolfe MM, Lichtenstein DR, Singh G. Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med. 1999;340(24):1888–99. Epub 1999/06/17.
15. Crofford LJ. COX-1 and COX-2 tissue expression: implications and predictions. J Rheumatol Suppl. 1997;49:15–9. Epub 1997/07/01.
16. Schoen RT, Vender RJ. Mechanisms of nonsteroidal anti-inflammatory drug-induced gastric damage. Am J Med. 1989;86(4):449–58. Epub 1989/04/01.
17. Lichtenberger LM, Wang ZM, Romero JJ, Ulloa C, Perez JC, Giraud MN, et al. Non-steroidal anti-inflammatory drugs (NSAIDs) associate with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. Nat Med. 1995;1(2):154–8. Epub 1995/02/01.
18. Lichtenberger LM, Zhou Y, Jayaraman V, Doyen JR, O'Neil RG, Dial EJ, et al. Insight into NSAID-induced membrane alterations, pathogenesis and therapeutics: characterization of interaction of NSAIDs with phosphatidylcholine. Biochim Biophys Acta. 2012;1821(7):994–1002. Epub 2012/04/24.
19. Hampton DD, Hale LP. Mast cells are critical for protection against peptic ulcers induced by the NSAID piroxicam. PLoS One. 2011;6(8), e23669. Epub 2011/08/23.
20. Reuter BK, Davies NM, Wallace JL. Nonsteroidal anti-inflammatory drug enteropathy in rats: role of permeability, bacteria, and enterohepatic circulation. Gastroenterology. 1997;112(1):109–17. Epub 1997/01/01.
21. Somasundaram S, Sigthorsson G, Simpson RJ, Watts J, Jacob M, Tavares IA, et al. Uncoupling of intestinal mitochondrial oxidative phosphorylation and inhibition of cyclooxygenase are required for the development of NSAID-enteropathy in the rat. Aliment Pharmacol Ther. 2000;14(5):639–50. Epub 2000/05/03.
22. Wallace JL. Mechanisms, prevention and clinical implications of nonsteroidal anti-inflammatory drug-enteropathy. World J Gastroenterol. 2013;19(12):1861–76. Epub 2013/04/10.
23. Robert A, Asano T. Resistance of germfree rats to indomethacin-induced intestinal lesions. Prostaglandins. 1977;14(2):333–41. Epub 1977/08/01.
24. Uejima M, Kinouchi T, Kataoka K, Hiraoka I, Ohnishi Y. Role of intestinal bacteria in ileal ulcer formation in rats treated with a nonsteroidal antiinflammatory drug. Microbiol Immunol. 1996;40(8):553–60. Epub 1996/01/01.
25. Henry D, Lim LL, Garcia Rodriguez LA, Perez Gutthann S, Carson JL, Griffin M, et al. Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. BMJ. 1996;312(7046):1563–6. Epub 1996/06/22.
26. Hernandez-Diaz S, Rodriguez LA. Association between nonsteroidal anti-inflammatory drugs and upper gastrointestinal tract bleeding/perforation: an overview of epidemiologic studies published in the 1990s. Arch Intern Med. 2000;160(14):2093–9. Epub 2000/07/25.
27. Masclee GM, Valkhoff VE, Coloma PM, de Ridder M, Romio S, Schuemie MJ, et al. Risk of upper gastrointestinal bleeding from different drug combinations. Gastroenterology. 2014;147(4):784-92 e9; quiz e13-4. Epub 2014/06/18.
28. Bhala N, Emberson J, Merhi A, Abramson S, Arber N, Baron JA, et al. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. Lancet. 2013;382(9894):769–79. Epub 2013/06/04.
29. Lanas A, Hunt R. Prevention of anti-inflammatory drug-induced gastrointestinal damage: benefits and risks of therapeutic strategies. Ann Med. 2006;38(6):415–28. Epub 2006/09/30.
30. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Med. 2000;343(21):1520–8. Epub 2000/11/23.
31. Ofman JJ, MacLean CH, Straus WL, Morton SC, Berger ML, Roth EA, et al. A metaanalysis of severe upper gastrointestinal complications of nonsteroidal antiinflammatory drugs. J Rheumatol. 2002;29(4):804–12. Epub 2002/04/13.
32. Silverstein FE, Graham DY, Senior JR, Davies HW, Struthers BJ, Bittman RM, et al. Misoprostol reduces serious gastrointestinal complications in patients with rheumatoid arthritis receiving nonsteroidal anti-inflammatory drugs. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 1995;123(4):241–9. Epub 1995/08/15.
33. Masso Gonzalez EL, Patrignani P, Tacconelli S, Garcia Rodriguez LA. Variability among nonsteroidal antiinflammatory drugs in risk of upper gastrointestinal bleeding. Arthritis Rheum. 2010;62(6):1592–601. Epub 2010/02/24.
34. Laine L, Smith R, Min K, Chen C, Dubois RW. Systematic review: the lower gastrointestinal adverse effects of non-steroidal anti-inflammatory drugs. Aliment Pharmacol Ther. 2006;24(5):751–67. Epub 2006/08/22.

35. Yeomans ND. The ulcer sleuths: The search for the cause of peptic ulcers. J Gastroenterol Hepatol. 2011;26 Suppl 1:35–41. Epub 2011/01/14.
36. Wilcox CM, Alexander LN, Cotsonis GA, Clark WS. Nonsteroidal antiinflammatory drugs are associated with both upper and lower gastrointestinal bleeding. Dig Dis Sci. 1997;42(5):990–7. Epub 1997/05/01.
37. Higuchi K, Umegaki E, Watanabe T, Yoda Y, Morita E, Murano M, et al. Present status and strategy of NSAIDs-induced small bowel injury. J Gastroenterol. 2009;44(9):879–88. Epub 2009/07/02.
38. Maiden L, Thjodleifsson B, Theodors A, Gonzalez J, Bjarnason I. A quantitative analysis of NSAID-induced small bowel pathology by capsule enteroscopy. Gastroenterology. 2005;128 (5):1172–8. Epub 2005/05/12.
39. Matsumoto T, Kudo T, Esaki M, Yano T, Yamamoto H, Sakamoto C, et al. Prevalence of non-steroidal anti-inflammatory drug-induced enteropathy determined by double-balloon endoscopy: a Japanese multicenter study. Scand J Gastroenterol. 2008;43(4):490–6. Epub 2008/03/28.
40. Watari I, Oka S, Tanaka S, Igawa A, Nakano M, Aoyama T, et al. Comparison of small-bowel mucosal injury between low-dose aspirin and non-aspirin non-steroidal anti-inflammatory drugs: a capsule endoscopy study. Digestion. 2014;89(3):225–31. Epub 2014/05/28.
41. Hall RI, Petty AH, Cobden I, Lendrum R. Enteritis and colitis associated with mefenamic acid. Br Med J (Clin Res Ed). 1983;287(6400):1182. Epub 1983/10/22.
42. Lanas A, Sekar MC, Hirschowitz BI. Objective evidence of aspirin use in both ulcer and nonulcer upper and lower gastrointestinal bleeding. Gastroenterology. 1992;103(3):862–9. Epub 1992/09/01.
43. Lanas A, Serrano P, Bajador E, Esteva F, Benito R, Sainz R. Evidence of aspirin use in both upper and lower gastrointestinal perforation. Gastroenterology. 1997;112(3):683–9. Epub 1997/03/01.
44. Smith JA, Pineau BC. Endoscopic therapy of NSAID-induced colonic diaphragm disease: two cases and a review of published reports. Gastrointest Endosc. 2000;52(1):120–5. Epub 2000/07/07.
45. Chan FK, Lanas A, Scheiman J, Berger MF, Nguyen H, Goldstein JL. Celecoxib versus omeprazole and diclofenac in patients with osteoarthritis and rheumatoid arthritis (CONDOR): a randomised trial. Lancet. 2010;376(9736):173–9. Epub 2010/07/20.
46. Chan FK, Cryer B, Goldstein JL, Lanas A, Peura DA, Scheiman JM, et al. A novel composite endpoint to evaluate the gastrointestinal (GI) effects of non-steroidal antiinflammatory drugs through the entire GI tract. J Rheumatol. 2010;37(1):167–74. Epub 2009/11/04.
47. Brun J, Jones R. Nonsteroidal anti-inflammatory drug-associated dyspepsia: the scale of the problem. Am J Med. 2001;110(1A):12S–3S. Epub 2001/02/13.
48. Cayla G, Collet JP, Silvain J, Thiefin G, Woimant F, Montalescot G. Prevalence and clinical impact of upper gastrointestinal symptoms in subjects treated with low dose aspirin: the UGLA survey. Int J Cardiol. 2012;156(1):69–75. Epub 2010/11/26.
49. Armstrong CP, Blower AL. Non-steroidal anti-inflammatory drugs and life threatening complications of peptic ulceration. Gut. 1987;28 (5):527–32. Epub 1987/05/01.
50. Gabriel SE, Jaakkimainen L, Bombardier C. Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs. A meta-analysis Ann Intern Med. 1991;115(10):787–96. Epub 1991/11/15.
51. Garcia Rodriguez LA, Jick H. Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs. Lancet. 1994;343(8900):769–72. Epub 1994/03/26.
52. Griffin MR, Piper JM, Daugherty JR, Snowden M, Ray WA. Nonsteroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elderly persons. Ann Intern Med. 1991;114(4):257–63. Epub 1991/02/15.
53. Langman MJ, Weil J, Wainwright P, Lawson DH, Rawlins MD, Logan RF, et al. Risks of bleeding peptic ulcer associated with individual non-steroidal anti-inflammatory drugs. Lancet. 1994;343(8905):1075–8. Epub 1994/04/30.
54. Huang JQ, Sridhar S, Hunt RH. Role of Helicobacter pylori infection and non-steroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. Lancet. 2002;359(9300):14–22. Epub 2002/01/26.
55. Lanza FL, Chan FK, Quigley EM. Guidelines for prevention of NSAID-related ulcer complications. Am J Gastroenterol. 2009;104(3):728–38. Epub 2009/02/26.
56. Bjorkman DJ. Nonsteroidal anti-inflammatory drug-induced gastrointestinal injury. Am J Med. 1996;101 (1A):25S–32S. Epub 1996/07/31.
57. Longstreth GF. Epidemiology of hospitalization for acute upper gastrointestinal hemorrhage: a population-based study. Am J Gastroenterol. 1995;90(2):206–10. Epub 1995/02/01.
58. Weil J, Colin-Jones D, Langman M, Lawson D, Logan R, Murphy M, et al. Prophylactic aspirin and risk of peptic ulcer bleeding. BMJ. 1995;310 (6983):827–30. Epub 1995/04/01.
59. Shorr RI, Ray WA, Daugherty JR, Griffin MR. Concurrent use of nonsteroidal anti-inflammatory drugs and oral anticoagulants places elderly persons at high risk for hemorrhagic peptic ulcer disease. Arch Intern Med. 1993;153 (14):1665–70. Epub 1993/07/26.
60. Harper P, Young L, Merriman E. Bleeding risk with dabigatran in the frail elderly. N Engl J Med. 2012;366(9):864–6. Epub 2012/03/02.
61. Sipahi I, Celik S, Tozun N. A comparison of results of the US Food and Drug Administration's mini-sentinel program with randomized clinical trials:

the case of gastrointestinal tract bleeding with dabigatran. JAMA Intern Med. 2014;174(1):150–1. Epub 2013/11/20.
62. Southworth MR, Reichman ME, Unger EF. Dabigatran and postmarketing reports of bleeding. N Engl J Med. 2013;368(14):1272–4. Epub 2013/03/15.
63. Holster IL, Valkhoff VE, Kuipers EJ, Tjwa ET. New oral anticoagulants increase risk for gastrointestinal bleeding: a systematic review and meta-analysis. Gastroenterology. 2013;145(1):105–12. Epub 2013/03/09.
64. Hernandez I, Baik SH, Pinera A, Zhang Y. Risk of bleeding with dabigatran in atrial fibrillation. JAMA Intern Med. 2015;175(1):18–24. Epub 2014/11/05.
65. Abraham NS, Singh S, Alexander GC, Heien H, Haas LR, Crown W, et al. Comparative risk of gastrointestinal bleeding with dabigatran, rivaroxaban, and warfarin: population based cohort study. BMJ. 2015;350:h1857. Epub 2015/04/26.
66. Chang HY, Zhou M, Tang W, Alexander GC, Singh S. Risk of gastrointestinal bleeding associated with oral anticoagulants: population based retrospective cohort study. BMJ. 2015;350:h1585. Epub 2015/04/26.
67. Rockey DC. Gastrointestinal bleeding. Gastrointestinal bleeding risk is increased by novel anticoagulants. Nat Rev Gastroenterol Hepatol. 2015;12(3):131–2. Epub 2015/01/21.
68. Conn HO, Blitzer BL. Nonassociation of adrenocorticosteroid therapy and peptic ulcer. N Engl J Med. 1976;294(9):473–9. Epub 1976/02/26.
69. Conn HO, Poynard T. Corticosteroids and peptic ulcer: meta-analysis of adverse events during steroid therapy. J Intern Med. 1994;236(6):619–32. Epub 1994/12/01.
70. Messer J, Reitman D, Sacks HS, Smith Jr H, Chalmers TC. Association of adrenocorticosteroid therapy and peptic-ulcer disease. N Engl J Med. 1983;309(1):21–4. Epub 1983/07/07.
71. Kremer D, Fraser R, Brown JJ, Lever AF, Davies DL, Robertson JI. Amiloride in primary hyperaldosteronism with chronic peptic ulceration. Br Med J. 1973;2(5860):216–7. Epub 1973/04/28.
72. Weil J, Langman MJ, Wainwright P, Lawson DH, Rawlins M, Logan RF, et al. Peptic ulcer bleeding: accessory risk factors and interactions with non-steroidal anti-inflammatory drugs. Gut. 2000;46(1):27–31. Epub 1999/12/22.
73. Garcia Rodriguez LA, Lin KJ, Hernandez-Diaz S, Johansson S. Risk of upper gastrointestinal bleeding with low-dose acetylsalicylic acid alone and in combination with clopidogrel and other medications. Circulation. 2011;123(10):1108–15. Epub 2011/03/02.
74. Piper JM, Ray WA, Daugherty JR, Griffin MR. Corticosteroid use and peptic ulcer disease: role of nonsteroidal anti-inflammatory drugs. Ann Intern Med. 1991;114(9):735–40. Epub 1991/05/01.
75. Curtis JR, Lanas A, John A, Johnson DA, Schulman KL. Factors associated with gastrointestinal perforation in a cohort of patients with rheumatoid arthritis. Arthritis Care Res (Hoboken). 2012;64(12):1819–28. Epub 2012/06/26.
76. Laine L, Curtis SP, Cryer B, Kaur A, Cannon CP. Assessment of upper gastrointestinal safety of etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: a randomised comparison. Lancet. 2007;369(9560):465–73. Epub 2007/02/13.
77. Vergara M, Catalan M, Gisbert JP, Calvet X. Meta-analysis: role of Helicobacter pylori eradication in the prevention of peptic ulcer in NSAID users. Aliment Pharmacol Ther. 2005;21(12):1411–8. Epub 2005/06/14.
78. Chan FK, Sung JJ, Chung SC, To KF, Yung MY, Leung VK, et al. Randomised trial of eradication of Helicobacter pylori before non-steroidal anti-inflammatory drug therapy to prevent peptic ulcers. Lancet. 1997;350(9083):975–9. Epub 1997/11/05.
79. Chan FK, To KF, Wu JC, Yung MY, Leung WK, Kwok T, et al. Eradication of Helicobacter pylori and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomised trial. Lancet. 2002;359(9300):9–13. Epub 2002/01/26.
80. Leung Ki EL, Chan FK. Interaction of Helicobacter pylori infection and low-dose aspirin in the upper gastrointestinal tract: implications for clinical practice. Best Pract Res Clin Gastroenterol. 2012;26(2):163–72. Epub 2012/05/01.
81. Chan FK, Chung SC, Suen BY, Lee YT, Leung WK, Leung VK, et al. Preventing recurrent upper gastrointestinal bleeding in patients with Helicobacter pylori infection who are taking low-dose aspirin or naproxen. N Engl J Med. 2001;344(13):967–73. Epub 2001/03/29.
82. Chan FK, Ching JY, Suen BY, Tse YK, Wu JC, Sung JJ. Effects of Helicobacter pylori infection on long-term risk of peptic ulcer bleeding in low-dose aspirin users. Gastroenterology. 2013;144(3):528–35. Epub 2013/01/22.
83. Chan FK, Ching JY, Hung LC, Wong VW, Leung VK, Kung NN, et al. Clopidogrel versus aspirin and esomeprazole to prevent recurrent ulcer bleeding. N Engl J Med. 2005;352(3):238–44. Epub 2005/01/22.
84. Chan FK, Hung LC, Suen BY, Wu JC, Lee KC, Leung VK, et al. Celecoxib versus diclofenac and omeprazole in reducing the risk of recurrent ulcer bleeding in patients with arthritis. N Engl J Med. 2002;347(26):2104–10. Epub 2002/12/27.
85. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial.

Celecoxib Long-term Arthritis Safety Study JAMA. 2000;284(10):1247–55. Epub 2000/09/09.
86. Rostom A, Dube C, Wells G, Tugwell P, Welch V, Jolicoeur E, et al. Prevention of NSAID-induced gastroduodenal ulcers. Cochrane Database Syst Rev. 2002;4, CD002296. Epub 2003/01/10.
87. Rostom A, Muir K, Dube C, Jolicoeur E, Boucher M, Joyce J, et al. Gastrointestinal safety of cyclooxygenase-2 inhibitors: a Cochrane Collaboration systematic review. Clin Gastroenterol Hepatol. 2007;5(7):818–28, 28 e1–5; quiz 768. Epub 2007/06/09.
88. Hudson N, Taha AS, Russell RI, Trye P, Cottrell J, Mann SG, et al. Famotidine for healing and maintenance in nonsteroidal anti-inflammatory drug-associated gastroduodenal ulceration. Gastroenterology. 1997;112(6):1817–22. Epub 1997/06/01.
89. Taha AS, Hudson N, Hawkey CJ, Swannell AJ, Trye PN, Cottrell J, et al. Famotidine for the prevention of gastric and duodenal ulcers caused by nonsteroidal antiinflammatory drugs. N Engl J Med. 1996;334(22):1435–9. Epub 1996/05/30.
90. Simon TJ, Berenson MM, Berlin RG, Snapinn S, Cagliola A. Randomized, placebo-controlled comparison of famotidine 20 mg b.d. or 40 mg b.d. in patients with erosive oesophagitis. Aliment Pharmacol Ther. 1994;8(1):71–9. Epub 1994/02/01.
91. Hooper L, Brown TJ, Elliott R, Payne K, Roberts C, Symmons D. The effectiveness of five strategies for the prevention of gastrointestinal toxicity induced by non-steroidal anti-inflammatory drugs: systematic review. BMJ. 2004;329(7472):948. Epub 2004/10/12.
92. Taha AS, McCloskey C, Prasad R, Bezlyak V. Famotidine for the prevention of peptic ulcers and oesophagitis in patients taking low-dose aspirin (FAMOUS): a phase III, randomised, double-blind, placebo-controlled trial. Lancet. 2009;374(9684):119–25. Epub 2009/07/07.
93. Chan FK, Sung JJ, Ching JY, Wu JC, Lee YT, Leung WK, et al. Randomized trial of low-dose misoprostol and naproxen vs. nabumetone to prevent recurrent upper gastrointestinal haemorrhage in users of non-steroidal anti-inflammatory drugs. Aliment Pharmacol Ther. 2001;15(1):19–24. Epub 2001/01/03.
94. Raskin JB, White RH, Jackson JE, Weaver AL, Tindall EA, Lies RB, et al. Misoprostol dosage in the prevention of nonsteroidal anti-inflammatory drug-induced gastric and duodenal ulcers: a comparison of three regimens. Ann Intern Med. 1995;123(5):344–50. Epub 1995/09/01.
95. Rostom A, Wells G, Tugwell P, Welch V, Dube C, McGowan J. Prevention of NSAID-induced gastroduodenal ulcers. Cochrane Database Syst Rev. 2000;4, CD002296. Epub 2000/10/18.
96. Raskin JB, White RH, Jaszewski R, Korsten MA, Schubert TT, Fort JG. Misoprostol and ranitidine in the prevention of NSAID-induced ulcers: a prospective, double-blind, multicenter study. Am J Gastroenterol. 1996;91(2):223–7. Epub 1996/02/01.
97. Yeomans ND, Tulassay Z, Juhasz L, Racz I, Howard JM, van Rensburg CJ, et al. A comparison of omeprazole with ranitidine for ulcers associated with nonsteroidal antiinflammatory drugs. Acid Suppression Trial: Ranitidine versus Omeprazole for NSAID-associated Ulcer Treatment (ASTRONAUT) Study Group. N Engl J Med. 1998;338(11):719–26. Epub 1998/03/12.
98. Hawkey CJ, Karrasch JA, Szczepanski L, Walker DG, Barkun A, Swannell AJ, et al. Omeprazole compared with misoprostol for ulcers associated with nonsteroidal antiinflammatory drugs. Omeprazole versus Misoprostol for NSAID-induced Ulcer Management (OMNIUM) Study Group. N Engl J Med. 1998;338(11):727–34. Epub 1998/03/12.
99. Goldstein JL, Johanson JF, Suchower LJ, Brown KA. Healing of gastric ulcers with esomeprazole versus ranitidine in patients who continued to receive NSAID therapy: a randomized trial. Am J Gastroenterol. 2005;100(12):2650–7. Epub 2006/01/06.
100. Graham DY, Agrawal NM, Campbell DR, Haber MM, Collis C, Lukasik NL, et al. Ulcer prevention in long-term users of nonsteroidal anti-inflammatory drugs: results of a double-blind, randomized, multicenter, active- and placebo-controlled study of misoprostol vs lansoprazole. Arch Intern Med. 2002;162(2):169–75. Epub 2002/02/13.
101. Scheiman JM, Yeomans ND, Talley NJ, Vakil N, Chan FK, Tulassay Z, et al. Prevention of ulcers by esomeprazole in at-risk patients using non-selective NSAIDs and COX-2 inhibitors. Am J Gastroenterol. 2006;101(4):701–10. Epub 2006/02/24.
102. Regula J, Butruk E, Dekkers CP, de Boer SY, Raps D, Simon L, et al. Prevention of NSAID-associated gastrointestinal lesions: a comparison study pantoprazole versus omeprazole. Am J Gastroenterol. 2006;101(8):1747–55. Epub 2006/07/05.
103. Lanas A, Garcia-Rodriguez LA, Arroyo MT, Bujanda L, Gomollon F, Forne M, et al. Effect of antisecretory drugs and nitrates on the risk of ulcer bleeding associated with nonsteroidal anti-inflammatory drugs, antiplatelet agents, and anticoagulants. Am J Gastroenterol. 2007;102(3):507–15. Epub 2007/03/07.
104. Janssen M, Dijkmans BA, Vandenbroucke JP, Biemond I, Lamers CB. Achlorhydria does not protect against benign upper gastrointestinal ulcers during NSAID use. Dig Dis Sci. 1994;39(2):362–5. Epub 1994/02/01.
105. Scarpignato C, Hunt RH. Nonsteroidal antiinflammatory drug-related injury to the gastrointestinal tract: clinical picture, pathogenesis, and prevention. Gastroenterol Clin North Am. 2010;39(3):433–64. Epub 2010/10/19.

106. Goldstein JL, Eisen GM, Lewis B, Gralnek IM, Zlotnick S, Fort JG. Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole, and placebo. Clin Gastroenterol Hepatol. 2005;3(2):133–41. Epub 2005/02/11.
107. Goldstein JL, Johanson JF, Hawkey CJ, Suchower LJ, Brown KA. Clinical trial: healing of NSAID-associated gastric ulcers in patients continuing NSAID therapy - a randomized study comparing ranitidine with esomeprazole. Aliment Pharmacol Ther. 2007;26(8):1101–11. Epub 2007/09/27.
108. Wallace JL, Syer S, Denou E, de Palma G, Vong L, McKnight W, et al. Proton pump inhibitors exacerbate NSAID-induced small intestinal injury by inducing dysbiosis. Gastroenterology. 2011;141 (4):1314–22. Epub 2011/07/13.
109. Schnitzer TJ, Burmester GR, Mysler E, Hochberg MC, Doherty M, Ehrsam E, et al. Comparison of lumiracoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET), reduction in ulcer complications: randomised controlled trial. Lancet. 2004;364(9435):665–74. Epub 2004/08/25.
110. Chan FK, Wong VW, Suen BY, Wu JC, Ching JY, Hung LC, et al. Combination of a cyclo-oxygenase-2 inhibitor and a proton-pump inhibitor for prevention of recurrent ulcer bleeding in patients at very high risk: a double-blind, randomised trial. Lancet. 2007;369(9573):1621–6. Epub 2007/05/15.
111. Laine L, Maller ES, Yu C, Quan H, Simon T. Ulcer formation with low-dose enteric-coated aspirin and the effect of COX-2 selective inhibition: a double-blind trial. Gastroenterology. 2004;127(2):395–402. Epub 2004/08/10.
112. Cannon CP, Curtis SP, FitzGerald GA, Krum H, Kaur A, Bolognese JA, et al. Cardiovascular outcomes with etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: a randomised comparison. Lancet. 2006;368(9549):1771–81. Epub 2006/11/23.
113. Goldstein JL, Lowry SC, Lanza FL, Schwartz HI, Dodge WE. The impact of low-dose aspirin on endoscopic gastric and duodenal ulcer rates in users of a non-selective non-steroidal anti-inflammatory drug or a cyclo-oxygenase-2-selective inhibitor. Aliment Pharmacol Ther. 2006;23(10):1489–98. Epub 2006/05/04.
114. Rahme E, Bardou M, Dasgupta K, Toubouti Y, Ghosn J, Barkun AN. Hospitalization for gastrointestinal bleeding associated with non-steroidal anti-inflammatory drugs among elderly patients using low-dose aspirin: a retrospective cohort study. Rheumatology (Oxford). 2007;46(2):265–72. Epub 2006/07/18.
115. Juni P, Nartey L, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. Lancet. 2004;364(9450):2021–9. Epub 2004/12/08.
116. Nussmeier NA, Whelton AA, Brown MT, Langford RM, Hoeft A, Parlow JL, et al. Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. N Engl J Med. 2005;352 (11):1081–91. Epub 2005/02/17.
117. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med. 2005;352 (11):1092–102. Epub 2005/02/17.
118. Solomon SD, Wittes J, Finn PV, Fowler R, Viner J, Bertagnolli MM, et al. Cardiovascular risk of celecoxib in 6 randomized placebo-controlled trials: the cross trial safety analysis. Circulation. 2008;117 (16):2104–13. Epub 2008/04/02.
119. Gladding PA, Webster MW, Farrell HB, Zeng IS, Park R, Ruijne N. The antiplatelet effect of six non-steroidal anti-inflammatory drugs and their pharmacodynamic interaction with aspirin in healthy volunteers. Am J Cardiol. 2008;101(7):1060–3. Epub 2008/03/25.
120. Renda G, Tacconelli S, Capone ML, Sacchetta D, Santarelli F, Sciulli MG, et al. Celecoxib, ibuprofen, and the antiplatelet effect of aspirin in patients with osteoarthritis and ischemic heart disease. Clin Pharmacol Ther. 2006;80(3):264–74. Epub 2006/09/06.
121. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclo-oxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. BMJ. 2006;332(7553):1302–8. Epub 2006/06/03.
122. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. JAMA. 2006;296(13):1633–44. Epub 2006/09/14.
123. Capone ML, Tacconelli S, Sciulli MG, Grana M, Ricciotti E, Minuz P, et al. Clinical pharmacology of platelet, monocyte, and vascular cyclooxygenase inhibition by naproxen and low-dose aspirin in healthy subjects. Circulation. 2004;109 (12):1468–71. Epub 2004/03/24.
124. Pearson TA, Blair SN, Daniels SR, Eckel RH, Fair JM, Fortmann SP, et al. AHA Guidelines for Primary Prevention of Cardiovascular Disease and Stroke: 2002 Update: Consensus Panel Guide to Comprehensive Risk Reduction for Adult Patients Without Coronary or Other Atherosclerotic Vascular Diseases. American Heart Association Science Advisory and Coordinating Committee. Circulation. 2002;106(3):388–91. Epub 2002/07/18.

125. MacDonald TM, Wei L. Effect of ibuprofen on cardioprotective effect of aspirin. Lancet. 2003;361 (9357):573–4. Epub 2003/02/25.
126. FitzGerald GA. Parsing an enigma: the pharmacodynamics of aspirin resistance. Lancet. 2003;361 (9357):542–4. Epub 2003/02/25.
127. Catella-Lawson F, Reilly MP, Kapoor SC, Cucchiara AJ, DeMarco S, Tournier B, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. N Engl J Med. 2001;345 (25):1809–17. Epub 2001/12/26.
128. Strand V. Are COX-2 inhibitors preferable to non-selective non-steroidal anti-inflammatory drugs in patients with risk of cardiovascular events taking low-dose aspirin? Lancet. 2007;370(9605):2138–51. Epub 2007/12/25.

Adverse Effects of Nonsteroidal Anti-inflammatory Drugs on the Cardiovascular System

5

Carmelo Scarpignato and Corrado Blandizzi

Abbreviations

ACCEPT	Assessing the Cardiovascular Risk Between CElecoxib and Nonselective Nonsteroidal Anti-inflammatory Drugs in Patients With Rheumatoid Arthritis and Osteoarthritis Trial
AF	Atrial fibrillation
AHA	American Heart Association
AKF	Acute kidney failure
AMI	Acute myocardial infarction
APC	Adenoma prevention with celecoxib
APPROVe	Adenomatous Polyp Prevention on Vioxx™
AS	Ankylosing spondylitis
ASA	Acetylsalicylic acid (aspirin)
BP	Blood pressure
CIs	Confidence intervals
CHD	Coronary heart disease
CHF	Congestive heart failure
CHF	Congestive heart failure
CHMP	Committee for medicinal products for human use
CLASS	Celecoxib Long-term Arthritis Safety Study
CNT	Coxib and Traditional NSAID Trialist
COX	Cyclooxygenase
CV	Cardiovascular
CVD	Cardiovascular disease
EDGE	Etoricoxib versus Diclofenac sodium Gastrointestinal tolerability and Effectiveness trial
EMA	European Medicines Agency
FDA	Food and Drug Administration
GCs	Glucocorticoids
GI	Gastrointestinal
MEDAL	Multinational Etoricoxib versus Diclofenac Arthritis Long-Term
NSAID	Nonsteroidal anti-inflammatory drugs
OA	Osteoarthritis
OD	Once daily
OR	Odd ratio
OTC	Over the counter
PG	Prostaglandin
PPI	Proton pump inhibitor
PRECISION	Prospective Randomized Evaluation of Celecoxib Integrated Safety versus Ibuprofen or Naproxen
PreSAP	Prevention of colorectal Sporadic Adenomatous Polyps
PsA	Psoriatic arthritis
RA	Rheumatoid arthritis
RCT	Randomized clinical trial

C. Scarpignato, M.D., D.Sc., Pharm.D., M.P.H. (✉)
Clinical Pharmacology and Digestive Pathophysiology Unit, Department of Clinical and Experimental Medicine, Maggiore University Hospital, University of Parma, Cattani Pavillon, Parma 43125, Italy
e-mail: scarpi@tin.it

C. Blandizzi, M.D., Ph.D.
Division of Pharmacology, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_5

REACH	REduction of Atherothrombosis for Continued Health
RR	Relative risk
SCOT	Standard Care versus Celecoxib Outcome Trial
VIGOR	Vioxx™ Gastrointestinal Outcomes Research
WHO	World Health Organization

The Need for NSAID Therapy in Patients with Cardiovascular Disease

The risk of developing cardiovascular disease (CVD) is largely (75–90 %) explained by the presence or absence of traditional CVD risk factors. Age is a well-known traditional risk factor, which is generally considered non-modifiable for obvious reasons. Indeed, even after adjusting for traditional risk factors in a multivariable CVD prediction model, age remains a fundamental predictor of CVD risk (*for review see* [1]).

It is intuitive that—if age is an independent risk factor for developing CVD—it is also a risk for other age-related diseases, like, for instance, musculoskeletal disorders. It is a primary risk factor for the development of osteoarthritis (OA), likely due to aging changes in cells and tissues that make the joint more susceptible to damage and less able to maintain homeostasis [2]. It is widely accepted that arthritis is the leading cause of disability among old adults [3]. Also, acute and chronic pain is a major clinical problem of OA [4] and represents a common reason for patients to visit their family physician [5].

Pain-activated spinal reflexes cause the activation of the sympathetic nervous system, which increases peripheral vascular resistance, heart rate, and stroke volume. The response also involves the neuroendocrine system and, in particular, the hypothalamic-pituitary-adrenal axis, in addition to further activation of the sympathetic system by adrenal glands [6]. All these pathophysiological changes are related to the intensity of pain, which represents a significant predictor of hypertensive status, independent of the effects of age, thus suggesting that chronic pain is associated with an increased risk of hypertension [6]. In addition, pain-related disability and restriction of mobility may subsequently increase the thromboembolism risk [7]. Moreover, the reduced efficacy of endogenous analgesic systems described in the elderly, together with a decreased tolerance to pain and the slower resolution of post-injury hyperalgesia, can make it more difficult for the older adult to cope with pain. Older persons are therefore likely to be particularly vulnerable to the negative impacts of pain and pain-associated pathophysiological changes [8]. It is therefore evident that persistent pain in patients with CVD may be hazardous and should be proactively managed.

Thirty eight percent of patients with CVD also have OA as a comorbid condition, which—in 71 % of cases—is associated with physical disabilities [9]. Conversely, patients with OA suffer from at least one comorbid disease, with cardiac disease being among the most prevalent [10]. Comorbidities can affect how patients experience pain associated with chronic disease. Indeed, comorbidity is closely associated with significantly worse pain and increases the likelihood of disability among OA patients regardless of age and gender [11].

Pain management should be multimodal and tailored to the individual patient, and it will likely include a combination of both non-pharmacological and pharmacological interventions. The use of any pharmacological agent in patients with CVD, especially the elderly, should be tempered with caution with particular attention to increased sensitivity to medications, drug-drug interactions, and associated comorbidities. Therefore, these patients will often require down-adjustment of dosage and careful attention to the risk/benefit ratio of treatment [12]. There is no single ideal

pain medication for management of rheumatic pain. The four broad categories of drugs, namely, simple analgesics (i.e., paracetamol), nonsteroidal anti-inflammatory drugs (NSAIDs), stronger analgesics (i.e., opioids), and adjuvant drugs, each have unique and particular concerns regarding their adverse effect profiles.

Over the recent years, guidelines on pain management issued by professional organizations have paid attention mainly to safety concerns, especially for chronic conditions (such as OA) that require long-term treatment. Hence, there is consensus that paracetamol should be the first choice agent due to its putative, favorable safety profile despite its lower analgesic effectiveness than NSAIDs (*for review see* [13]). A recent meta-analysis of randomized, placebo-controlled trials [14] has shown that paracetamol is ineffective in the treatment of low back pain and provides minimal short-term benefit for patients with OA. Despite being recommended by the American Heart Association (AHA) [15], paracetamol may not be as safe as traditionally believed. Indeed, a systematic review of observational studies [16] showed that the dose-response estimated for most endpoints (cardiovascular, CV, renal, and gastrointestinal, GI, adverse events) suggests a considerable degree of paracetamol toxicity, especially at the upper end of standard analgesic doses. All the above results support the reconsideration of recommendation by clinical practice guidelines to choose paracetamol as a first-line treatment.

Although initially considered as cartilage driven, OA is much more complex than previously believed and low-grade (local and systemic) inflammation is the hallmark of this chronic and progressive condition [17]. In this connection, cyclooxygenase-2 (COX-2) selective or nonselective (ns) NSAIDs, which display both analgesic and anti-inflammatory properties, represent a pathophysiologically and pharmacologically sound approach. Although NSAIDs are very effective drugs, their use is associated with a broad spectrum of adverse reactions. GI adverse effects are the most common and include a wide clinical spectrum ranging from dyspepsia, heartburn, and abdominal discomfort to more serious events such as peptic ulcer with life-threatening complications of bleeding and perforation. The appreciation that CV risk is also increased further complicates the choices for physicians prescribing anti-inflammatory therapy in patients with CVD [18, 19].

Cardiovascular Adverse Effects of NSAIDs

Myocardial Infarction

The best-characterized mechanism of action of NSAIDs is the inhibition of COX activity of prostaglandin H (PGH) synthase-1 and PGH synthase-2 (also referred to as COX-1 and COX-2). Given the role that prostanoids, such as prostaglandin (PG) E_2, PGI_2, and thromboxane A_2, play in the local modulation of many important cellular functions, this mechanism of action is probably sufficient to explain the pharmacologic (i.e., the anti-inflammatory, analgesic, and antipyretic) effects of NSAIDs [20]. There are, however, a number of other COX-independent actions, often molecule-related and not shared by all the members of this class of drugs [21].

With exception of low-dose aspirin, (acetylsalicylic acid, ASA) which—for both pharmacokinetic and pharmacodynamic reasons—can be considered COX-1 selective [22], traditional NSAIDs are all nonselective toward COX isoenzyme inhibition. However, after the discovery of selective COX-2 inhibitors [23], some old compounds (such as nimesulide, meloxicam, and etodolac) have been found to be *preferential* COX-2 inhibitors [24].

Soon after the introduction of the selective COX-2 inhibitors into the market, concerns were raised that coxibs might be prothrombotic and increase the risk of acute myocardial infarction (AMI) [25]. This issue arose because, *theoretically*, these compounds may affect the balance between prothrombotic and antithrombotic PGs [26]. And indeed, the CV safety of these agents has, since then, repeatedly been questioned. A sub-analysis of the VIGOR trial [27], performed in rheumatoid arthritis (RA) patients to asses GI safety, demonstrated a

significant increase in the risk of AMI for rofecoxib users as compared to naproxen users. The absence of a placebo group in this trial and the low event rate in this subgroup analysis make the interpretation of these findings difficult. Possible explanations for these observations include an increased risk of AMI for rofecoxib, a cardioprotective effect of naproxen, or both. Alternatively, the findings of the VIGOR trial with respect to AMI may have simply occurred by chance and neither rofecoxib nor naproxen truly affects the risk of‘ AMI. A thoughtful review discussing these issues was published by Baigent and Patrono [28].

The increase in CV risk with rofecoxib was further reported in the APPROVe trial [29], a placebo-controlled study, designed to evaluate the efficacy of this selective COX-2 inhibitor in preventing adenomatous polyp recurrence. An extended analysis [30] showed that data were compatible with an early increase in CV risk that persisted for one year after stopping treatment. However, the CLASS study [31], carried out in OA patients to evaluate GI tolerability of celecoxib, was unable to find any increase in the rate of AMI with this agent, compared to ibuprofen or diclofenac. However, a meta-analysis of Mukherjee et al. [25] extended the CV safety concerns to celecoxib and, potentially, to all the selective COX-2 inhibitors.

On September 23, 2004, just 2 months before the APPROVe trial was scheduled to end, Merck withdrew Vioxx® from all markets worldwide without consulting the Food and Drug Administration (FDA). It did so because rofecoxib appeared unique among selective COX-2 inhibitors in its CV risk profile. On February 16–18, 2005, the FDA convened a joint meeting of its advisory committees on arthritis and on drug safety and risk management [32]. The members unanimously concluded that CV risk was a class effect, affecting all members to some extent. Surprisingly, the panel not only voted in favor of maintaining celecoxib and valdecoxib on the market but also in favor of allowing the reintroduction of rofecoxib back to the market. In each case, the panel recommended the FDA's strongest warnings ("black box" warnings) about CV risk, along with "other measures" to limit the drugs' use. The panel also recommended new warnings for traditional NSAIDs and physicians' caution in prescribing traditional NSAIDs [33, 34], which—with the exception of naproxen—were subsequently found to share the same CV risk [35, 36]. On February 17, 2005, European Medicines Agency (EMA) introduced a contraindication for all the selective COX-2 inhibitors in patients with ischemic heart disease or stroke and a warning for physicians to exercise caution when prescribing selective COX-2 inhibitors to patients with CV risk factors [37, 38]. Two adenoma prevention trials (APC and PreSAP) [39] showed a nearly twofold-increased CV risk. The trend for a dose-related increase in CV events and blood pressure (BP) raised the possibility that lower doses or alternative dosing intervals might be associated with less CV risk. Taking into account both clinical data and regulatory decisions, the American Heart Association (AHA) issued a position paper [15] that discouraged the use of selective COX-2 inhibitors in patients at CV risk.

Randomized Trials and Meta-analyses Focused on Cardiovascular Events

Although the above mentioned trials did raise awareness about the CV risk of selective COX-2 inhibitors, it is worth emphasizing that none of them was specifically designed to assess the risk of CV events, which were then evaluated in post hoc analyses.

The only published, large, randomized trial on the CV risk comparing a selective COX-2 inhibitor with a ns-NSAID is the MEDAL program [40]. In this setting, etoricoxib (60 or 90 mg daily) or diclofenac (150 mg daily) were given to more than 34.000 patients for an average of 18 months. The primary end point (i.e., occurrence of thrombotic CV events) was similar between the two groups, but etoricoxib was associated with a lower rate of GI events (albeit not of GI complications due to the allowance of free PPI use). This study was criticized for the use of diclofenac as comparator and for its experimental design, which included the results from three different trials (EDGE I, EDGE II, and

MEDAL), although they were all randomized and had the same prespecified CV end point (thrombotic CV events). Notwithstanding these limitations, the study demonstrated a similar CV risk of both etoricoxib and diclofenac, whose CV toxicity has been recently pointed out by the CNT Collaboration meta-analysis [41] as well as a review of RCTs and observational studies [42]. Like for COX-2 selective NSAIDs [43], the increase in CV risk with diclofenac appears to be dose-dependent [44, 45]. However, at doses below the maximal over the counter (OTC) dose and for durations recommended for OTC use, this NSAID (and ibuprofen as well) seem not to be associated with an increased CV risk [45].

A trial that might shed light on the safety of COX-2 selective and ns-NSAIDs is the PRECISION study [46]. This is the first study evaluating more than 20,000 RA/OA patients with high CV risk, chronically treated with anti-inflammatory agents, and will define the relative CV safety profile of celecoxib (100–200 mg bid), ibuprofen (600–800 mg tid), and naproxen (375–500 mg bid), providing data to help guide NSAID use for pain management in this population. While the results of this trial are eagerly awaited, to gain insights into the relative CV safety of currently available drugs, we must rely on a number of meta-analyses, performed on the topic.

Meta-analyses

The first meta-analysis of published and unpublished RCTs, with indirect estimates of the effects of traditional NSAIDs, was published by Kerney et al. [36]. This showed that selective COX-2 inhibitors are associated with a moderate increase in the risk of CV events, as are *high-dose* regimens of ibuprofen and diclofenac, while *high-dose* naproxen is not associated with such an excess risk (Fig. 5.1). Similar results were provided by a meta-analysis of observational studies, published in the same year [35] and later updated [47] (Fig. 5.2).

Further insights into the CV risk of both COX-2 selective and ns-NSAIDs were gained from the *network* meta-analysis of Trelle et al. [48]. These authors selected all large-scale, RCTs comparing *any* NSAID with other NSAIDs or placebo. The primary outcome was AMI. Secondary outcomes included stroke, death from CV disease, and death from any cause. This analysis provided risk estimates for all the selective COX-2 inhibitors and many traditional NSAIDs evaluated in comparative trials, showing that none of the drugs studied is safe in CV terms, with naproxen being the least harmful.

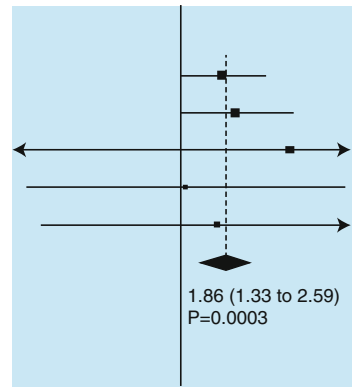
The most recent and authoritative meta-analysis is that performed by the so-called CNT Collaboration [41]. The authors assembled over 600 clinical trials that included more than 300,000 participants and used direct and indirect meta-analysis techniques to add certainty and precision to estimates of NSAID-associated CV and GI adverse events. Their results indicate that *high doses* of all the selective COX-2 inhibitors, diclofenac and ibuprofen increase the risk of major CV events (nonfatal AMI, nonfatal stroke, or CV death) and that high-dose naproxen is not associated with either an increased risk or significant aspirin-like protection for these outcomes. When compared to naproxen, the currently available COX-2 selective inhibitors (namely, celecoxib and etoricoxib) behaved differently, with only etoricoxib showing a significant increase in the risk of major CV events (Fig. 5.3). In addition, in this analysis, all NSAIDs (both COX-2 selective and ns-NSAIDs, including naproxen) double the risk of congestive heart failure (CHF) and increase the risk of peptic ulcer complications and other GI bleeding. Less information was available on other NSAIDs, but there is presently no evidence that any compound is safer than the more studied drugs in the class. In line with these results, a recent meta-analysis of observational studies, performed by the investigators of the SOS[1] project [49], showed that the most frequently used NSAIDs (except naproxen) are associated with an increased risk of AMI, *at high doses* or in patients with coronary heart

[1] *SOS* project = *S*afety *O*f non-*S*teroidal anti-inflammatory drugs project http://www.sos-nsaids-project.org.

a

COX 2 inhibitor	No of trials	Events/person years: Allocated COX 2 inhibitor	Events/person years: Allocated placebo
Rofecoxib	37	54/6638	30/6415
Celecoxib	41	44/8976	9/4953
Etoricoxib	17	2/753	0/414
Lumiracoxib	12	5/1375	2/584
Valdecoxib	14	8/748	1/273
Subtotal	121	113/18 490	42/12 639
		(0.6%/year)	(0.3%/year)

Heterogeneity between five drugs: χ^2=1.0, df=4, P=0.9



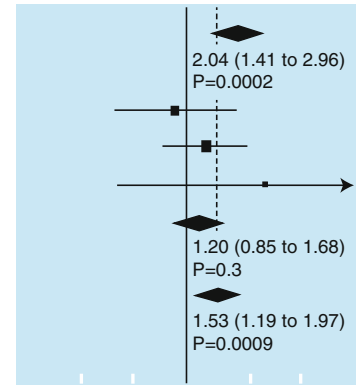
Event numbers and person years of exposure, with corresponding mean annual event rates in parenthesis, are presented for patients allocated to selective COX-2 inhibitor and placebo. Event rate ratios for subtotals, with 95% confidence intervals, are indicated by a diamond; rate ratios for individual selective COX-2 inhibitors, with 99% confidence intervals, are indicated by a square and horizontal line. Diamonds to the right of the solid line indicate hazard with a selective COX 2 inhibitor compared with placebo, but this is conventionally significant only if the diamond does not overlap the solid line

b

COX 2 inhibitor versus:	No of trials	Events/person years: Allocated COX 2 inhibitor	Events/person years: Allocated NSAID
(a) Naproxen	42	99/16 360	30/10 978
		(0.6%/year)	(0.3%/year)
Ibuprofen	24	21/5848	21/5160
Diclofenac	26	61/10 886	32/6913
Other non-naproxen	7	5/166	2/274
(b) Any non-naproxen	51	87/16 900	55/12 347
		(0.5%/year)	(0.4%/year)
Any NSAID	91	186/33 260	85/23 325
		(0.6%/year)	(0.4%/year)

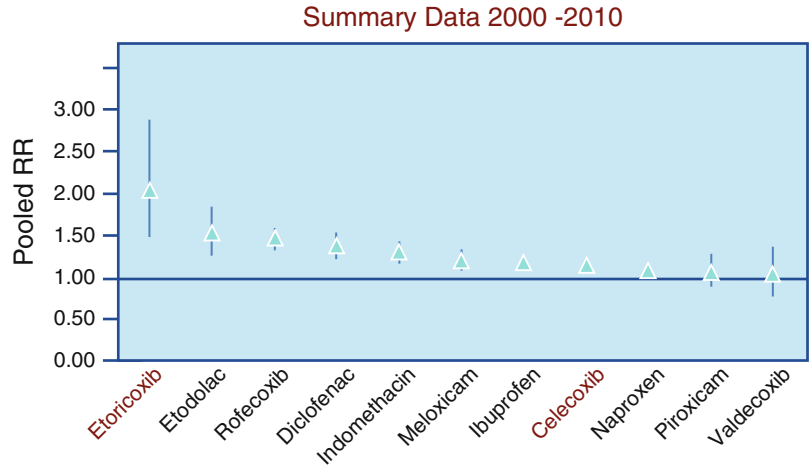
Heterogeneity between (a) and (b): χ^2=4.3, df=1, P=0.04

Between non-naproxen NSAIDs: χ^2=2.6, df=2, P=0.3



Symbols and conventions are as in **Fig. 5.1a**

Fig. 5.1 (**a**) Comparison of the effects of different selective COX-2 inhibitors versus placebo on myocardial infarction (from Kearney et al. [36]). Event numbers and person years of exposure, with corresponding mean annual event rates in parenthesis, are presented for patients allocated to selective COX-2 inhibitor or placebo. Event rate ratios for subtotals, with 95 % confidence intervals (CIs), are indicated by a *diamond*; rate ratios for individual selective COX-2 inhibitors, with 99 % CIs, are indicated by a *square* and *horizontal line*. *Diamonds* to the *right* of the *solid line* indicate hazard with a selective COX-2 inhibitor compared with placebo, but this is conventionally significant only if the diamond does not overlap the solid line. (**b**) Comparison of effects of different selective COX-2 inhibitors versus ns-NSAIDs on myocardial infarction (from Kearney et al. [36]). *Symbols* and conventions are as in Fig. 5.1(**a**)



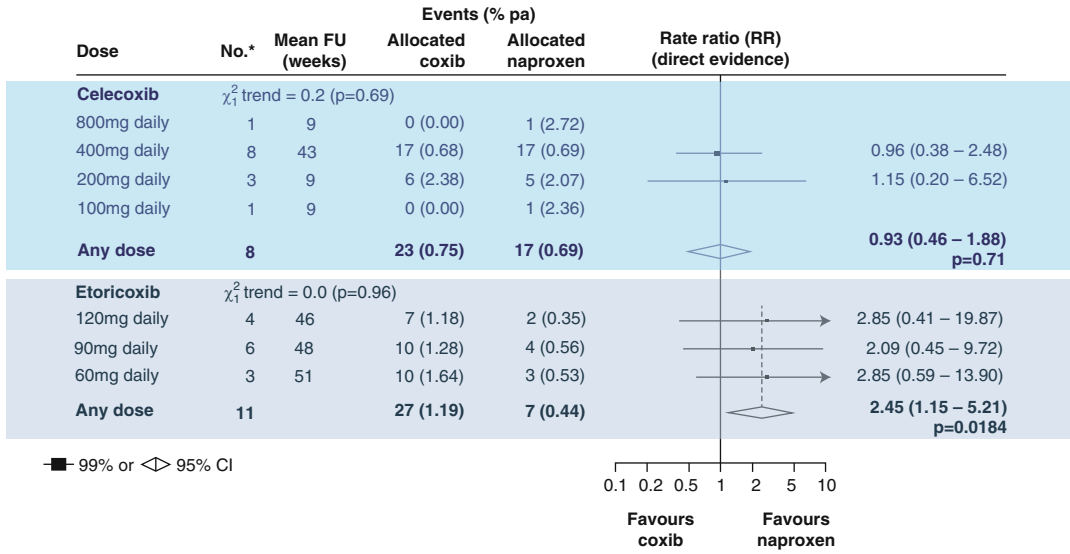


Fig. 5.2 Meta-analysis of observational studies on CV risk of COX-2 selective and ns-NSAIDs: Pooled relative risk (RR) for individual drugs (from McGettigan and Henry [47])

Fig. 5.3 Effects of different regimens of the currently available COX-2 selective inhibitors on major CV events: trials of celecoxib or etoricoxib versus naproxen (from the CNT Collaboration meta-analysis [41])

disease (CHD). For diclofenac and rofecoxib, the risk was increased both at low and high doses.

It should be emphasized, however, that the intrinsic patient's CV risk factors are of paramount importance. While confirming that frequent NSAID use significantly increases the CV risk in a dose-dependent fashion, Chan et al. [50] also found that the elevated risk was particularly evident among current smokers and was absent among those who had never smoked (Table 5.1). Therefore, the lower the risk factors, the lower the propensity for NSAIDs to cause CV adverse events. Drugs are indeed only one of the several concomitant risk factors and likely not the most important. The interaction of the expected baseline CV and thrombotic risk with components of drug exposure and duration of treatment with a selective COX-2 inhibitor is illustrated in Fig. 5.4 [51]. In this connection, the preliminary results of the SCOT Trial [52] showed that–in patients with arthritis but *without* known CVD–CV event rates were low and the CV

outcomes did not differ significantly between celecoxib and ns-NSAIDs.

Time Dependence of NSAID-Associated CV Risk

Although it might appear intuitive that the shorter the duration of drug exposure, the lower the risk, there is little information on this critical issue.

Brief (3–5 days) perioperative use of NSAIDs was not associated with increased risk for postoperative AMI after total hip and knee replacement [53]. However, this patient population, albeit relatively old (mean age 65), was not at high CV risk since only 12 % of the subjects had a history of coronary heart disease. In addition, NSAIDs were given on the top of patient-controlled epidural analgesia.

In a random population of 125,000 Canadian NSAID users [54], it was shown that a small proportion of patients using rofecoxib for the first time had their AMI shortly after starting the drug (a median of 9 days after the first exposure). This risk did not increase with the length of treatment and returned to baseline shortly after treatment was discontinued (Fig. 5.5). On the contrary, Nussmeier et al. [55] reported that orally administered valdecoxib (preceded by either placebo or parecoxib) resulted—during the 10 days of treatment—in an increased risk of CV events, which persisted for additional 30 days. This was, however, a peculiar patient population undergoing cardiac surgery, in whom the extent of inflammation and endothelial stress is very high.

A recent systematic review [56], evaluating specifically the evidence regarding the adverse effects of NSAID short-term use, concluded that—when prescribed at the most effective dose for 10 days or fewer—these drugs may be considered relatively safe. Along the same lines, another systematic review of RCTs and observational studies [42] found little or no increase in CV risk, associated with exposures shorter than 30 days.

Solomon et al. [57]–in a case-control study of 54,475 patients 65 years of age or older–evidenced

Table 5.1 CV risk in NSAID and paracetamol users: effect of smoking and frequency of drug intake (from data in Chan et al. [50])

Treatment	RR	95 % CIs
NSAIDs	1.44	1.27–1.65
• Current smokers	1.82	1.38–2.42
• Past smokers	1.58	1.28–1.95
• Never smokers	1.11	0.88–1.41
• >15 tables/week	1.86	1.27–2.73
Paracetamol	1.35	1.14–1.59
• >15 tablets/week	1.68	1.10–2.58

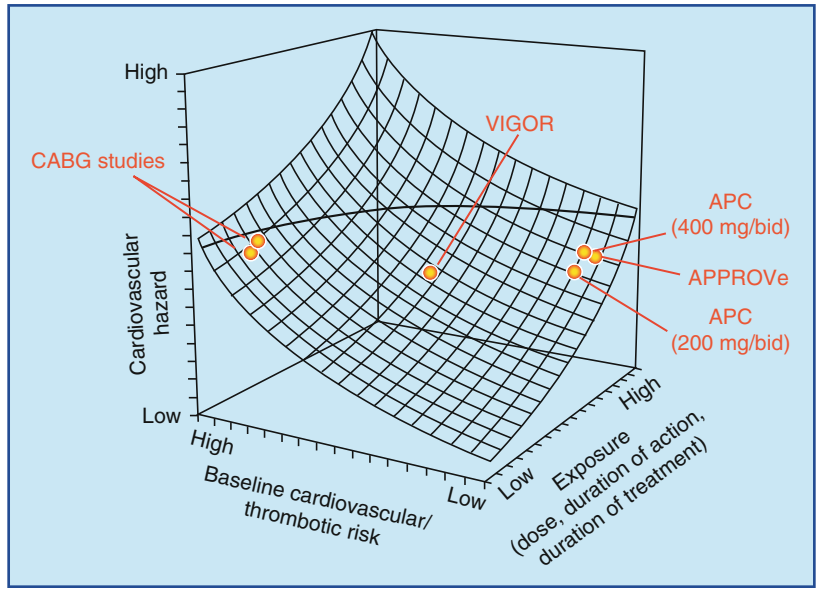


Fig. 5.4 Illustration of the expected interaction of baseline CV an thrombotic risk with components of drug exposure including dose, duration of action, and duration of treatment with a selective COX-2 inhibitor (from Grosser et al. [51]). The approximate relationship of CV hazard detected in controlled studies within this interaction are indicated (*see text*)

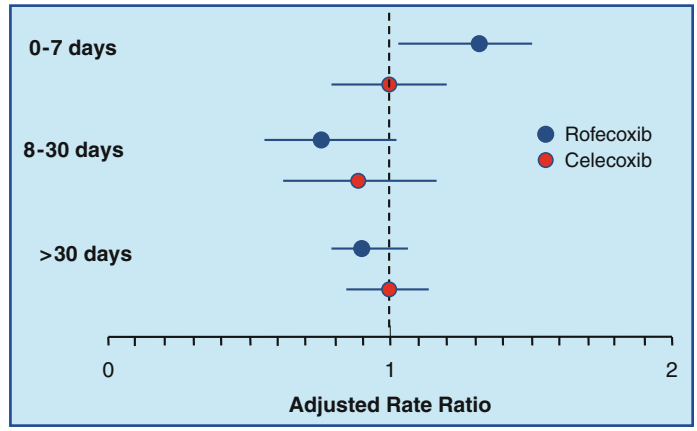


Fig. 5.5 Risk of myocardial infarction among elderly users of rofecoxib and celcoxib. Adjusted rate ratios as a function of time after treatment is discontinued (modified from reference [54])

that rofecoxib, but not celecoxib, was associated with a significant increase in the risk of AMI, which remained constant during the first 90 days and vanished thereafter.

The above studies, albeit heterogeneous, seem to challenge the EMA, FDA, and AHA recommendations, according to which physicians should prescribe the lowest effective dose for the *shortest* possible duration of treatment. Indeed, the risk does appear to increase early after starting treatment, but can decrease even with continuous treatment, perhaps owing to development of tolerance.

CV Risk of NSAID Use After First-Time Myocardial Infarction

The risks of CV mortality and morbidity are well-known complications after AMI. This elevated CV risk is most prominent soon after the AMI but declines with time, reaching the risk level of the background population after 5–10 years [58]. However, knowledge about the CV safety of NSAIDs in the years following AMI is limited.

To determine whether a history of past AMI modified the risk of AMI recurrence associated with the use of NSAIDs, Gislason et al. [59] analyzed the risk of rehospitalization for AMI and death related to NSAID use in patients with prior AMI, and found that selective COX-2 inhibitors at all dosages and ns-NSAIDs at high dosages increase mortality.

In a population-based cohort study of 122,079 elderly people with and without previous AMI and newly treated with an NSAID, only rofecoxib use was associated with an increased risk of AMI in those without a previous event [60]. However, both rofecoxib and celecoxib were associated with an excess risk of AMI for current users with a history of AMI. On the contrary, no increased risk was observed for celecoxib users in a population-based cohort of Canadian patients aged 66 years and older, who survived a hospitalization for AMI [61]. A large cohort study [62] examined patients recently discharged from hospital with CHD, tabulating the rates of subsequent serious CV events with ns-NSAIDs and COX-2 selective agents. In this patient population, naproxen showed better CV safety than did diclofenac and ibuprofen as well as high doses of celecoxib and rofecoxib. When the incidence of serious CHD was assessed according to the duration of NSAID therapy, the adjusted rates for any drug (with the exception of naproxen) were increased with durations of use <90 days (Fig. 5.6). In contrast, there was no significant increase in the risk for use of longer duration [62].

A large observational study (44,095 patients from the REACH[2] registry) found that—in patients with established atherothrombosis (CHD, cerebrovascular disease, or peripheral artery disease) or multiple (>3) risk factors for atherothrombotic disease)—NSAID use is associated with a higher

[2] REACH = REduction of Atherothrombosis for Continued Health

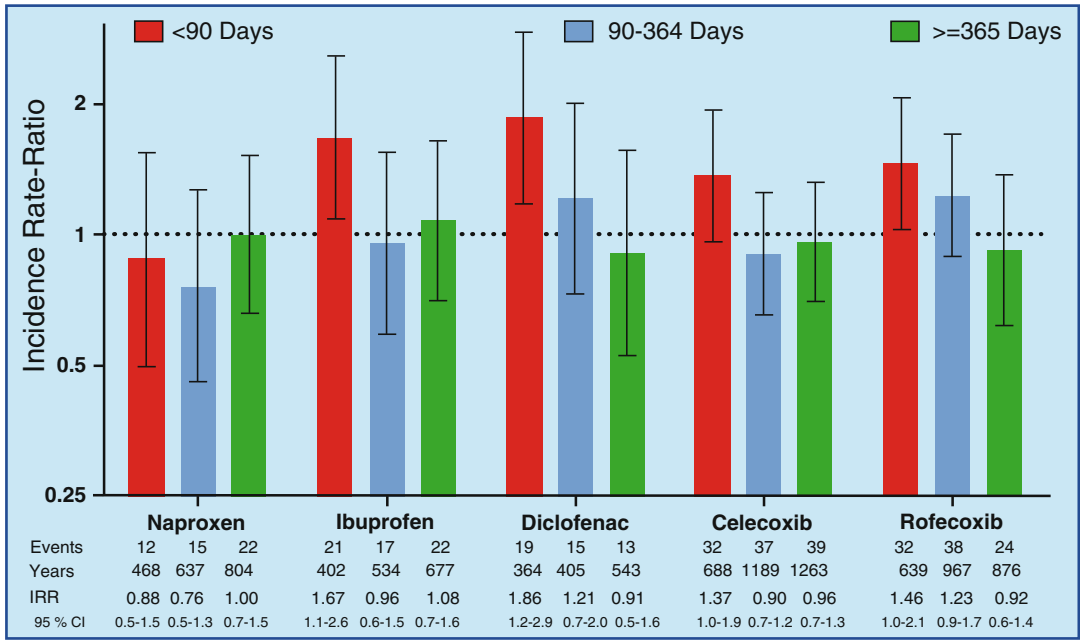


Fig. 5.6 Occurrence of CHD by total duration of NSAID current use in patients after hospitalization for serious coronary events. Reference category is non use of any NSAID (from Ray et al. [62])

risk of AMI (as well as stroke) and hospitalizations for both ischemia and heart failure when compared to nonusers [63].

Despite the fact that NSAIDs are contraindicated among patients with established CV disease, many receive NSAID treatment for a short period of time. However, even short-term treatment with most NSAIDs was associated with increased risk of death and recurrent AMI in patients with prior AMI [64]. Actually, ongoing treatment with NSAIDs, particularly selective COX-2 inhibitors and diclofenac, is associated with worsened prognosis in patients admitted with first-time AMI [65], by increasing the risk of CV death [66].

All the above studies definitely show that even short-term treatment with most NSAIDs is associated with an increased CV risk in patients with AMI. Furthermore, NSAID use among patients with first-time AMI is associated with persistently increased risk of all-cause mortality and of a composite end point of coronary death or nonfatal recurrent AMI, respectively, for at least 5 years thereafter. NSAIDs have therefore no apparent well-tolerated treatment window among patients with AMI.

CV Risk of NSAIDs in Special Patient Populations

Inflammatory Arthritides

Cardiovascular disease (CVD) comorbidity is a significant issue for the inflammatory arthritides (IA). There is a wealth of mortality studies showing an increased CV mortality in RA and the evidence suggests that the same is likely to be true for psoriatic arthritis (PsA) and ankylosing spondylitis (AS) [67, 68]. The pathophysiological understanding of the mechanisms that promote the development of vascular disease has changed over the last few decades, leading to the recognition that inflammation is a major player, involved both in the impairment of the classic CV risk factors and as directly in the process of endothelial injury, dysfunction, and ultimately atherosclerosis [69]. *The use of NSAIDs could therefore be beneficial*. In this connection, Lindhardsen et al. [70] showed—in

a nationwide cohort study—that the CV risk associated with *overall* NSAID use was significantly lower in RA patients than in controls (HR, 1.22; 95 % CIs, 1.09–1.37), despite the finding that a few NSAIDs (namely, rofecoxib and diclofenac) did confer an increased risk. Along the same lines, a case-control study [71], while confirming that AS patients display an increased CV risk, showed that long-term (>12 months) use of both COX-2 selective and nonselective NSAIDs can decrease the risk up to 10 times. However, short-term (6 months) use of NSAID was found to increase the CV risk, as it happens in the general population.

Osteoarthritis

Initially considered cartilage driven, OA, the prototypic age-related disease [72], is much more complex than previously thought and low-grade (local and systemic) inflammation is the hallmark of this chronic and progressive condition [17]. However, despite the prevalence of CVD in patients with OA is significant, the relationship between OA and CVD is not straightforward [73]. Indeed, a nested, case-control study [74] found no significant increased risk associated with the current use of NSAIDs as a group. A dose effect was not identified, but there was a significant increased risk associated with long-term use (>1 year) (Odd ratio, OR, 1.43; 95 % CIs, 1.11–1.83). While in patients at low or intermediate background risk NSAIDs did not show any association with AMI, the analysis revealed a moderate positive association among those at high background CV risk. The strongest association with AMI was obtained in patients at high background CV risk exposed to NSAIDs for long term (OR, 1.80; 1.26–2.58). However, in high-risk population, even short-term treatments were associated with an increased AMI risk (OR, 1.32; 1.01–1.71).

Diabetes Mellitus

It is well known that CVD is a major cause of mortality in patients with diabetes mellitus. CV risk factors and diabetes overlap, leading to the hypothesis that both share an inflammatory basis. Therefore, targeting CV risk factors, including the subclinical inflammatory status, is critical to minimize the long-term CV complications of the disease [75]. Despite this, NSAID use in diabetic elderly patients increased the CV risk, which was higher in those with CHD or heart failure and in users of anticoagulants or antiplatelet agents, likely because of the higher probability of developing myocardial infarction or stroke among this patient population [76]. The same holds true for diabetic patients with OA [74].

CV Adverse Effects of NSAIDs: Is There a Genetic Predisposition?

In an attempt to provide a reliable mechanistic explanation for the increase in CV adverse events observed in patients treated with selective COX-2 inhibitors in clinical trials, several authors have advocated the so-called "COX-2 hypothesis," according to which the selective blockade of COX-2 would result in an *imbalance* between the impaired intravascular COX-2-dependent production of antithrombotic prostacyclin, without any interference with the COX-1-dependent biosynthesis of prothrombotic thromboxane in platelets [51].

The *imbalance* hypothesis, supported by human studies, has received renewed attention due to the relevance that vascular prostacyclin may have in the regulation of normal CV functions and fostered intensive research on pathophysiological mechanisms linking prostacyclin to CV disease, with particular regard for the underlying genetic polymorphisms that might hamper the biological activities of this prostanoid and its IP receptor [77]. Much information in this context have been retrieved from mice lacking the IP receptor ($IP^{-/-}$), which have substantiated the relevance of prostacyclin activity in the CV system and supported a strong correlation of altered IP receptor activity with several atherothrombotic CV disorders, including stroke, myocardial infarction, and hypertension. Of greater importance, $IP^{-/-}$ mice have provided convincing evidence that prostacyclin counteracts the in vivo actions of thromboxane A_2, thus lending credibility to the notion that adverse CV events

associated with selective COX-2 inhibitors might depend, at least in part, on the inhibition of prostacyclin biosynthesis [77].

Findings in $IP^{-/-}$ mice have prompted the implementation of genetic studies to test the hypothesis that polymorphisms in the human IP (hIP) gene, causing a loss of receptor response to prostacyclin, might behave as a sort of "endogenous COX-2 inhibition" or "functional human IP receptor knockout." Consistently with this expectation, in a cohort study on high-risk CV patients ($n = 1063$), both the disease severity and frequency of adverse CV events were found to be significantly increased over a 3-year follow-up in patients with a hIP polymorphism (Arg212Cys) associated with a loss of receptor function, as compared with age- and risk factor-matched controls with normal alleles. Of note, in the same study, patients carrying the hIP Arg212Cys polymorphism displayed also a significant impairment of platelet aggregation [78]. A subsequent case-control study, that compared the results of coronary angiographies from patients with hIP polymorphisms associated with defective (Arg212Cys, Arg215Cys, Leu104Arg) or normal (Val15Aal, Val25Met, Pro226Thr, Ser319Trp, Gly181Ala) receptor function, showed more severe coronary artery obstruction in patients with dysfunctional hIP receptor mutations [79].

Overall, the consistency of evidence from several lines of research, including early trials on the safety and efficacy of selective COX-2 inhibitors, investigations on mice with genetic deletion of the prostacyclin IP receptor ($IP^{-/-}$), and human studies on hIP receptor gene polymorphisms, taken together with the results of a number of recent meta-analyses, point out the important concept that a decrease in intravascular prostacyclin activity, arising from a blockade of its biosynthesis (as a consequence of pharmacological COX-2 inhibition) and/or a dysfunctional hIP receptor activity (as a consequence of receptor gene polymorphism or deletion), can support an increased risk of adverse CV outcomes. This body of knowledge, if adequately substantiated, might foster the advent of CV pharmacogenetics pursuing the personalized management of NSAID therapy. For example, it might be envisaged that in the clinical management of patients with known genetic variants affecting the activity of prostacyclin pathway, the use of selective COX-2 inhibitors should likely be avoided [77].

CV Adverse Effects of NSAIDs: Is COX-2-Mediated Immunomodulation Responsible?

The view that a prothrombotic effect explains the increase in MI, associated with both COX-2 selective and ns-NSAIDs, has been increasingly questioned, with the renal effects, especially increase in BP, gaining more acceptance as CV risk factors (*see below*).

COX inhibition is associated with an established anti-inflammatory action, which is systemic and neither site nor organ specific. Reduced vessel wall inflammation is likely to result in an improvement in arterial wall compliance but with the potential for destabilization of plaque and increased risk of fragmentation, detachment, and embolization. None of these pathophysiological events involve an increase in thrombosis per se. Rather, they suggest that the observed drug effects are based on the nature of the inflammation of the arterial wall, stemming from the Th_1 type immune response, which may be patient-dependent. Indeed, inhibition of PG synthesis results in augmentation of the Th_1 response by limiting prostanoid synthesis. Although the role of prostanoids as mediators of inflammation in the periphery is well understood, the systemic immunomodulatory role of prostanoids shifting the immune response away from a Th_1 type is less appreciated [80]. It is well established that atherosclerosis is an inflammatory arterial disease driven by a Th_1 type immune response. However, while the vulnerable phenotype of atheroma is associated with the cellular Th_1 immune response, the stable plaque phenotype is associated with a Th_2 type response. By interpreting all above evidences, Padol and Hunt [80] proposed the augmentation of Th_1-mediated atherogenesis/production of pro-atherogenic cytokines associated with detrimental plaque remodeling, instability, rupture, and

embolization as a plausible explanation for the increased CV events observed with COX-2 selective and ns-NSAIDs.

Stroke

The same mechanisms responsible for the increased risk of AMI and CV death associated with COX-2 selective and ns-NSAID use could also be responsible for the risk of ischemic stroke. However, the available data show that the risk of stroke conferred by these drugs is much smaller, if any, than the risk of AMI.

In the meta-analysis by Kearney et al. [36], neither selective COX-2 inhibitors nor ns-NSAIDs did increase the risk of stroke when compared to placebo. However, selective COX-2 inhibitors were associated with a significantly lower incidence of stroke than any non-naproxen traditional NSAID (rate ratio 0.62, 0.41–0.95; $p = 0.03$). The lack of increase in stroke risk by both classes of anti-inflammatory drugs, when compared to placebo, has recently been confirmed by the large individual data meta-analysis, performed by the CNT Collaboration [41]. However, the network meta-analysis of Trelle et al. [48] found a trend toward an increase in the risk for all the drugs studied, with a significant effect for ibuprofen (RR: 3.36, 1.00–11.6), diclofenac (RR: 2.86, 1.09–8.36), and lumiracoxib (RR: 2.81, 1.05–7.48). The adverse effect of diclofenac (but not that of ibuprofen) was confirmed by Garcia-Poza et al. [81] in a population-based, case-control study, in which NSAID dose, duration of treatment, and baseline CV risk appeared to modulate the risk.

It is difficult to reconcile the different results of the above analyses, none of which provides proof of causality but only (presence or lack of) association. However, it is worth emphasizing that the network meta-analysis, finding an increased stroke risk for NSAIDs, used *indirect* treatment comparisons and some modeling assumptions. Although interesting, its results should be considered complementary to traditional meta-analyses and interpreted with caution. In addition, the different studies selected in the different meta-analyses might have included patients with different kinds of stroke (thrombotic, cardio-embolic, or hemorrhagic), with the majority of strokes being ischemic [82]. Since the mechanisms are different, one would infer that the risk of stroke with NSAIDs should be differentiated by subtype. As the overall numbers of events have been limited within the prospective trials, the majority of stroke events quoted in various studies were not differentiated with respect to stroke subtypes. A study by Johnsen et al. [83] found no increased risk of intracranial hemorrhage with NSAID exposure when subjects were stratified by gender, age, and history of hypertension. Additionally, two other studies [84, 85] found no association between either ischemic or hemorrhagic stroke, with any NSAID.

Non-ischemic CV Adverse Effects of NSAIDs

Although the major safety issues for NSAIDs have mainly been the GI tolerability and ischemic CV risk, a number of other adverse effects, largely affecting directly or indirectly the vascular system, have been described.

Congestive Heart Failure

The renal and vascular effects of NSAIDs (*see below*) may explain the increased risk of CHF associated with their use. All studies that have analyzed this issue have consistently found an increase in CHF risk, which was roughly doubled when compared to non-exposure.

It has been long known that users of NSAIDs have an increased risk of hospitalization for CHF and that this effect is larger among patients with preexisting CV disease [*for review see* [86]). Initiation of NSAID therapy may double the risk of developing heart failure in susceptible individuals [87]. Patients with renal failure, diabetes, or hypertension when taking NSAIDs

might be at a greater risk of developing CHF than patients without those conditions. It is less likely, however, that these drugs could also induce heart failure in otherwise healthy individuals [86]. The large CNT Collaboration meta-analysis [41] confirmed that all NSAIDs (be they COX-2 selective or not) doubled the risk of CHF (rate ratio 1.85–2.28).

Although, as a class, selective COX-2 inhibitors share the same CHF risk of traditional (i.e. ns) NSAIDs, some comparative studies have shown differences among individual drugs. Indeed, a large population-based retrospective cohort study [88] found a higher risk of admission for CHF in users of rofecoxib and ns-NSAIDs, but not celecoxib compared to nonusers (Table 5.2). These results are in line with the findings of the celecoxib trial database, in which no statistically significant increases in CHF incidence were observed among high-risk patients, such as those receiving concomitant diuretics [89], as well as among the patient population from the large ACCEPT study, performed in Japan [90].

An echocardiographic investigation [91] evaluated the effect of NSAIDs on cardiac function in elderly people, who recently started drug intake. Current NSAID use for <14 days was associated with a significantly higher left ventricular end-systolic dimension (+1.74 mm; 95 % CI, 0.20–3.28), left ventricular end-diastolic dimension (+3.69 mm, 95 % CI, 1.08–6.31), and significantly lower fractional shortening (−6.03 %, 95 % CI, -9.81–2.26 %), compared with nonusers. Current NSAID use for >14 days was associated with a higher left end-diastolic dimension (+1.96 mm, 95 % CI, 0.82–3.11), but there was no change in the other

Table 5.2 Risk of hospitilization for CHF in patients taking COX-2 selective or ns-NSAIDs (from data in Mandani et al. [88]))

	Study cohort			
	Non-NSAID users	Colecoxib users	Rofecoxib users	ns-NSAID users
Stratified CHF analysis				
Patients without a recent history of CHF-related admission				
Sample size	98,409	18,517	14,317	11,424
Number of admissions	248	84	111	30
Days of follow-up (mean, SD)	139 (77)	170 (97)	147 (90)	94 (69)
Total follow-up (person-years)	37,507	8642	5749	2944
Crude CHF rate per 1000 person-years	6-6	9-7	19-3	10-2
Model-based risk ratios				
Unadjusted rate ratio (95 %CI)	1-0 (reference)	1.5 (1.2–1.9)	3.0 (2.4–3.7)	1.5 (1.1–2.3)
Adjusted rate ratio (95 %CI)	1-0 (reference)	0.9 (0.7–1.2)	1.8 (1.4–2.3)	1.1 (0.7–1.6)
Numbers needed to treat to harm	n/a	n/a	434	n/a
Patients with a recent history of CHF-related admission				
Sample size	1591	391	266	182
Number of admissions	100	32	32	17
Days of follow-up (mean, SD)	136 (78)	148 (96)	133 (86)	97 (77)
Total follow-up (person-years)	593	159	97	49
Crude CHF rate per 1000 person-years	169	202	330	350
Model-based risk ratios				
Unadjusted rate ratio (95 % CI)	1-0 (reference)	1.2 (0.8–1.8)	2.0 (1.3–2.9)	2.0 (1.2–3.3)
Adjusted rate ratio (95 % CI)	1-0 (reference)	1.2 (0.8–1.7)	1.8 (1.2–2.7)	2.2 (1.3–3.7)
Numbers needed to treat to harm	n/a	n/a	19	12

The estimated numbers needed to harm in patients with a history of recent admission for heart failure were significantly lower than those of individuals with no previous history

echocardiographic parameters. This study provides for the first time an objective evaluation of the NSAID effects on cardiac function.

Atrial Fibrillation

Inflammation is strongly associated with cardiac dysrhythmia, either as a cause or a consequence. Anti-inflammatory drugs are widely prescribed, and some of them, including corticosteroids and NSAIDs, have been associated with an increased CV risk. Therefore, the eventual pro- or anti-arrhythmic effect of these drugs is of high interest for clinical practice. While anti-inflammatory drugs have demonstrated anti-arrhythmic properties in postoperative atrial fibrillation (AF), NSAIDs increase the risk of AF [92].

A nested case-control study [93] showed that current use of NSAIDs is associated with an increased risk of chronic AF (RR, 1.44; 95 % CI, 1.08–1.91). Such risk was further increased among long-term users with treatment duration longer than 1 year (RR, 1.80; 95 % CI, 1.20–2.72). The increased risk of chronic AF was not explained by the presence of heart failure. These findings were further confirmed by a population-based case-control study [94], which also showed that the use of non aspirin NSAIDs was associated with an increased risk of atrial flutter. Compared with nonusers, the association was strongest for new users, with a 40–70 % increase in RR (lowest for ns-NSAIDs and highest for selective COX-2 inhibitors). A more recent population-based study [95] found that new NSAID use may predispose patients to AF, and the risk is almost doubled in CHF patients. Also, recent use (within 30 days after discontinuation of NSAIDs) was associated with an increased risk of AF compared with those who had never used these drugs (HR, 1.84; 95 % CIs, 1.34–2.51) [96]. However, in one such study [95], use of selective COX-2 inhibitors was not significantly related to AF occurrence, except in patients with chronic kidney or pulmonary diseases.

A recent meta-analysis of the available studies [97] confirmed that – overall – NSAID use is associated with a 12 % increased risk of AF. The risk appeared larger for new users (RR, 1.53; 95 % CIs, 1.37–1.70) compared with long-term users (RR, 1.09; 95 % CIs, 1.04–1.14) and in presence of CHF and CKD.

These studies thus add evidence that AF (and flutter) need to be added to the CV risks to be considered when prescribing NSAIDs.

Effects on Renal Function and Blood Pressure

Several studies have reported that NSAID use is associated with adverse effects on renal function, estimated glomerular filtration rate (eGFR) alteration, fluid and electrolyte disturbances, and BP elevation.

The renal effect of COX-2 selective and ns-NSAIDs was evaluated in a retrospective, nested, case-control study, involving 1,459,271 new users [98]. Acute kidney failure (AKF) was defined as a creatinine increase greater than 50 %. A higher risk of AKF was found in new users of any single NSAID (OR, 1.82; 95%CI, 1.68, 1.98) compared to nonusers, without recent use. The risk of AKF varied among different NSAIDs, with risk generally increasing with decrease in COX-2 selectivity (rofecoxib < celecoxib < meloxicam < etodolac < diclofenac < piroxicam < salsalate < sulindac < ibuprofen < naproxen < high-dose aspirin < indomethacin < ketorolac). Those using multiple NSAIDs appeared to have the highest risk (OR, 2.90; 95 % CIs, 2.62, 3.22).

In patients with RA, the prevalence of [99] and/or risk of developing [100] CKD is high. In this population, therefore, the risk for renal adverse effects of NSAIDs could be higher. However, a prospective, cohort study [101] showed that eGFR was not significantly altered by NSAID use, unless a baseline impairment of renal function (i.e., eGFR<30 mL/min) was present.

A longitudinal cohort study [102] evaluated the risk of CKD in hypertensive patients taking NSAIDs. After controlling for confounding factors, the analysis showed that – compared with nonusers – NSAID use was associated with a 1.18-fold increased risk of CKD in

subjects taking NSAIDs for 1 to 89 days and a 1.32-fold increased risk of CKD in those taking NSAIDs for ≥90 days.

The effect of NSAID therapy on CKD progression was specifically investigated in a systematic review [103]. Regular-dose NSAID use did not significantly affect the risk of accelerated CKD progression, but high-dose NSAID use significantly increased the risk (OR, 1.26 (95%CI, 1.06–1.50). Therefore, avoidance of NSAIDs in the medium term seems unnecessary in patients with moderate to severe CKD, if not otherwise contraindicated. Since the definition of high-dose of NSAID use remains unclear, the lowest effective dose of NSAIDs should be prescribed where indicated.

An association between NSAID therapy and elevated BP has been found in several epidemiologic studies. This BP effect, which is consistent with the important role of endogenous prostanoids (namely, PGs and thromboxanes) in BP homeostasis [104], appears to be of particular relevance in patients with preexisting hypertension. These arachidonic acid-derived mediators exert a wide range of biological actions, including regulation of vasomotor tone and renal sodium excretion as well as renin secretion [104].

A large meta-analysis of 54 studies including 1234 patients [105] showed that NSAID use is associated with increase in BP, an effect found solely in hypertensive subjects. Of the drugs studied, indomethacin and naproxen were associated with the largest increases in BP. The average effects of piroxicam, aspirin, ibuprofen, and sulindac were negligible. These findings were confirmed in a subsequent meta-analysis [106], which also showed that NSAIDs antagonize the BP-lowering effect of antihypertensive medications to an extent that may potentially increase hypertension-related morbidity [107]. A more recent systematic review [108] confirmed this trend, but found a consistent effect only for ibuprofen. Among the antihypertensive drugs, it appears that dihydropyridine calcium channel blockers are more effective at lowering and maintaining BP control (Table 5.3) and should therefore be the preferred medication in NSAID users [107, 109, 110].

Table 5.3 Interaction between NSAIDs and different classes of anti hypertensive medications (modified from Kalafutova et al. [107])

Antihypertensive classes	NA^+ and H_2O excretion	RAAS	Brady-kinen	Decrease of anti-hypertensive effects of NSAIDs	Δ systolic BP after NSAIDs	Studies
Diuretics	↑	–	–	+/++	6.11[a]	Ishiguro et al. (2008)
					2.1	MacDonald et al. (2010)
Beta-blocker	↑	↓	–	+/++	6.2	Johnsen et al. (1994)
					2.8	Macdonald (2010)
Ace inhibitors	↑	↓	↑	+++	10.3	Morgan and Anderson (1993)
					6.8	Palonia et al. (1995)
					3.7	MacDonald et al. (2010)
					4.6	Conlin et al. (2009)
					11.7[a]	Morgan and Anderson (1993)
Angiotensin II blockers	↑	↑	↑	+/+++	4.6[a]	MacDonald et al. (2010)
					3.8[a]	Conlin et al. (2009)
Calcium channel blockers	↑	–	–	–	0.3	Palonia et al. (1995)
					1.3	Morgan and Anderson (1993)
					1.1	Morgan and Anderson (2003)

RAAS renin-angiotensin-aldosterone system, *ACE* angiotensin-converting-enzyme, + ++ +++ measure of antihypertensive effect. The quoted publications are all reported in the Ref. [107]

[a]Significant effect

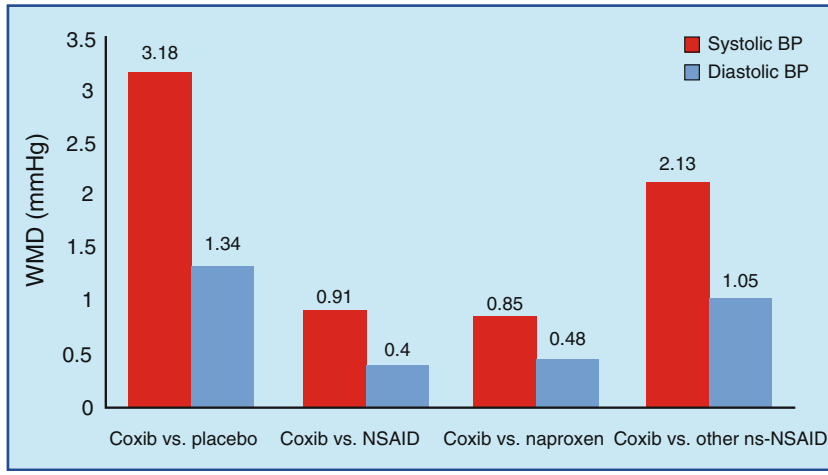


Fig. 5.7 Weighted mean difference (WWd) for systolic BP in coxib trials versus placebo and ns-NSAID (from Chan et al. [112])

An original meta-analysis [111], subsequently updated [112], showed that selective COX-2 inhibitors appear to produce greater hypertension than either ns-NSAIDs or placebo (Fig. 5.7). However, this response was heterogeneous, with markedly raised BP associated with rofecoxib and etoricoxib, whereas celecoxib, valdecoxib and lumiracoxib appeared to have little effect on BP.

Among the different mechanisms by which COX-2 selective and ns-NSAIDs may increase CV risk, the BP effects of these medications appear relevant [30]. However, the significant correlation in placebo-controlled trials among the five agents' elevations in BP values and their rate ratios for CV events strongly suggests that the BP effects of NSAIDs are an important determinant of the increase in CV risk (*see below*) [113]. The best current evidence comes from the centrally adjudicated placebo-controlled trials of celecoxib for colorectal adenoma prevention (APC and PreSAP trials): *if the blood pressure was not raised at 1 or 3 years after randomization, CV risk was not significantly increased* [39]. Further insights into this puzzle will be gained when the full results of the PRECISION trial [46] become available.

NSAID-Induced Fluid and Electrolyte Disturbances

Fluid retention is the most common renal manifestation of NSAID therapy [114]. Renal PGE_2 regulates sodium homeostasis by decreasing sodium reabsorption in the ascending limb of the loop of Henle [115]. NSAIDs inhibit the synthesis of PGE_2 leading to an increase in sodium reabsorption. Although sodium balance is altered during NSAID treatment in most patients, the effects are generally mild and not clinically relevant [114, 116]. However, a small percentage of patients develop clinical *sequelae* including weight gain, peripheral edema, hypertension, and, rarely, pulmonary edema [114, 116–118]. In most NSAID studies, the incidence of these renal effects is 3–5 % [114, 116]. Because changes in sodium balance occur rapidly, clinical manifestations of sodium retention tend to occur shortly after initiating or increasing NSAID therapy [114, 116].

Clinically significant fluid retention is more likely to develop in patients with underlying renal disease, CHF, hepatic insufficiency, or in patients receiving diuretics [114, 116]. As a result, NSAIDs should be used with caution in these patients. It is

advisable to monitor body weight when treating high-risk patients with NSAIDs. There is some evidence that longer-acting NSAIDs (half-life >4 h) may be associated with more sodium retention as compared with shorter-acting NSAIDs [119].

Hyperkalemia is a rare, but potentially life-threatening, consequence of NSAID therapy [114, 116]. NSAIDs inhibit renal prostacyclin formation blunting renin release, aldosterone formation, and potassium excretion. Patients at risk for hyperkalemia include those taking potassium supplements, potassium-sparing diuretics, and ACE inhibitors or those with underlying renal insufficiency (e.g., diabetic nephropathy) [114, 116]. Hyperkalemia tends to occur early in the course of therapy and is reversible with discontinuation of the NSAID.

Animal experiments and clinical trials with preferential and selective COX-2 inhibitors revealed that COX-2 is the critical enzyme for sodium excretion, renin release, and likely antagonism of antidiuretic hormone. Both COX-2 selective and ns-NSAIDs upregulate the Na-K-2Cl cotransporter type 2 (NKCC2) in the thick ascending limb of Henle's loop and aquaporin-2 in the collecting duct [120]. For renal hemodynamics, evidence points to COX-1 as the predominant enzyme [121]. As a consequence, the gain in renal safety by use of selective COX-2 inhibitors is small or negligible with respect to sodium retention or hyperkalemia.

Peripheral Edema

Peripheral edema is an occasional adverse effect associated with all NSAIDs [114, 116]. The degree of edema is typically moderate and reversible with discontinuation of the drug. Ns-NSAIDs and selective COX-2 inhibitors have similar effects on sodium retention-mediated processes, such as edema, because the increased sodium retention that has been associated with all NSAIDs appears to be mediated mainly by COX-2 [114, 116]. Similar frequencies of edema, as an adverse event reported in several studies of NSAIDs and selective COX-2 inhibitors, support this hypothesis [114, 116].

The incidence of edema in four large studies, comparing either rofecoxib or celecoxib with several ns-NSAIDs, was within the same range. It is worth mentioning, however, that the occurrence of edema in these large multicenter studies was an investigator-reported outcome, and therefore the definition of edema differed between the rofecoxib studies and the celecoxib studies, limiting the utility of comparisons among data.

Celecoxib (200 mg daily) and rofecoxib (25 mg daily) were compared with each other as regards the rates of edema in two trials [122, 123] performed in elderly OA patients with drug-controlled hypertension. After 6 weeks of coxib treatment, the incidence of edema was significantly higher in the rofecoxib-treated patients than in those receiving celecoxib (9.5 % versus 4.9 % in the first study and 7.7 % versus 4.7 % in the second one). These data are in line with the findings from an analysis of more than 50 clinical studies [89] with celecoxib showing a low (i.e., 2.1 %) overall incidence of peripheral edema. However, in a study [124] performed in *healthy* elderly subjects on a sodium-replete diet, no subject reported edema after either rofecoxib or celecoxib.

Although, in the etoricoxib program database [125], the incidence of lower extremity edema with the drug (60–120 mg daily) was similar to that observed with comparator NSAIDs, discontinuation rates because of edema (as well as hypertension) were higher [126].

A meta-analysis by Zhang et al. [127] examined data from 114 RCTs, including 127 trial populations (40 rofecoxib, 37 celecoxib, 29 valdecoxib + parecoxib, 15 etoricoxib, and 6 lumiracoxib). Compared with control, rofecoxib was associated with an increased risk of composite renal events (RR, 1.53; 95 % CI, 1.33–1.76); adverse renal effects increased with greater dose and duration of treatment (both $p \leq 0.05$). For all individual renal endpoints, rofecoxib was associated with increased risk of peripheral edema (RR, 1.43; 95 % CI,

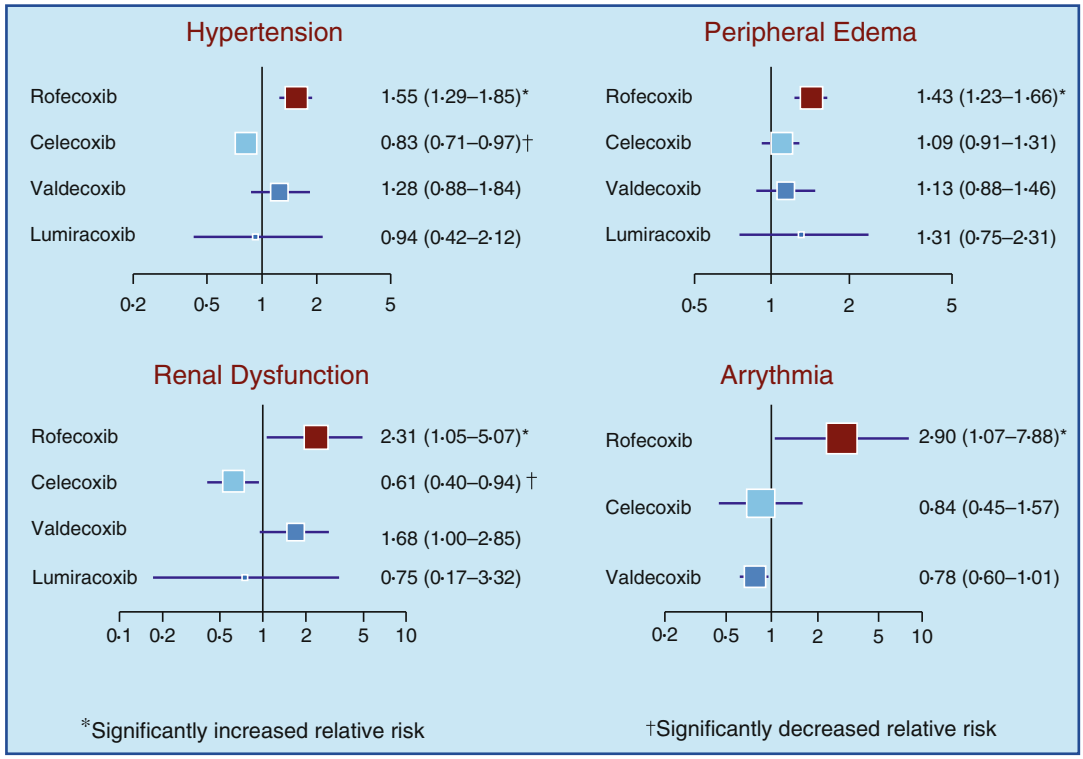


Fig. 5.8 Meta-analysis of RCTs by RR (95 % CIs) of adverse events hypertension, peripheral oedema, renal dysfunction, and arrythmia (from Strand [129])

1.23–1.66), hypertension (RR, 1.55; 95 % CI, 1.29–1.85), and renal dysfunction (RR, 2.31; 95 % CI, 1.05–5.07). In contrast, celecoxib was associated with lower risk of both renal dysfunction (RR, 0.61; 95 % CI, 0.40–0.94) and hypertension (RR, 0.83; 95 % CI, 0.71–0.97) compared with controls. The risk for peripheral edema was also not significant (RR, 1.09, 091–1-31). Other agents were not significantly associated with risk. While belonging to the same class, the different selective COX-2 inhibitors differ in terms of potency and COX-2 selectivity as well as in their respective pharmacokinetics and metabolism [128]. These differences may explain their different renal and CV adverse event profiles [129] (Fig. 5.8).

Despite the relevant CV and renal risks associated with the use of NSAIDs, as mentioned above, there is a largely inappropriate use of these drugs in patients with CVD both in Italy [130] and elsewhere [63, 131–133]. To reverse this alarming trend, a specific project (namely, CardioPain™) was started in a region of Southern Italy with the aim of reducing the inappropriate use of NSAIDs, by indicating clearly in the hospital discharge letter that this class of drugs is contraindicated in patients with CVD [134]. CardioPain™ was highly appreciated by the Italian Medicines Agency (AIFA), which recommended this initiative be implemented also by other Italian regions.

Prevention of Cardiovascular Adverse Events in Patients with Single or Dual Antiplatelet Therapy

Aspirin, a prototypic NSAID, acts also as an antiplatelet agent when administered at low dose (75–325 mg once daily), by virtue of its inhibitory effect on thromboxane A biosynthesis

by COX-1 isoenzyme. It represents a standard antithrombotic treatment in CV diseases, with particular regard for ischemic heart disease [135]. The benefits of low-dose aspirin in secondary prevention clearly outweigh the risk [136]. However, this is not the case for primary prevention [137], where recommendations for aspirin use should be individualized, taking into account the balance between benefits and risks, as well as individual patient values and preferences [138]. Given that aspirin is a life-saving drug, discontinuing it or not adhering to the correct administration schedule enhances the risk of CV and cerebral events more than three-fold [139–141]. This risk can be magnified by up to 90-fold in patients with intracoronary stents [139].

The majority of NSAIDs are known to interfere negatively with the antiplatelet action of aspirin, and therefore combined therapy with a selective COX-2 inhibitor plus a PPI should be offered to patients with high levels of both GI and CV risk, as discussed below in detail. Current evidence suggests that this interaction is attributable to COX-1 inhibition, since selective COX-2 inhibitors do not possess antiplatelet activity. The binding sites for ASA and NSAIDs on COX-1 are located in a relatively narrow hydrophobic channel. ASA initially acetylates COX-1 with a reversible binding and then irreversibly acetylates a Ser residue. NSAIDs, on the other hand, bind reversibly within the hydrophobic channel or at the active site of the enzyme. NSAIDs can compete with ASA for these binding sites and, in doing so, they, protect platelet COX-1 from permanent inactivation by ASA-induced acetylation [142]. Thus, the inhibition of aspirin antiplatelet activity cannot be considered a class effect for all NSAIDs. For example, docking studies provide evidence that only NSAIDs that form hydrogen bonds with Ser530, Arg120, Tyr385, and other amino acids associated with the COX-1 hydrophobic channel will interfere with aspirin antiplatelet activity [142].

While the pharmacodynamic, negative interaction between ns-NSAIDs and low-dose aspirin has been clearly established by studies in healthy volunteers and patients, the clinical consequences of such interaction remain unclear [143]. Indeed, epidemiological studies have provided conflicting results, with only three out of six reports showing a reduction of the cardioprotective effect of aspirin. However, the few available RCTs are consistent in their findings that ns-NSAID use does worsen the CV outcome in patients taking low-dose aspirin. In the Physician Health Study [144], aspirin was effective for primary prevention except in patients taking NSAIDs. A recent epidemiological study [145] found that–in patients taking antithrombotic therapy–NSAID use increased the rate of CV events (CV death, nonfatal AMI, and stroke), regardless of the antiplatelet drug, types of NSAID, or duration of use. Celecoxib (as well as other selective COX-2 inhibitors) does not affect platelet aggregation and interfere with the anti-aggregant activity of low-dose aspirin, alone or in combination with clopidogrel (Fig. 5.9) [18, 146].

This lack of interference with the antithrombotic action of antiplatelet drugs–together with a better tolerability throughout the entire GI tract–would make this COX-2 selective agent a suitable anti-inflammatory drug for patients receiving low-dose aspirin for CV or cerebrovascular prevention [129], despite the contrary opinion of the EMA [37, 38]. However, at the time when the Committee for Medicinal Products for Human Use (CHMP) issued its recommendations, much of the current evidence was not yet available.

Although data support the conclusion that COX-2 inhibitors are preferable to ns-NSAIDs in patients with chronic pain and CV risk, requiring low-dose aspirin, the RRs and benefits should be assessed in the individual patient. Celecoxib, given in low doses (200 mg once daily) with low-dose aspirin, can provide the same pain and inflammatory relief as ns-NSAIDs, with less upper and lower GI and CV risk [147]. The lower GI [148] and CV [41] safety profile of etoricoxib seems to be less favorable.

Several epidemiologic studies and meta-analyses have shown that naproxen appears to

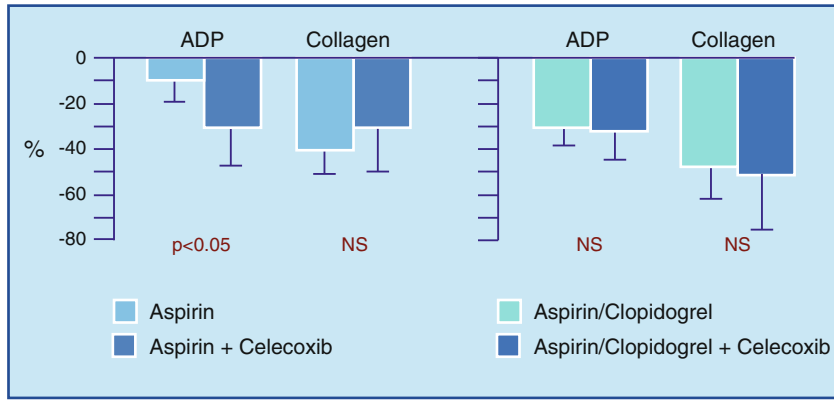


Fig. 5.9 Reductions in platelet aggregation (pretreatment inhibition %−post-treatment inhibition %) by antiplatelet agents (alone or in combination), with or without addition of celecoxib (from Lee et al. [146])

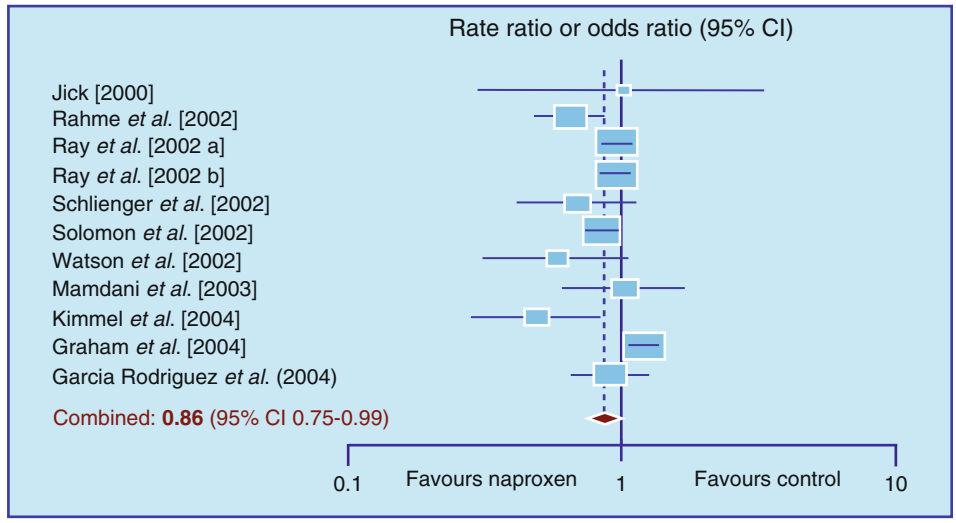


Fig. 5.10 Meta-analysis of observational studies (8 case-control studies and 3 retrospective cohort studies) of naproxen and risk of myocardial infarction (from Jüni et al. [149])

be the least CV harmful NSAID (Fig. 5.10) [36, 149], but this advantage has to be weighed against GI toxicity. It must also be considered that the absence of an increased CV risk, as observed in RCTs and meta-analysis with naproxen when compared to placebo, was based on a high naproxen dose (500 mg b.i.d) [36], which displays an antiplatelet activity, similar, albeit reversible, to that of low-dose aspirin.

Are There Safer Alternatives to NSAIDs in Patients with CVD?

NSAIDs exert–as a class–analgesic, anti-inflammatory, and antipyretic activities [150]. Taking into account their wide range of adverse events, especially those at the level of GI tract and CV system, opioids could be used instead

when only the analgesic action is sought [151, 152]. As matter of fact, guidelines issued by both the American Geriatric Society [153] and the British Geriatric Society [154] emphasize that–in carefully selected and monitored old patients, who generally hold CV comorbidity–opioids may be preferable to NSAIDs. However, intersecting with the upward trajectory in opioid use [155] are the increasing trends in opioid-related adverse effects, especially prescription drug abuse, addiction, and overdose deaths. And indeed, despite limited evidence and variable development methods, recent guidelines on chronic pain agree on several opioid risk mitigation strategies, including upper dosing thresholds, cautions with certain medications, attention to drug-drug and drug-disease interactions, and use of risk assessment tools [156].

When the anti-inflammatory action is sought and NSAID treatment is contraindicated, short-term use of glucocorticoids (GCs) may be an alternative therapeutic option [157]. GCs have, in addition, a significant analgesic effect in neuropathic and bone pain [158]. And indeed, they are considered adjuvant analgesics [159] within the pain World Health Organization (WHO) ladder [160].

Safe NSAID Therapy in CVD: Conclusions

Navigating through the different GI and CV risk factors in patients with CVD and balancing them with the potential benefits of NSAID therapy is a difficult task. Like any pharmacologic therapy, appropriateness is a must. Being analgesic and anti-inflammatory drugs, NSAID prescribing is mainly indicated when both pharmacologic activities are required. When pain relief is the main target of therapy, the choice will depend on its nature (acute versus chronic as well as nociceptive versus neuropathic) and severity and should take into account the relative efficacy and safety of each class of drugs (NSAIDs, weak opioids, strong opioids, or their combination), together with underlying risk factors. In this connection, the knowledge about the benefit/risk ratio of opioids in CVD should be improved and their use not dogmatically discouraged. Along the same lines, provided there are no absolute contraindications, a short course of GCs could be an alternative to NSAID therapy when the desired pharmacologic activity is the anti-inflammatory one.

A recent Cochrane systematic review [161] evaluated the scientific evidence on the efficacy and safety of using pain pharmacotherapy in patients with RA and CV or renal comorbidities. There were no trials that specifically compared the efficacy and safety of pain pharmacotherapies for patients with RA, with and without comorbid CV or renal conditions. In the absence of specific evidence, current guidelines suggest that pain management drugs (be they NSAIDs, opioids, or adjuvant steroids) should be used with caution. Further research is therefore required to guide clinicians when treating pain and/or inflammation in patients with CVD.

Acknowledgments We would like to thank Dr. Anna Bertelé (Laboratory of GI Functional Investigations, Maggiore University Hospital, Parma, Italy) for her precious help during the preparation of this manuscript. We are also indebted to Professor Richard H Hunt (MacMaster University, ON, CA) for his critical reading of the chapter and his valuable suggestions.

References

1. Dhingra R, Vasan RS. Age as a risk factor. Med Clin North Am. 2012;96:87–91.
2. Loeser RF. Age-related changes in the musculoskeletal system and the development of osteoarthritis. Clin Geriatr Med. 2010;26:371–86.
3. McDonough CM, Jette AM. The contribution of osteoarthritis to functional limitations and disability. Clin Geriatr Med. 2010;26:387–99.
4. Schaible HG. Mechanisms of chronic pain in osteoarthritis. Curr Rheumatol Rep. 2012;14:549–56.
5. Mäntyselkä P, Kumpusalo E, Ahonen R, Kumpusalo A, Kauhanen J, Viinamäki H, Halonen P, Takala J. Pain as a reason to visit the doctor: a study in Finnish primary health care. Pain. 2001;89:175–80.
6. Saccó M, Meschi M, Regolisti G, Detrenis S, Bianchi L, Bertorelli M, Pioli S, Magnano A, Spagnoli F, Giuri PG, Fiaccadori E, Caiazza A. The

relationship between blood pressure and pain. J Clin Hypertens. 2013;15:600–5.
7. Sellier E, Labarere J, Sevestre MA, Belmin J, Thiel H, Couturier P, Bosson JL, Association pour la Promotion de l'Angiologie Hospitalière. Risk factors for deep vein thrombosis in older patients: a multicenter study with systematic compression ultrasonography in postacute care facilities in France. J Am Geriatr Soc. 2008;56:224–30.
8. Gibson SJ, Farrell M. A review of age differences in the neurophysiology of nociception and the perceptual experience of pain. Clin J Pain. 2004;20:227–39.
9. Slater M, Perruccio AV, Badley EM. Musculoskeletal comorbidities in cardiovascular disease, diabetes and respiratory disease: the impact on activity limitations; a representative population-based study. BMC Public Health. 2011;11:77.
10. van Dijk GM, Veenhof C, Schellevis F, Hulsmans H, Bakker JP, Arwert H, Dekker JH, Lankhorst GJ, Dekker J. Comorbidity, limitations in activities and pain in patients with osteoarthritis of the hip or knee. BMC Musculoskelet Disord. 2008;9:95.
11. Caporali R, Cimmino MA, Sarzi-Puttini P, Scarpa R, Parazzini F, Zaninelli A, Ciocci A, Montecucco C. Comorbid conditions in the AMICA study patients: effects on the quality of life and drug prescriptions by general practitioners and specialists. Semin Arthritis Rheum. 2005;35 Suppl 1:31–7.
12. Fitzcharles MA, Lussier D, Shir Y. Management of chronic arthritis pain in the elderly. Drugs Aging. 2010;27:471–90.
13. Scarpignato C. Piroxicam-β-cyclodextrin: a GI safer piroxicam. Curr Med Chem. 2013;20:2415–37.
14. Machado GC, Maher CG, Ferreira PH, Pinheiro MB, Lin CW, Day RO, McLachlan AJ, Ferreira ML. Efficacy and safety of paracetamol for spinal pain and osteoarthritis: systematic review and meta-analysis of randomised placebo controlled trials. BMJ. 2015;350.
15. Antman EM, Bennett JS, Daugherty A, Furberg C, Roberts H, Taubert KA, Association AH. Use of nonsteroidal antiinflammatory drugs: an update for clinicians: a scientific statement from the American Heart Association. Circulation. 2007;115:1634–42.
16. Roberts E, Delgado Nunes V, Buckner S, Latchem S, Constanti M, Miller P, Doherty M, Zhang W, Birrell F, Porcheret M, Dziedzic K, Bernstein I, Wise E, Conaghan PG. Paracetamol: not as safe as we thought? A systematic literature review of observational studies. Ann Rheum Dis. 2016;75:552–9.
17. Berenbaum F. Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). Osteoarthritis Cartilage. 2013;21:16–21.
18. Hunt RH, Lanas A, Stichtenoth DO, Scarpignato C. Myths and facts in the use of anti-inflammatory drugs. Ann Med. 2009;41:423–37.
19. Scarpignato C, Hunt RH. Nonsteroidal antiinflammatory drug-related injury to the gastrointestinal tract: clinical picture, pathogenesis, and prevention. Gastroenterol Clin North Am. 2010;39:433–64.
20. Harirforoosh S, Asghar W, Jamali F. Adverse effects of nonsteroidal antiinflammatory drugs: an update of gastrointestinal, cardiovascular and renal complications. J Pharm Sci. 2013;16:821–47.
21. Süleyman H, Demircan B, Karagöz Y. Anti-inflammatory and side effects of cyclooxygenase inhibitors. Pharmacol Rep. 2007;59:247–58.
22. Patrono C, Baigent C. Nonsteroidal anti-inflammatory drugs and the heart. Circulation. 2014;129:907–16.
23. FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med. 2001;345:433–42.
24. Blandizzi C, Tuccori M, Colucci R, Fornai M, Antonioli L, Ghisu N, Del Tacca M. Role of coxibs in the strategies for gastrointestinal protection in patients requiring chronic non-steroidal anti-inflammatory therapy. Pharmacol Res. 2009;59:90–100.
25. Mukherjee D, Nissen SE, Topol RJ. Risk of cardiovascular events associated with selective COX-2 inhibitors. JAMA. 2001;286:954–9.
26. Catella-Lawson F, Crofford LJ. Cyclooxygenase inhibition and thrombogenicity. Am J Med. 2001;110(Suppl 3A):28S–32.
27. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJ, VIGOR Study Group. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N Engl J Med. 2000;343:1520–8.
28. Baigent C, Patrono C. Selective cyclooxygenase 2 inhibitors, aspirin, and cardiovascular disease: a reappraisal. Arthritis Rheum. 2003;48:12–20.
29. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, Konstam MA, Baron JA, Adenomatous Polyp Prevention on Vioxx (APPROVe) Trial Investigators. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med. 2005;352:1092–102.
30. Baron JA, Sandler RS, Bresalier RS, Lanas A, Morton DG, Riddell R, Iverson ER, Demets DL. Cardiovascular events associated with rofecoxib: final analysis of the APPROVe trial. Lancet. 2008;372:1756–64.
31. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Verburg KM, Geis GS. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. JAMA. 2000;284:1247–55.
32. Food and Drug Administration (FDA). Materials from the FDA Joint Meeting with the Drug Safety and Risk Management Advisory Committee,

February 16–18, 2005. www.fda.gov/ohrms/dockets/ac/cder05.html#DrugSafetyRiskMgmt
33. Okie S. Raising the safety bar—the FDA's coxib meeting. N Engl J Med. 2005;352:1283–5.
34. Kuehn BM. FDA panel: keep COX-2 drugs on market: black box for COX-2 labels, caution urged for all NSAIDs. JAMA. 2005;293:1571–2.
35. McGettigan P, Henry D. Cardiovascular risk and inhibition of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxygenase 2. JAMA. 2006;296:1633–44.
36. Kearney PM, Baigent C, Godwin J, Halls H, Emberson JR, Patrono C. Do selective cyclooxygenase-2 inhibitors and traditional non-steroidal anti-inflammatory drugs increase the risk of atherothrombosis? Meta-analysis of randomised trials. BMJ. 2006;332:1302–8.
37. European Medicines Agency (EMA). European Medicines Agency announces regulatory action on COX-2 inhibitors, 2005. http://www.ema.europa.eu/docs/en_GB/document_library/Public_statement/2009/11/WC500014818.pdf
38. European Medicines Agency (EMA). Press Release: European Medicines Agency concludes action on COX-2 inhibitors, 2005. http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2010/01/news_detail_000969.jsp&mid=WC0b01ac058004d5c1
39. Solomon SD, Pfeffer MA, McMurray JJ, Fowler R, Finn P, Levin B, Eagle C, Hawk E, Lechuga M, Zauber AG, Bertagnolli MM, Arber N, Wittes J, APC and PreSAP Trial Investigators. Effect of celecoxib on cardiovascular events and blood pressure in two trials for the prevention of colorectal adenomas. Circulation. 2006;114:1028–35.
40. Cannon CP, Curtis SP, FitzGerald GA, Krum H, Kaur A, Bolognese JA, Reicin AS, Bombardier C, Weinblatt ME, van der Heijde D, Erdmann E, Laine L, Steering Committee MEDAL. Cardiovascular outcomes with etoricoxib and diclofenac in patients with osteoarthritis and rheumatoid arthritis in the Multinational Etoricoxib and Diclofenac Arthritis Long-term (MEDAL) programme: a randomised comparison. Lancet. 2006;368:1771–81.
41. Coxib and traditional NSAID Trialists' (CNT) Collaboration. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. Lancet. 2013;382:769–79.
42. Salvo F, Antoniazzi S, Duong M, Molimard M, Bazin F, Fourrier-Réglat A, Pariente A, Moore N. Cardiovascular events associated with the long-term use of NSAIDs: a review of randomized controlled trials and observational studies. Expert Opin Drug Saf. 2014;13:573–85.
43. Fosbøl EL, Gislason GH, Jacobsen S, Folke F, Hansen ML, Schramm TK, Sørensen R, Rasmussen JN, Andersen SS, Abildstrom SZ, Traerup J, Poulsen HE, Rasmussen S, Køber L, Torp-Pedersen C. Risk of myocardial infarction and death associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs) among healthy individuals: a nationwide cohort study. Clin Pharmacol Ther. 2009;85:190–7.
44. Odom DM, Mladsi DM, Saag KG, Sherif BN, Miles L, Ronquest N, Wang J. Relationship between diclofenac dose and risk of gastrointestinal and cardiovascular events: meta-regression based on two systematic literature reviews. Clin Ther. 2014;36:906–17.
45. Moore N, Salvo F, Duong M, Blin P, Pariente A. Cardiovascular risks associated with low-dose ibuprofen and diclofenac as used OTC. Expert Opin Drug Saf. 2014;13:167–79.
46. Becker MC, Wang TH, Wisniewski L, Wolski K, Libby P, Lüscher TF, Borer JS, Mascette AM, Husni ME, Solomon DH, Graham DY, Yeomans ND, Krum H, Ruschitzka F, Lincoff AM, Nissen SE, Investigators PRECISION. Rationale, design, and governance of Prospective Randomized Evaluation of Celecoxib Integrated Safety versus Ibuprofen Or Naproxen (PRECISION), a cardiovascular end point trial of nonsteroidal antiinflammatory agents in patients with arthritis. Am Heart J. 2009;157:606–12.
47. McGettigan P, Henry D. Cardiovascular risk with non-steroidal anti-inflammatory drugs: systematic review of population-based controlled observational studies. PLoS Med. 2011;8, e1001098.
48. Trelle S, Reichenbach S, Wandel S, Hildebrand P, Tschannen B, Villiger PM, Egger M, Jüni P. Cardiovascular safety of non-steroidal anti-inflammatory drugs: network meta-analysis. BMJ. 2011;342:c7086.
49. Varas-Lorenzo C, Riera-Guardia N, Calingaert B, Castellsague J, Salvo F, Nicotra F, Sturkenboom M, Perez-Gutthann S. Myocardial infarction and individual nonsteroidal anti-inflammatory drugs meta-analysis of observational studies. Pharmacoepidemiol Drug Saf. 2013;22:559–750.
50. Chan AT, Manson JE, Albert CM, Chae CU, Rexrode KM, Curhan GC, Rimm EB, Willett WC, Fuchs CS. Nonsteroidal antiinflammatory drugs, acetaminophen, and the risk of cardiovascular events. Circulation. 2006;113:1578–87.
51. Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. J Clin Invest. 2006;116:4–15.
52. MacDonald TM, Hawkey C, Ford I, McMurray J, Scheiman J, Hallas J, Findlay E, Grobbee RE, Hobbs R, Ralston S, Reid D, Walters M, Webster J, Ruschitzka F, Lewis Ritchie L, Perez-Gutthann S, Connolly E, Greenlaw N, Wilson A, Wei L, Mackenzie IS. The Standard Care versus Celecoxib Outcome Trial (SCOT): A randomized, trial comparing the cardiovascular safety of celecoxib versus traditional non-steroidal anti-inflammatory drugs. European Society of Cardiology 2015 Congress; August 31, 2015; London. Abstract 3156.

53. Liu SS, Bae JJ, Bieltz M, Ma Y, Memtsoudis S. Association of perioperative use of nonsteroidal anti-inflammatory drugs with postoperative myocardial infarction after total joint replacement. Reg Anesth Pain Med. 2012;37:45–50.
54. Lévesque LE, Brophy JM, Zhang B. Time variations in the risk of myocardial infarction among elderly users of COX-2 inhibitors. CMAJ. 2006; 174:1563–9.
55. Nussmeier NA, Whelton AA, Brown MT, Langford RM, Hoeft A, Parlow JL, Boyce SW, Verburg KM. Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. N Engl J Med. 2005;352:1081–91.
56. Aminoshariae A, Kulild JC, Donaldson M. Short-term use of nonsteroidal anti-inflammatory drugs and adverse effects: an updated systematic review. J Am Dent Assoc. 2015;147:98–110.
57. Solomon DH, Schneeweiss S, Glynn RJ, Kiyota Y, Levin R, Mogun H, Avorn J. Relationship between selective cyclooxygenase-2 inhibitors and acute myocardial infarction in older adults. Circulation. 2004;109:2068–73.
58. Solomon SD, Zelenkofske S, McMurray JJ, Finn PV, Velazquez E, Ertl G, Harsanyi A, Rouleau JL, Maggioni A, Kober L, White H, Van de Werf F, Pieper K, Califf RM, Pfeffer MA, Valsartan in Acute Myocardial Infarction Trial (VALIANT) Investigators. Sudden death in patients with myocardial infarction and left ventricular dysfunction, heart failure, or both. N Engl J Med. 2005;352:2581–8.
59. Gislason GH, Jacobsen S, Rasmussen JN, Rasmussen S, Buch P, Friberg J, Schramm TK, Abildstrom SZ, Køber L, Madsen M, Torp-Pedersen C. Risk of death or reinfarction associated with the use of selective cyclooxygenase-2 inhibitors and nonselective nonsteroidal antiinflammatory drugs after acute myocardial infarction. Circulation. 2006;113:2906–13.
60. Brophy JM, Lévesque LE, Zhang B. The coronary risk of cyclo-oxygenase-2 inhibitors in patients with a previous myocardial infarction. Heart. 2007; 93:189–94.
61. Vanasse A, de Brum-Fernandes AJ, Courteau J. Cardiovascular safety of celecoxib in acute myocardial infarction patients: a nested case-control study. Heart Int. 2009;4, e10.
62. Ray WA, Varas-Lorenzo C, Chung CP, Castellsague J, Murray KT, Stein CM, Daugherty JR, Arbogast PG, García-Rodríguez LA. Cardiovascular risks of nonsteroidal antiinflammatory drugs in patients after hospitalization for serious coronary heart disease. Circ Cardiovasc Qual Outcomes. 2009;2:155–63.
63. Kohli P, Steg PG, Cannon CP, Smith Jr SC, Eagle KA, Ohman EM, Alberts MJ, Hoffman E, Guo J, Simon T, Sorbets E, Goto S, Bhatt DL, Registry Investigators REACH. NSAID use and association with cardiovascular outcomes in outpatients with stable atherothrombotic disease. Am J Med. 2014; 127:53–60.
64. Schjerning Olsen AM, Fosbøl EL, Lindhardsen J, Folke F, Charlot M, Selmer C, Lamberts M, Bjerring Olesen J, Køber L, Hansen PR, Torp-Pedersen C, Gislason GH. Duration of treatment with nonsteroidal anti-inflammatory drugs and impact on risk of death and recurrent myocardial infarction in patients with prior myocardial infarction: a nationwide cohort study. Circulation. 2011;123:2226–35.
65. Lamberts M, Fosbøl EL, Olsen AM, Hansen ML, Folke F, Kristensen SL, Olesen JB, Hansen PR, Køber L, Torp-Pedersen C, Gislason GH. Ongoing treatment with non-steroidal anti-inflammatory drugs at time of admission is associated with poorer prognosis in patients with first-time acute myocardial infarction. Int J Cardiol. 2013; 168:832–7.
66. Olsen AM, Fosbøl EL, Lindhardsen J, Andersson C, Folke F, Nielsen MB, Køber L, Hansen PR, Torp-Pedersen C, Gislason GH. Cause-specific cardiovascular risk associated with nonsteroidal anti-inflammatory drugs among myocardial infarction patients—a nationwide study. PLoS One. 2013;8, e54309.
67. John H, Kitas G. Inflammatory arthritis as a novel risk factor for cardiovascular disease. Eur J Intern Med. 2012;23:575–9.
68. Nurmohamed MT, Heslinga M, Kitas GD. Cardiovascular comorbidity in rheumatic diseases. Nat Rev Rheumatol. 2015;11:693–704.
69. Schuett KA, Lehrke M, Marx N, Burgmaier M. High-risk cardiovascular patients: clinical features, comorbidities, and interconnecting mechanisms. Front Immunol. 2015;6:591.
70. Lindhardsen J, Gislason GH, Jacobsen S, Ahlehoff O, Olsen AM, Madsen OR, Torp-Pedersen-C, Hansen PR. Non-steroidal anti-inflammatory drugs and risk of cardiovascular disease in patients with rheumatoid arthritis: a nationwide cohort study. Ann Rheum Dis. 2014;73:1515–21.
71. Tsai WC, Ou TT, Yen JH, Wu CC, Tung YC. Long-term frequent use of non-steroidal anti-inflammatory drugs might protect patients with ankylosing spondylitis from cardiovascular diseases: a nationwide case-control study. PLoS One. 2015;10, e0126347.
72. Loeser RF. Aging and osteoarthritis. Curr Opin Rheumatol. 2011;23:492–6.
73. Hall AJ, Stubbs B, Mamas MA, Myint PK, Smith TO. Association between osteoarthritis and cardiovascular disease: Systematic review and meta-analysis. Eur J Prev Cardiol. 2015;23:938–46.
74. de Abajo FJ, Gil MJ, García Poza P, Bryant V, Oliva B, Timoner J, García-Rodríguez LA. Risk of nonfatal acute myocardial infarction associated with non-steroidal antiinflammatory drugs, non-narcotic analgesics and other drugs used in osteoarthritis: a

nested case-control study. Pharmacoepidemiol Drug Saf. 2014;23:1128–38.
75. Leon BM, Maddox TM. Diabetes and cardiovascular disease: Epidemiology, biological mechanisms, treatment recommendations and future research. World J Diabetes. 2015;6:1246–58.
76. Kim J, Lee J, Shin CM, Lee DH, Park BJ. Risk of gastrointestinal bleeding and cardiovascular events due to NSAIDs in the diabetic elderly population. BMJ Open Diabetes Res Care. 2015;3, e000133.
77. Stitham J, Vanichakarn P, Ying L, Hwa J. Cardiovascular pharmacogenetics of anti-thrombotic agents and non-steroidal anti-inflammatory drugs. Curr Mol Med. 2014;14:909–31.
78. Arehart E, Stitham J, Asselbergs FW, Douville K, MacKenzie T, Fetalvero KM, Gleim S, Kasza Z, Rao Y, Martel L, Segel S, Robb J, Kaplan A, Simons M, Powell RJ, Moore JH, Rimm EB, Martin KA, Hwa J. Acceleration of cardiovascular disease by a dysfunctional prostacyclin receptor mutation: potential implications for cyclooxygenase-2-inhibition. Circ Res. 2008;102:986–93.
79. Stitham J, Arehart E, Elderon L, Gleim SR, Douville K, Kasza Z, Fetalvero K, MacKenzie T, Robb J, Martin KA, Hwa J. Comprehensive biochemical analysis of rare prostacyclin receptor variants: study of association of signaling with coronary artery obstruction. J Biol Chem. 2011; 286:7060–9.
80. Padol IT, Hunt RH. Association of myocardial infarctions with COX-2 inhibition may be related to immunomodulation towards a Th_1 response resulting in atheromatous plaque instability: an evidence-based interpretation. Rheumatology (Oxford). 2010;49:837–43.
81. García-Poza P, de Abajo FJ, Gil MJ, Chacón A, Bryant V, García-Rodríguez LA. Risk of ischemic stroke associated with non-steroidal anti-inflammatory drugs and paracetamol: a population-based case-control study. J Thromb Haemost. 2015; 13:708–18.
82. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2012 update: a report from the American Heart Association. Circulation 2012;125:e2–e220.
83. Johnsen SP, Pedersen L, Friis S, Blot WJ, McLaughlin JK, Olsen JH, Sørensen HT. Nonaspirin nonsteroidal anti-inflammatory drugs and risk of hospitalization for intracerebral hemorrhage: a population-based case-control study. Stroke. 2003;34:387–91.
84. Bak S, Andersen M, Tsiropoulos I, García Rodríguez LA, Hallas J, Christensen K, Gaist D. Risk of stroke associated with nonsteroidal anti-inflammatory drugs: a nested case-control study. Stroke. 2003; 34:379–86.
85. Choi NK, Park BJ, Jeong SW, Yu KH, Yoon BW. Nonaspirin nonsteroidal anti-inflammatory drugs and hemorrhagic stroke risk: the Acute Brain Bleeding Analysis study. Stroke. 2008;39:845–9.
86. Bleumink GS, Feenstra J, Sturkenboom MC, Stricker BH. Nonsteroidal anti-inflammatory drugs and heart failure. Drugs. 2003;63:525–34.
87. Garcia Rodriguez LA, Hernandez-Diaz S. Nonsteroidal antiinflammatory drugs as a trigger of clinical heart failure. Epidemiology. 2003; 14:240–6.
88. Mamdani M, Juurlink DN, Lee DS, Rochon PA, Kopp A, Naglie G, Austin PC, Laupacis A, Stukel TA. Cyclo-oxygenase-2 inhibitors versus non-selective non-steroidal anti-inflammatory drugs and congestive heart failure outcomes in elderly patients: a population-based cohort study. Lancet. 2004;363:1751–6.
89. Whelton A, Maurath CJ, Verburg KM, Geis GS. Renal safety and tolerability of celecoxib, a novel cyclooxygenase-2 inhibitor. Am J Ther. 2000;7:159–75.
90. Hirayama A, Tanahashi N, Daida H, Ishiguro N, Chachin M, Sugioka T, Kawai S, all ACCEPT study investigators in Japan. Assessing the cardiovascular risk between celecoxib and nonselective nonsteroidal antiinflammatory drugs in patients with rheumatoid arthritis and osteoarthritis. Circ J. 2013;78:194–205.
91. van den Hondel KE, Eijgelsheim M, Ruiter R, Witteman JC, Hofman A, Stricker BH. Effect of short-term NSAID use on echocardiographic parameters in elderly people: a population-based cohort study. Heart. 2011;97:540–3.
92. Granier M, Massin F, Pasquié JL. Pro- and anti-arrhythmic effects of anti-inflammatory drugs. Antiinflamm Antiallergy Agents Med Chem. 2013;12:83–93.
93. De Caterina R, Ruigómez A, Rodríguez LA. Long-term use of anti-inflammatory drugs and risk of atrial fibrillation. Arch Intern Med. 2010;170:1450–5.
94. Schmidt M, Christiansen CF, Mehnert F, Rothman KJ, Sørensen HT. Non-steroidal anti-inflammatory drug use and risk of atrial fibrillation or flutter: population based case-control study. BMJ. 2011; 343:d3450.
95. Chao TF, Liu CJ, Chen SJ, Wang KL, Lin YJ, Chang SL, Lo LW, Hu YF, Tuan TC, Wu TJ, Chen TJ, Tsao HM, Chen SA. The association between the use of non-steroidal anti-inflammatory drugs and atrial fibrillation: a nationwide case-control study. Int J Cardiol. 2013;168:312–6.

96. Krijthe BP, Heeringa J, Hofman A, Franco OH, Stricker BH. Non-steroidal anti-inflammatory drugs and the risk of atrial fibrillation: a population-based follow-up study. BMJ Open. 2014;4, e004059.
97. Liu G, Yan YP, Zheng XX, Xu YL, Lu J, Hui RT, Huang XH. Meta-analysis of nonsteroidal anti-inflammatory drug use and risk of atrial fibrillation. Am J Cardiol. 2014;114:1523–9.
98. Lafrance JP, Miller DR. Selective and non-selective non-steroidal anti-inflammatory drugs and the risk of acute kidney injury. Pharmacoepidemiol Drug Saf. 2009;18:923–31.
99. Couderc M, Tatar Z, Pereira B, Tiple A, Gilson M, Fautrel B, Pouplin S, Dernis E, Gossec L, Gaujoux-Viala C, Soubrier M, Dougados M. The prevalence of renal impairment in patients with rheumatoid arthritis: Results from the COMEDRA study. Arthritis Care Res. 2015;68:638–44.
100. Chiu HY, Huang HL, Li CH, Chen HA, Yeh CL, Chiu SH, Lin WC, Cheng YP, Tsai TF, Ho SY. Increased risk of chronic kidney disease in rheumatoid arthritis associated with cardiovascular complications—a national population-based cohort study. PLoS One. 2015;10, e0136508.
101. Möller B, Pruijm M, Adler S, Scherer A, Villiger PM, Finckh A, Swiss Clinical Quality Management in Rheumatic Diseases (SCQM) Foundation, CH-8048 Zurich, Switzerland. Chronic NSAID use and long-term decline of renal function in a prospective rheumatoid arthritis cohort study. Ann Rheum Dis. 2015;74:718–23.
102. Hsu CC, Wang H, Hsu YH, Chuang SY, Huang YW, Chang YK, Liu JS, Hsiung CA, Tsai HJ. Use of nonsteroidal anti-inflammatory drugs and risk of chronic kidney disease in subjects with hypertension: nationwide longitudinal cohort study. Hypertension. 2015;66:524–33.
103. Nderitu P, Doos L, Jones PW, Davies SJ, Kadam UT. Non-steroidal anti-inflammatory drugs and chronic kidney disease progression: a systematic review. Fam Pract. 2013;30:247–55.
104. Francois H, Coffman TM. Prostanoids and blood pressure: which way is up? J Clin Invest. 2004;114:757–9.
105. Pope JE, Anderson JJ, Felson DT. A meta-analysis of the effects of nonsteroidal anti-inflammatory drugs on blood pressure. Arch Intern Med. 1993; 153:477–84.
106. Johnson AG, Nguyen TV, Day RO. Do nonsteroidal anti-inflammatory drugs affect blood pressure? A meta-analysis. Ann Intern Med. 1994;121:289–300.
107. Kalafutova S, Juraskova B, Vlcek J. The impact of combinations of non-steroidal anti-inflammatory drugs and anti-hypertensive agents on blood pressure. Adv Clin Exp Med. 2014;23:993–1000.
108. Morrison A, Ramey DR, van Adelsberg J, Watson DJ. Systematic review of trials of the effect of continued use of oral non-selective NSAIDs on blood pressure and hypertension. Curr Med Res Opin. 2007;23:2395–404.
109. Morgan T, Anderson A. The effect of nonsteroidal anti-inflammatory drugs on blood pressure in patients treated with different antihypertensive drugs. J Clin Hypertens. 2003;5:53–7.
110. Khatchadourian ZD, Moreno-Hay I, de Leeuw R. Nonsteroidal anti-inflammatory drugs and antihypertensives: how do they relate? Oral Surg Oral Med Oral Pathol Oral Radiol. 2014; 117:697–703.
111. Aw TJ, Haas SJ, Liew D, Krum H. Meta-analysis of cyclooxygenase-2 inhibitors and their effects on blood pressure. Arch Intern Med. 2005;165:490–6.
112. Chan CC, Reid CM, Aw TJ, Liew D, Haas SJ, Krum H. Do COX-2 inhibitors raise blood pressure more than nonselective NSAIDs and placebo? An updated meta-analysis. J Hypertens. 2009;27:2332–41.
113. Elliott WJ. Do the blood pressure effects of nonsteroidal antiinflammatory drugs influence cardiovascular morbidity and mortality? Curr Hypertens Rep. 2010;12:258–66.
114. Brater DC. Anti-inflammatory agents and renal function. Semin Arthritis Rheum. 2002;32(3 Suppl 1): 33–42.
115. Harris RC. Cyclooxygenase-2 and the kidney: functional and pathophysiological implications. J Hypertens. 2002;20 Suppl 6:S3–9.
116. Whelton A. Renal aspects of treatment with conventional nonsteroidal anti-inflammatory drugs versus cyclooxygenase-2-specific inhibitors. Am J Med. 2001;110(Suppl 3A):33S–42.
117. Epstein M. Non-steroidal anti-inflammatory drugs and the continuum of renal dysfunction. J Hypertens. 2002;20 Suppl 6:S17–23.
118. Brater DC, Harris C, Redfern JS, Gertz BJ. Renal effects of COX-2-selective inhibitors. Am J Nephrol. 2001;21:1–15.
119. Murray MD, Black PK, Kuzmik DD, Haag KM, Manatunga AK, Mullin MA, Hall SD, Brater DC. Acute and chronic effects of nonsteroidal antiinflammatory drugs on glomerular filtration rate in elderly patients. Am J Med Sci. 1995;310:188–97.
120. Kim GH. Renal effects of prostaglandins and cyclooxygenase-2 inhibitors. Electrolyte Blood Press. 2008;6:35–41.
121. Stichtenoth DO, Frölich JC. COX-2 and the kidneys. Curr Pharm Des. 2000;6:1737–53.
122. Whelton A, Fort JG, Puma JA, Normandin D, Bello AE, Verburg KM, Study Group SUCCESSVI. Cyclooxygenase-2-specific inhibitors and cardiorenal function: a randomized, controlled trial of celecoxib and rofecoxib in older hypertensive osteoarthritis patients. Am J Ther. 2001;8:85–95.
123. Whelton A, White WB, Bello AE, Puma JA, Fort JG, Investigators SUCCESS-VII. Effects of celecoxib and rofecoxib on blood pressure and edema in patients > or =65 years of age with systemic hypertension and osteoarthritis. Am J Cardiol. 2002;90:959–63.
124. Schwartz JI, Vandormael K, Malice MP, Kalyani RN, Lasseter KC, Holmes GB, Gertz BJ,

Gottesdiener KM, Laurenzi M, Redfern KJ, Brune K. Comparison of rofecoxib, celecoxib, and naproxen on renal function in elderly subjects receiving a normal-salt diet. Clin Pharmacol Ther. 2002;72:50–61.
125. Curtis SP, Ng J, Yu Q, Shingo S, Bergman G, McCormick CL, Reicin AS. Renal effects of etoricoxib and comparator nonsteroidal anti-inflammatory drugs in controlled clinical trials. Clin Ther. 2004;26:70–83.
126. Moore RA, Derry S, McQuay HJ. Discontinuation rates in clinical trials in musculoskeletal pain: meta-analysis from etoricoxib clinical trial reports. Arthritis Res Ther. 2008;10:R53.
127. Zhang J, Ding EL, Song Y. Adverse effects of cyclooxygenase 2 inhibitors on renal and arrhythmia events: meta-analysis of randomized trials. JAMA. 2006;296:1619–32.
128. Shi S, Klotz U. Clinical use and pharmacological properties of selective COX-2 inhibitors. Eur J Clin Pharmacol. 2008;64:233–52.
129. Strand V. Are COX-2 inhibitors preferable to non-selective non-steroidal anti-inflammatory drugs in patients with risk of cardiovascular events taking low-dose aspirin? Lancet. 2007;370:2138–51.
130. AIFA 2013. L'uso dei farmaci in Italia—Rapporto OsMed 2012. http://www.agenzia farmaco.gov.it/sites/default/files/Rapporto_OsMed_2012.pdf.
131. Lanas A, Garcia-Tell G, Armada B, Oteo-Alvaro A. Prescription patterns and appropriateness of NSAID therapy according to gastrointestinal risk and cardiovascular history in patients with diagnoses of osteoarthritis. BMC Med. 2011;9:38.
132. Varga Z, Kriška M, Kristová V, Petrová M. Analysis of non-steroidal anti-inflammatory drug use in hospitalized patients and perception of their risk. Interdiscip Toxicol. 2013;6:141–4.
133. Koffeman AR, Valkhoff VE, Jong GW, Warlé-van Herwaarden MF, Bindels PJ, Sturkenboom MC, Luijsterburg PA, Bierma-Zeinstra SM. Ischaemic cardiovascular risk and prescription of non-steroidal anti-inflammatory drugs for musculoskeletal complaints. Scand J Prim Health Care. 2014;32:90–8.
134. CardioPain. http://cardiopain.it/8-ecm/14-cardiopain-in-roccadaspide-l-isola-che-c-e-1
135. Patrono C, García Rodríguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med. 2005;353:2373–83.
136. Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, Peto R, Buring J, Hennekens C, Kearney P, Meade T, Patrono C, Roncaglioni MC, Zanchetti A. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373:1849–60.
137. Barnett H, Burrill P, Iheanacho I. Don't use aspirin for primary prevention of cardiovascular disease. BMJ. 2010;340:c1805.
138. Hennekens CH, Dalen JE. Aspirin in the primary prevention of cardiovascular disease: current knowledge and future research needs. Trends Cardiovasc Med. 2014;24:360–6.
139. Maulaz AB, Bezerra DC, Michel P, Bogousslavsky J. Effect of discontinuing aspirin therapy on the risk of brain ischemic stroke. Arch Neurol. 2005;62:1217–20.
140. Biondi-Zoccai GG, Lotrionte M, Agostoni P, Abbate A, Fusaro M, Burzotta F, Testa L, Sheiban I, Sangiorgi G. A systematic review and meta-analysis on the hazards of discontinuing or not adhering to aspirin among 50,279 patients at risk for coronary artery disease. Eur Heart J. 2006;27:2667–74.
141. Biondi-Zoccai G, Wu Y, Serrano Jr CV, Frati G, Agostoni P, Abbate A. Aspirin underuse, non-compliance or cessation: definition, extent, impact and potential solutions in the primary and secondary prevention of cardiovascular disease. Int J Cardiol. 2015;182:148–54.
142. Saxena A, Balaramnavar VM, Hohlfeld T, Saxena AK. Drug/drug interaction of common NSAIDs with antiplatelet effect of aspirin in human platelets. Eur J Pharmacol. 2013;721:215–24.
143. Corman SL, Fedutes BA, Ansani NT. Impact of nonsteroidal antiinflammatory drugs on the cardioprotective effects of aspirin. Ann Pharmacother. 2005;39:1073–9.
144. Kurth T, Glynn RJ, Walker AM, Chan KA, Buring JE, Hennekens CH, Gaziano JM. Inhibition of clinical benefits of aspirin on first myocardial infarction by nonsteroidal antiinflammatory drugs. Circulation. 2003;108:1191–5.
145. Schjerning Olsen AM, Gislason GH, McGettigan P, Fosbøl E, Sørensen R, Hansen ML, Køber L, Torp-Pedersen C, Lamberts M. Association of NSAID use with risk of bleeding and cardiovascular events in patients receiving antithrombotic therapy after myocardial infarction. JAMA. 2015;313:805–14.
146. Lee W, Suh JW, Yang HM, Kwon DA, Cho HJ, Kang HJ, Kim HS, Oh BH. Celecoxib does not attenuate the antiplatelet effects of aspirin and clopidogrel in healthy volunteers. Korean Circ J. 2010;40:321–7.
147. Scarpignato C, Lanas A, Blandizzi C, Lems WF, Hermann M, Hunt RH, for the International NSAID Consensus Group. Safe prescribing of non-steroidal anti-inflammatory drugs in patients with osteoarthritis—an expert consensus addressing benefits as well as gastrointestinal and cardiovascular risks. BMC Med. 2015;13:55.
148. Laine L, Curtis SP, Langman M, Jensen DM, Cryer B, Kaur A, Cannon CP. Lower gastrointestinal events in a double-blind trial of the cyclo-

oxygenase-2 selective inhibitor etoricoxib and the traditional nonsteroidal anti-inflammatory drug diclofenac. Gastroenterology. 2008;135:1517–25.
149. Jüni P, Nartey L, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. Lancet. 2004;364:2021–9.
150. Brooks PM, Day RO. Nonsteroidal antiinflammatory drugs--differences and similarities. N Engl J Med. 1991;324:1716–25.
151. Chou R, Fanciullo GJ, Fine PG, Miaskowski C, Passik SD, Portenoy RK. Opioids for chronic noncancer pain: prediction and identification of aberrant drug-related behaviors: a review of the evidence for an American Pain Society and American Academy of Pain Medicine clinical practice guideline. J Pain. 2009;10:131–46.
152. Provenzano DA, Viscusi ER. Rethinking the role of opioids in the outpatient management of chronic nonmalignant pain. Curr Med Res Opin. 2014;30:2051–62.
153. American Geriatrics Society Panel on Pharmacological Management of Persistent Pain in Older Persons. Pharmacological management of persistent pain in older persons. J Am Geriatr Soc. 2009;57:1331–46.
154. Abdulla A, Adams N, Bone M, Elliott AM, Gaffin J, Jones D, Knaggs R, Martin D, Sampson L, Schofield P, British Geriatric Society. Guidance on the management of pain in older people. Age Ageing. 2013;42 Suppl 1:i1–57.
155. van Ojik AL, Jansen PA, Brouwers JR, van Roon EN. Treatment of chronic pain in older people: evidence-based choice of strong-acting opioids. Drugs Aging. 2012;29:615–25.
156. Nuckols TK, Anderson L, Popescu I, Diamant AL, Doyle B, Di Capua P, Chou R. Opioid prescribing: a systematic review and critical appraisal of guidelines for chronic pain. Ann Intern Med. 2014;160:38–47.
157. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. N Engl J Med. 2005;353:1711–23.
158. Leppert W, Buss T. The role of corticosteroids in the treatment of pain in cancer patients. Curr Pain Headache Rep. 2012;16:307–13.
159. Vyvey M. Steroids as pain relief adjuvants. Can Fam Phys. 2010;56:1295–7.
160. World Health Organization. WHO's cancer pain ladder for adults. http://www.who.int/cancer/palliative/painladder/en.
161. Marks JL, Colebatch AN, Buchbinder R, Edwards CJ. Pain management for rheumatoid arthritis and cardiovascular or renal comorbidity. Cochrane Database Syst Rev. 2011;10, CD008952.

Safe Prescription of NSAIDs in Clinical Practice

6

Mohammad Yaghoobi and Richard H. Hunt

Introduction

NSAIDs are among the most frequently prescribed medications around the world and are given for many different medical conditions, including but not limited to osteoarthritis, rheumatological diseases, and pain syndromes such as migraine headache. Their clinical utility has continued despite known gastrointestinal adverse effects including mucosal damage throughout the gastrointestinal tract and more recently cardiovascular adverse events. NSAIDs can cause damage in both the upper and lower gastrointestinal tracts including the small intestine and colon. Selective COX-2 inhibitors have a safer gastrointestinal profile than nonselective NSAIDs, but the cardiovascular adverse effects are similar. Adverse events carry a significant health and economic burden on patients and the healthcare system. Therefore, clinicians should familiarize themselves with the approach to safe prescription of NSAIDs in clinical practice. Over recent years, there has been significant improvement in our knowledge of the pharmacological and non-pharmacological characteristics to improve the safety profile of NSAIDs. These approaches are effective and based on strong evidence from randomized controlled trials (RCTs) and meta-analyses. The mechanism of action of NSAIDs and aspirin and the pathophysiology of the adverse events caused by these drugs are discussed in other chapters and therefore will not be further explored here.

Proposing safer prescription of NSAIDs would not be possible without understanding the mechanisms of mucosal defenses and how they act to protect the gastrointestinal mucosa against the toxic effects of NSAIDs and the pathophysiology of damage resulting from NSAID use. There are several known mechanisms by which the human body is protected against the adverse effects of NSAIDs. Maintaining gastric blood flow and secretion of bicarbonate as well as inhibition of the adhesion of leukocytes in the microvasculature of the stomach and duodenum have been recognized as critically important mechanisms of the natural mucosal defense mechanisms against damage caused by NSAIDs [1].

Physicians should be aware of the risk factors for toxicity and adverse events when prescribing NSAIDs. The prescribers should explain the potential risk to their patients when advising or prescribing NSAIDs. (This is fully discussed in

M. Yaghoobi, M.D., M.S. (Epi.), A.F.S. (✉)
Division of Gastroenterology, McMaster University, 1280 Main Street West, Rm-3V3, Hamilton, ON, Canada L8S 4K1
e-mail: Yaghoobi@mcmaster.ca

R.H. Hunt, F.R.C.P., F.R.C.P.Ed., F.R.C.P.C.
Division of Gastroenterology, Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_6

Table 6.1 Risk factors for NSAID-induced adverse events

Upper gastrointestinal tract	Lower gastrointestinal tract
History of peptic ulcer disease	Hypertension
Older age	Obesity
Helicobacter pylori infection	Older age
Smoking	Proton pump inhibitors
Alcohol	H_2-RAs
Coadministration of aspirin, antiplatelet agents, or corticosteroids	

Chap. 4.) These risk factors are much better understood for the upper than for the lower gastrointestinal tracts and are summarized in Table 6.1.

**The mechanisms by which NSAIDs cause lower gastrointestinal damage are less well understood and are deemed to differ from the circumstances in the upper gastrointestinal tract. Bjarnason and colleagues proposed that NSAIDs first solubilize lipids of the phospholipid layer on mucosal surface cells and trigger mitochondrial damage [2]. This depletes intracellular energy, releases intracellular calcium, and generates free radicals, leading to a decrease of adhesion between epithelial cells which increase the permeability of the small intestinal epithelium. This increased permeability allows toxins and luminal contents, including bacteria and their degradation products, bile acids, and pancreatic secretions, to enter the mucosa and induce inflammation by activation of neutrophils [2]. This then leads to intramural damage and reduced mucosal blood flow.

The risk factors for enterotoxicity are less well explored but hypertension and obesity are known to be important. Interestingly, while PPIs and H_2-receptor antagonists (H_2-RAs) decrease adverse events by NSAIDs in the upper gastrointestinal tract, they might increase the rate of damage in the lower gastrointestinal tract [3]. However, clinicians should be cautious in interpreting the data on the role of PPI and H_2-RAs in the lower gastrointestinal tract since this is an observed association and there is insufficient evidence from RCTs or cohort studies to show causality. One study in rats showed that PPIs might change the intestinal microbial population [4]. The investigators transferred jejunal bacteria from PPI-treated rats to germ-free mice and observed increased NSAID-induced damage in the small intestine as compared to controls. The results need to be confirmed in human studies, but it implies the potential benefits of probiotics in reducing the rate of NSAID-induced enteropathy in patients receiving PPI [5]. In 113 patients with rheumatoid arthritis who were taking NSAIDs, a cross-sectional video-capsule endoscopy study using multivariate regression analysis showed that the use of a PPI (RR 5.22), the age over 65 years (RR 4.16), and the use of an H_2-RA (RR 3.95) were the main risk factors for significant intestinal damage and a fall in hemoglobin [3]. Most patients in this study used loxoprofen, which is not widely used, and therefore, one should be cautious in generalizing the results to the clinical practice. On the other hand, the cross-sectional design of the study did not allow excluding baseline enteropathy in included patients. Although the quality of evidence from this study is not strong, one possible explanation might be that PPIs alter the nature of the bile, the gut microbiota, and the enterohepatic circulation of NSAIDs [1]. The rate of lower gastrointestinal events with NSAIDs has been increasing over the recent years. Lanas and colleagues showed that the rate of upper gastrointestinal adverse events due to NSAIDs decreased between 1996 and 2005 in Spain, while the rate of adverse events in the lower gastrointestinal tract increased [5]. A study in the USA showed similar results between 1998 and 2006 [6]. Possible explanations for these findings include increased physician awareness of adverse events and the strategies to avoid them. During this period in the USA, however, hospital admissions for lower GI bleeding increased by 8 %, while the number of upper GI bleeding events fell by 14 % [6].

Strategies to Improve the Safety Profile of NSAIDs

Substituting NSAIDs with an Alternative Treatment

The best strategy to avoid NSAID-induced toxicity is to avoid prescribing NSAIDs or substituting them with a less toxic medication such as acetaminophen or a low-dose opioid. Although this might not be possible in every patient, physicians should explore alternatives to NSAIDs and inform patients of available interventions and medications. This should be particularly emphasized in patients who carry significant risk factors for NSAID toxicity. It is not uncommon to see patients who carry multiple risk factors, and clinicians should specifically discuss the additive risk of adverse events in this situation and emphasize on the use of the protective measures. The additive effect of risk factors was proven in a randomized, double-blind, placebo-controlled trial in 8843 patients in the USA and Canada. Patients receiving continuous therapy with NSAIDs were randomized to receive misoprostol or placebo [7]. The logistic regression model showed that at 6 months, patients with no risk factors had a 0.4 % risk of gastrointestinal complications, while this number was 1 % for those with one of the risk factors including age over 75, history of peptic ulcer or gastrointestinal bleeding, or history of cardiovascular disease and was 9 % for patients with all four risk factors. Non-pharmacological treatment including exercise, appliances, and weight reduction for overweight patients should be considered for patients with osteoarthritis [8]. Alternatives to NSAID therapy include other non-pharmacological pain reliefs such as cognitive behavioral therapy, acupuncture, alternative medicine, and more recently transcutaneous electrical nerve stimulation (TENS), which activates native opioid receptors, or pharmacological pain relief such as acetaminophen (paracetamol) which is less effective but safer than NSAIDs when taken within the recommended dose range [8]. However, one might argue that acetaminophen carries its own risk of adverse events especially related to hepatotoxicity. Acetaminophen has been shown also to carry some cardiovascular risk and caused an increase in blood pressure in a randomized crossover study when compared to placebo [9].

Another alternative is to choose an opioid analgesic with or without paracetamol. The efficacy of opioids is similar to that of paracetamol but is less effective than NSAIDs alone [10]. However, the adverse effects of opioids exceed those of NSAIDs or paracetamol, particularly in elderly patients, and include constipation, central nervous system reactions, increased risk of falls, and death [10]. Slow-acting drugs for OA including glucosamine sulfate, chondroitin sulfate, diacerein, avocado/soybean unsaponifiable, and hyaluronic acid provide symptomatic relief and have reasonable safety profile, but the indication for using these agents is not well defined and the effect sizes are small. On the other hand, the evidence supporting these agents in rheumatological diseases and specifically in osteoarthritis is not strong, and most trials provided inconclusive results [8]. Intra-articular steroid injections, topical NSAIDs, osteotomy, and joint-preserving or joint-replacing surgical procedures may also be considered [8]. However, most patients will eventually require an NSAID at some time during the clinical course of their disease. The use of alternative pain relief should be individualized based on the patient's characteristics, risk factors, indication, duration of use, previous exposure, and availability.

Choosing an NSAID with the Least Gastrointestinal Toxicity

There are three subclasses of NSAIDs each with a different gastrointestinal toxicity profile.

Nonselective (ns)-NSAIDs

These medications inhibit both isoforms of the COX enzyme and are the most toxic to the gastrointestinal tract. Cardiovascular adverse events of NSAIDs should be considered at the same time as the gastrointestinal safety profile is

discussed with the patient. Renal adverse effects of nonselective NSAIDs are rarely seen in patients without renal risk factors [11] but can reach 20 % in at-risk patients [12] and include edema, hypertension, hyperkalemia, decreased GFR, and acute renal failure [13]. NSAIDs raise blood pressure in both normotensive and hypertensive individuals [14]. The cardiovascular and non-GI adverse events of NSAIDs are discussed extensively in chapter 2c.

A systematic review and meta-regression analysis of randomized controlled trial (RCT) studies and controlled cohort studies showed a significantly higher risk of gastrointestinal complications related to NSAID use than for nonusers: indomethacin, RR = 2.25 (1.00, 5.08); naproxen, RR = 1.83 (1.25, 2.68); diclofenac, RR = 1.73 (1.21, 2.46); piroxicam, RR = 1.66 (1.14, 2.44); tenoxicam, RR = 1.43 (0.40, 5.14); meloxicam, RR = 1.24 (0.98, 1.56); and ibuprofen, RR = 1.19 (0.93, 1.54) [15]. Meta-regression analysis showed that the significant risk factors were age, drug dose, and rheumatoid arthritis or osteoarthritis as the indication for taking NSAIDs.

Selective COX-2 Inhibitors

According to a meta-analysis, any dose of celecoxib was associated with significantly less clinical ulcers and gastrointestinal bleeds than ns-NSAIDs [16]. One should remember that selective COX-2 inhibitors may have some degree of COX-1 inhibitory activity. The SUCCESS I study also confirmed a better safety profile with celecoxib (200–400 mg/day) when compared with diclofenac and naproxen in 13,274 patients with osteoarthritis. There were significantly less upper gastrointestinal complications in the celecoxib-treated patients (0.1/100 patient-years) compared with the nonselective NSAIDs (0.8/100 patient-years) [17]. Celecoxib versus omeprazole and diclofenac in patients with osteoarthritis and rheumatoid arthritis (CONDOR) was a multicenter randomized double-blind trial comparing celecoxib and diclofenac in combination with omeprazole in patients with osteoarthritis and rheumatoid arthritis in 4484 patients with arthritis for 6 months [18]. The risk of clinically significant adverse events in the gastrointestinal tract was lower in patients treated with celecoxib than in those treated with diclofenac combined with omeprazole. Most complications were observed in patients who experienced a drop in hemoglobin level.

Newer coxibs including rofecoxib (now withdrawn) and etoricoxib (Arcoxia) which have greater selectivity for COX-2 also show a better gastrointestinal profile compared with nonselective NSAIDs [19–21].

Etoricoxib is a selective cyclooxygenase inhibitor, which is not approved in the USA but is used in many countries. A randomized, parallel-group, double-blind study where upper gastrointestinal endoscopy was performed at intervals over 12 weeks was conducted in 680 patients taking etoricoxib 120 mg once daily, ibuprofen 800 mg three times daily, or placebo [21]. The cumulative incidence of ulcers >3 mm at 12 weeks in the ibuprofen group (17 %) was significantly higher than in the etoricoxib group (8.1 %, $p = 0.007$) or placebo (1.86 %, $p < 0.001$); similar results were seen for ulcers >5 mm.

Post hoc analysis of serious lower gastrointestinal clinical events in a prospective, double-blind, randomized trial was performed in 8076 rheumatoid arthritis patients who were randomly assigned to naproxen 500 mg twice daily or rofecoxib 50 mg daily [22]. Bleeding with >2 g/dL drop in hemoglobin or hospitalization for perforation, obstruction, ulceration, or diverticulitis per 100 patient-years was 0.41 for rofecoxib and 0.89 for naproxen (relative risk, 0.46; 95 % CI, 0.22–0.93; $p = 0.032$).

The risk of lower gastrointestinal damage in association with selective COX-2 inhibitors and their role in reducing enterotoxicity is less well studied than that in the upper gastrointestinal tract. In the Vioxx Gastrointestinal Outcomes Research (VIGOR) trial, the incidence of severe lower gastrointestinal events including bleeding, perforation, obstruction, and complicated diverticular disease was lower in patients using rofecoxib than in those taking naproxen [23]. However, the

MEDAL trial showed no difference in adverse events between etoricoxib and diclofenac [24].

Celecoxib was associated with significantly less small intestinal mucosal damage than naproxen or ibuprofen combined with a PPI in healthy volunteers [20, 25]. There is some evidence that the selective COX-2 inhibitors may have a better profile in the lower gastrointestinal tract. Lumiracoxib 100 mg once daily induced less damage to the small bowel than naproxen 500 mg twice daily and omeprazole 20 mg once daily [26]. However, in another study, no differences were found in the proportion and type of small bowel lesions in patients taking nonselective NSAIDs in comparison with selective COX-2 inhibitors [27].

A recent network meta-analysis included randomized controlled trials comparing newer coxibs including celecoxib, etoricoxib, parecoxib, and lumiracoxib with relatively selective COX-2 inhibitors including nabumetone, meloxicam, and etodolac with a study duration of 4 weeks [28]. The primary outcomes were ulcer complications and symptomatic ulcer. The authors included 36 trials with a total of 112,351 patients. The analyses indicated no significant difference between relatively selective COX-2 inhibitors and coxibs regarding ulcer complications, symptomatic ulcer, or endoscopic ulcer. There was no change in the results after adjusting for potential influential factors including age, sex, previous ulcer disease, and follow-up time. However, this study did not take into account clinical efficacy with respect to reduction of pain and inflammation.

To our knowledge, there is no study to investigate the preventive measures for the colonic adverse events with NSAIDs. The renovascular effects of nonselective NSAIDs and coxibs are also similar [29, 30].

In summary, there is strong evidence that the selective COX-2 inhibitors are associated with less adverse events in the upper gastrointestinal tract, but controversy remains about their role in reducing adverse events in the lower gastrointestinal tract, although most evidence supports some benefit.

Other Newer NSAIDs

The newer NSAIDs include nitric oxide (NO)-releasing and hydrogen sulfide (H2S)-releasing NSAIDs which possess improved safety profiles in the upper gastrointestinal tract and also a better cardiovascular profile. Both NO and H2S have potent vasodilator, antioxidant, and anti-inflammatory effects which are believed to protect the mucosal microvasculature and maintain mucosal integrity in the gastrointestinal tract [31]. These drugs remain under investigation but early clinical studies indicate promising results.

The COX inhibiting NO-releasing class provides a multipathway mechanism of action of COX inhibition and controlled nitric oxide donation. Nitric oxide mediates many processes that contribute to gastric mucosal integrity by protection from the adverse consequences of COX inhibition [32]. A randomized double-blind study of an NO-releasing NSAID evaluated the gastrointestinal safety and efficacy of AZD3582 in 970 patients with hip or knee osteoarthritis. Patients were randomized to AZD3582 750 mg twice daily, naproxen 500 mg twice daily, or placebo twice daily [33]. At six weeks, the incidence of endoscopic gastroduodenal ulcers with AZD3582 was 9.7 % versus 13.7 % with naproxen, but the difference was not statistically significant. One might argue that the release of NO occurs very shortly after consumption and thus the protective effect might be limited due to short half-life of the NO component. The medication has not gained regulatory approval mainly because the safety advantage over naproxen has not been well established [34].

The effects of an H2S-releasing derivative of naproxen (ATB-346) have been evaluated in several models of arthritis, obesity, and hypertension. In one study, ATB-346 did not cause significant gastrointestinal damage in rats [35]. In addition, its coadministration with low-dose aspirin and/or PPI did not show detectable small intestinal damage, whereas naproxen alone or in combination with low-dose aspirin and PPI caused severe small intestinal ulceration

and bleeding [35]. Although these results are promising, studies in human are needed to confirm effectiveness and safety of administration.

Using Minimal Effective Dose of NSAIDs

The evidence from randomized clinical trials and observational studies shows that both short-term and long-term use of NSAIDs carry the same risk of gastrointestinal complications. Therefore, even a short course of NSAID therapy, especially in patients with high gastrointestinal risk, should be considered for strategies to prevent gastrointestinal complications [36].

Avoiding Combination Therapy

The combination of ASA and a nonselective NSAID, such as naproxen, increases the small bowel toxicity with a synergistic effect [34, 36]. Furthermore, special attention should always be paid to taking a detailed medication history, since NSAIDs may interact with other medications causing increased adverse effects. A common example includes angiotensin-converting enzyme (ACE) inhibitors, angiotensin-type receptor antagonists, diuretics, and selective serotonin reuptake inhibitors (SSRIs). SSRIs are associated with an increased risk of gastrointestinal bleeding [37], leading to hospital admission [38]. Impairment of platelet aggregation through depletion of serotonin is the likely mechanism [39]. A systematic review reported an RR of 1.3–3.6 for UGIB for SSRIs alone and 12.2–15.6 for combination of SSRIs and nonselective NSAIDs [37].

Self-Medication

About 38 % of NSAID-related complications and dyspeptic symptoms occur in patients who self-medicate with NSAIDs [39, 40]. It is important to take a careful medication history at every visit to properly identify at-risk patients. Gastroprotective strategies should be advised in high-risk patients even when considering short periods of treatment.

Non-pharmacological Prevention

Treatment of *H. pylori* Infection

H. pylori infection and NSAIDs are both independent risk factors for gastroduodenal toxicity, and the current evidence supports an additive effect in patients who carry both risk factors [41, 42]. Eradication of *H. pylori* infection before NSAID therapy significantly reduces the rate of complicated and uncomplicated peptic ulcers [43]. The effect of eradication is established in naïve users of NSAIDs but has not been shown effective in those who have been using NSAIDs for the long term [44–47]. Eradication of *H. pylori* infection should not replace using a PPI or misoprostol to prevent gastrointestinal toxicity. A meta-analysis has shown that eradication of *H. pylori* infection is not as effective as PPI treatment in preventing gastrointestinal complications [48]. The majority of randomized controlled trials show that eradication of *H. pylori* infection is associated with a significant reduction in the incidence of endoscopic ulcers in patients commencing NSAIDs [43–45, 49, 50]. This benefit is not sufficiently evident in patients who are already on long-term NSAIDs or in those with an ulcer history or history of ulcer complications, where co-therapy with a PPI is necessary [47–49]. Based on the current evidence, the Maastricht IV/Florence Consensus Report for the management of *Helicobacter pylori* recommends testing for and treating *H. pylori* infection if found before prescribing NSAIDs for short- or long-term treatment. However, it suggests individualizing this decision in those who are already on NSAIDs [50, 51]. A recent expert consensus also recognized infection with *H. pylori* as a risk factor for the development of NSAID-induced gastropathy and supported the recommendation by the Maastricht IV/Florence Consensus Report [52].

Patient Education

Physicians need to educate their patients before prescribing the medications to ensure their adherence to the instructions. One report analyzed

several strategies to increase PPI prescription rates in patients considered to be at increased risk for NSAID-related gastrointestinal complications after admission to a cardiology or intensive cardiac unit and who were receiving ulcerogenic medication at discharge [53]. The authors reported that a 10-min physician educational intervention, a computer alert, or a combination of both could improve the use of appropriate gastroprotection treatment. After the intervention, the use of gastroprotection treatment increased from 43 to 61 %.

Nonadherence to either NSAIDs (taking higher than prescribed dose or dose creep) or a gastrointestinal protective agent (not taking it properly) increases the risk of adverse events [54]. Inadequate or suboptimal prevention puts patients at increased risk and is thus expensive [55]. In a prospective, multicenter, observational, longitudinal study, patients attending rheumatology/orthopedic clinics who had risk factors for gastrointestinal complications and were prescribed an NSAID and a gastroprotective agent for at least 15 days were followed by a telephone call to assess their adherence and the rate of side effects. More adverse events occurred in patients with suboptimal adherence to the gastroprotective drugs than in those with optimal adherence to gastroprotection (22.1 % versus 1.9 %, $p < 0.0001$) [56]. Patient education and monitoring for adherence to gastroprotection strategies/treatments should therefore be undertaken by the physician at every visit.

Pharmacological Prevention

Reducing the rate of gastrointestinal adverse events has major economical advantage in addition to a reduction of the sickness burden since over 80 % of the total costs attributable to NSAID use are related to the treatment or prevention of gastrointestinal complications [55]. Pharmacological prevention has gained significant attention in clinical practice, and the following treatment strategies have been proposed for gastrointestinal protection purposes.

Proton Pump Inhibitors (PPIs)

PPIs are the mainstay of pharmacological prevention of the gastrointestinal adverse events of NSAIDs. In a multinational, randomized, blinded, parallel-group, placebo-controlled (OBERON) trial, 2426 *H. pylori*-negative patients taking daily low-dose ASA (75–325 mg) were randomized to once-daily esomeprazole 40 mg ($n = 817$), 20 mg ($n = 804$), or placebo ($n = 805$) for 26 weeks [56]. The primary outcome was the occurrence of endoscopy-confirmed peptic ulcers. Esomeprazole 40 and 20 mg significantly reduced the rate of peptic ulcers [1.5 % with esomeprazole 40 mg and 1.1 % with esomeprazole 20 mg, compared with 7.4 % on placebo ($p < 0.0001$)]. Another randomized, placebo-controlled trial evaluated omeprazole 20 mg daily in preventing NSAID-induced endoscopic ulcers [57]. The main outcome measure was the development of gastric or duodenal ulcers detected endoscopically, the development of multiple erosions in the stomach or duodenum, or the onset of moderate or severe dyspeptic symptoms. The estimated probability of remaining free of ulcer or erosions for 6 months in patients taking omeprazole was 0.78 compared to 0.53 for placebo ($p = 0.004$). Fourteen patients receiving placebo (16.5 %) developed 15 ulcers (nine gastric and six duodenal ulcers), compared to three patients (3.6 %) receiving omeprazole (all gastric ulcers). A similar randomized, placebo-controlled trial assessed omeprazole 20 mg once daily in preventing NSAID-induced PUD in patients with a history of dyspepsia or uncomplicated peptic ulcer disease and with a need for continuous NSAID treatment [58]. The outcome measure was the development of gastric or duodenal ulcers, erosions, and dyspeptic symptoms at 1 and 3 months. At 3 months, 4.7 % of omeprazole-treated patients developed peptic ulcer, compared with 16.7 % of those treated with placebo. This effect was independent of previous peptic ulcer history or *H. pylori* status. The development of dyspeptic symptoms requiring active

treatment, either alone or in combination with ulcer(s) or erosions, occurred in 15.3 % (15 of 85) of patients treated with omeprazole and 35.6 % of those who received placebo. In 2049 cases of upper gastrointestinal bleeding and 20,000 controls, a further study reported that co-prescription of PPI and nonselective NSAIDs significantly reduced the risk of UGIB (RR 0.51, 95 % CI 0.34–0.78) [59]. A meta-analysis of all studies which included arms comparing PPI and placebo for the prevention of NSAID-induced upper gastrointestinal ulcers found that PPIs significantly reduced the risk of endoscopic duodenal ulcers [$n = 840$, OR 0.18 (0.10–0.34)] between 3 and 12 months [60]. More controversial, however, is the role of PPIs in preventing enterotoxicity. PPIs inhibit gastric acid secretion by binding to the H^+/K^+-ATPase and are significantly more effective than H_2-RAs in the treatment of NSAID-related upper gastrointestinal ulceration and the prevention of gastric and duodenal mucosal damage with NSAIDs [61, 62]. Despite this, the protection afforded by antisecretory treatment against NSAID injury in the upper gastrointestinal tract does not extend to the small intestine. Omeprazole did not prevent small intestinal lesions induced by short-term administration of naproxen or ibuprofen [20]. However, in another study, lansoprazole did reduce small intestinal lesions induced by indomethacin [63]. In summary, there is not enough evidence to clarify the role of PPIs in decreasing the rate of the lower gastrointestinal complications of NSAIDs.

Several authorities have expressed concerns on the potential interaction between PPI and clopidogrel in patients with cardiovascular comorbidities based on observational studies [64]. Metabolism through cytochrome P-450 was presumed to be the theoretical pathway that could affect the response to clopidogrel [65]. Two observational retrospective studies showed increased rate of adverse cardiac effect in patients taking clopidogrel and PPI [64, 66]. Both studies were criticized on their methodology and their retrospective nature which limited the assessment of compliance to clopidogrel and genetic variability regarding the cytochrome P-450. However, the Clopidogrel and the Optimization of Gastrointestinal Events Trial (COGENT) showed no clinically significant cardiovascular interaction between omeprazole and clopidogrel [67]. 3873 patients who were candidates for dual antiplatelet therapy were randomized to receive omeprazole or placebo. The rate of major cardiovascular events defined as death, myocardial infarction, and stroke was not significantly different in two groups (hazard ratio with omeprazole, 0.99; 95 % CI, 0.68–1.44; $p = 0.96$), while the rate of overall gastrointestinal events including bleeding was significantly higher in the placebo group. The benefits of PPIs in the prevention of gastrointestinal complications in patients taking low-dose aspirin and clopidogrel outweigh the damage caused by increased cardiovascular risk.

Histamine H_2-Receptor Antagonists (H_2-RAs)

The use of H_2-RAs has significantly decreased after the introduction of PPIs. It is unclear if H_2-RA could substitute for a PPI in preventing adverse events. So far, only famotidine twice daily has been shown to reduce the rate of both duodenal and gastric ulcers [60, 61]. Investigators in one RCT compared omeprazole 20 mg daily with ranitidine 150 mg twice daily in 425 patients [68]. In this study, PPI was superior to standard-dose ranitidine for the prevention of both gastric and duodenal ulcers [RR 0.32 (0.17–0.62) and 0.11 (0.01–0.89), respectively]. However, clinically significant ulcer-related events and dropouts due to adverse effects were comparable for both the PPI and H_2-RA [RR 3.07 (0.13–74.97) and 1.90 (0.77–4.67), respectively].

Misoprostol

Misoprostol is a synthetic prostaglandin E_1 analogue. Although the side effects which include abdominal cramps, diarrhea, and electrolyte loss make it difficult and a less desirable treatment for patients especially the elderly, misoprostol is associated with a lower rate of gastric and duodenal ulcer by 74 % and 53 %, respectively, in NSAID users [60]. Misoprostol also reduced gastrointestinal complications by 40 %. The

recommended dose of misoprostol is 800 mg daily, a dose at which significant reduction in peptic ulcer complications is seen (odds ratio 0.598, $p = 0.049$) although adverse events are higher [60]. There is insufficient evidence to determine the effect of misoprostol in the prevention of NSAID-associated small intestinal damage although this represents an important question which should be prospectively studied, although the adverse effects would likely still limit its use [69, 70].

Misoprostol compared with PPI treatment. A study comparing low-dose misoprostol (400 μg daily) with omeprazole (20 mg daily) showed a nonsignificant trend toward greater benefit with misoprostol over omeprazole for the prevention of gastric ulcer [71]. A comparison of high-dose misoprostol (800 μg daily) with lansoprazole (15 or 30 mg daily) showed no significant difference in the prevention of gastric ulcer, but the PPI was statistically better than misoprostol in preventing duodenal ulcer [RR 0.29 (0.15–0.56)] [72].

A systematic review found PPIs to be superior to placebo and H_2-RAs in reducing the risk of NSAID-induced endoscopic gastric and duodenal ulcer [60]. PPIs are also superior to misoprostol in the prevention of duodenal ulcer, but there is no evidence for this in preventing gastric ulcers [60]. Therefore, in the absence of contraindication, PPIs should be chosen over H_2-RA or misoprostol in preventing the adverse events of NSAIDs in the gastrointestinal tract.

There are several newer agents currently under investigation to protect the gastrointestinal tract against the potential adverse events of the NSAIDs.

Rebamipide

Rebamipide or (2-(4-chlorobenzoylamino)-3-[2 (1H)-quinolinon-4-yl]propionic acid) is a cytoprotective antiulcer agent that enhances defense of the gastric mucosa by increasing gastric mucus and stimulating the production of endogenous prostaglandins [73]. It also has anti-inflammatory properties and stimulates gastric mucus and the production of prostaglandins [73]. Rebamipide decreases the rate of diclofenac-induced small intestinal mucosal injury compared with placebo in a prospective RCT [73]. However, further studies are needed since the evidence is not enough to recommend its use in routine practice.

DA-9601 (Stillen)

DA-9601 is an antioxidant which prevents the formation of reactive oxygen species and is a new agent under investigation to prevent NSAID-associated gastrointestinal complications [74]. DA-9601 and misoprostol were studied as protection from NSAID-associated gastroduodenal injury in healthy volunteers in a randomized, double-blinded, multicenter non-inferiority study [75]. No placebo was used. DA-9601 was not inferior to misoprostol in terms of endoscopic gastroduodenal protection rates. However, the adverse event rate with DA-9601 was higher than with misoprostol. These adverse effects included diarrhea, abdominal pain, bloating, and nausea. In another multicenter, double-blind, stratified randomized non-inferiority study by the same group, 520 patients who were taking an NSAID (aceclofenac, 100 mg, twice daily) over a 4-week period were randomly assigned to receive either DA-9601 (60 mg, TID) ($n = 236$) or misoprostol (200 g, TID) ($n = 242$) [76]. The primary endpoint was the gastric protection rate, and the secondary endpoints were duodenal protection and ulcer incidence. At week 4, the gastric protection rates with DA-9601 and misoprostol were 81.4 % and 89.3 %, respectively. DA-9601 was not inferior to misoprostol. Adverse event rates were not different between the two groups. However, it is too early to conclude if DA-9601 could be introduced into clinical practice as a preventive agent before more studies prove safety and efficacy and compare it with the current strategies.

β-D-Glucuronidase Inhibitors

One study has shown that pretreatment of rats with an inhibitor of β-D-glucuronidase significantly protected the small intestine against damage induced by diclofenac, indomethacin, or

ketoprofen [77]. However, when the new agent was given after the administration of NSAIDs, it did not show clinical benefit.

Recommendations from Guidelines

Some guideline or consensus statements have addressed strategies in preventing adverse events with prescription of NSAIDs. The Maastricht IV/Florence Consensus Report for the management of *Helicobacter pylori* provides the following statements [67]:

- "*H. pylori* infection is associated with an increased risk of uncomplicated and complicated gastroduodenal ulcers in NSAID and low-dose aspirin (acetylsalicylic acid (ASA)) users. Evidence level: 2a Grade of recommendation: B
- Eradication reduces the risk of complicated and uncomplicated gastroduodenal ulcers associated with either NSAID or low-dose ASA use. Evidence level: 1b Grade of recommendation: A
- *H. pylori* eradication is beneficial before starting NSAID treatment. It is mandatory in patients with a peptic ulcer history. Evidence level: 1b Grade of recommendation: A. However, *H. pylori* eradication alone does not reduce the incidence of gastroduodenal ulcers in patients already receiving long-term NSAID treatment. They require continued PPI treatment as well as eradication treatment. Evidence level: 1b Grade of recommendation: A
- Testing for *H. pylori* should be performed in ASA users with a history of gastroduodenal ulcer. The long-term incidence of peptic ulcer bleeding is low in these patients after receiving eradication even in the absence of gastroprotective treatment. Evidence level: 2b Grade of recommendation: B"

A joint expert consensus document by the American College of Gastroenterology, American Heart Association, and American College of Cardiology Foundation Task Force on Reducing the Gastrointestinal Risks of Antiplatelet Therapy and NSAID Use in 2008 made the following recommendations [78]:

- PPIs are the preferred agents for the therapy and prophylaxis of NSAID- and ASA-associated GI injury.
- Testing for and eradicating *H. pylori* in patients with a history of ulcer disease is recommended before starting chronic antiplatelet therapy.

Conclusions

Strategies to reduce the rate of complications when prescribing NSAIDs in clinical practice include non-pharmacological interventions including patient education, monitoring the adherence to the protective measures, and alternative pain management strategies. Administering the lowest effective dose, choosing selective COX-2 inhibitors and considering the gastrointestinal and cardiovascular status of each patient should be the standard of care. Test and treat strategy for infection with *H. pylori* is required before initiating therapy with NSAIDs. Pharmacological treatments including coadministration of PPIs, H_2-RAs, and misoprostol should be used especially in patients with previous gastrointestinal risk factors. A new treatment to protect small bowel injury due to anti-inflammatory drugs is much needed. Further research should focus on newer classes of NSAIDs or novel medications since there is insufficient data to recommend these in clinical practice.

References

1. Blackler RW, Gemici B, Manko A, Wallace JL. - NSAID-gastroenteropathy: new aspects of pathogenesis and prevention. Curr Opin Pharmacol. 2014;19:11–6.
2. Bjarnason I, Williams P, So A, Zanelli G, Levi A, Gumpel J, et al. Intestinal permeability and inflammation in rheumatoid arthritis: effects of non-steroidal anti-inflammatory drugs. Lancet. 1984;24:1171–4.

3. Watanabe T, Tanigawa T, Nadatani Y, Nagami Y, Sugimori S, Okazaki H, Yamagami H. Risk factors for severe nonsteroidal anti-inflammatory drug-induced small intestinal damage. Dig Liver Dis. 2013;45:390–5.
4. Wallace JL, Syer S, Denou E, de Palma G, Vong L, McKnight W, Jury J, Bolla M, Bercik P, Collins SM, Verdu E, Ongini E. Proton pump inhibitors exacerbate NSAID-induced small intestinal injury by inducing dysbiosis. Gastroenterology. 2011;141(4):1314–22.
5. Lanas A, García-Rodríguez LA, Polo-Tomás M, Ponce M, Alonso-Abreu I, Perez-Aisa MA, et al. Time trends and impact of upper and lower gastrointestinal bleeding and perforation in clinical practice. Am J Gastroenterol. 2009;104:1633–41.
6. Zhao Y, Encinosa W. Hospitalizations for gastrointestinal bleeding in 1998 and 2006. Healthcare Cost and Utilization Project (HCUP) Statistical Brief #65 [report on the Internet]. Agency for Healthcare Research and Quality, Rockville, MD. [Last Accessed Nov 7, 2015.]. http://www.hcup-us.ahrq.gov/reports/statbriefs/sb65.jsp
7. Silverstein FE, Graham DY, Senior JR, Davies HW, Struthers BJ, Bittman RM, et al. Misoprostol reduces serious gastrointestinal complications in patients with rheumatoid arthritis receiving nonsteroidal anti-inflammatory drugs. A randomized, double-blind, placebo-controlled trial. Ann Intern Med. 1995; 123:241–9.
8. Zhang W, Doherty M, Arden N, Bannwarth B, Bijlsma J, Gunther KP, et al. EULAR evidence based recommendations for the management of hip OA: report of a task force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis. 2005;64:669–81.
9. Sudano I, Flammer AJ, Périat D, Enseleit F, Hermann M, Wolfrum M, Hirt A, Kaiser P, Hurlimann D, Neidhart M, Gay S, Holzmeister J, Nussberger J, Mocharla P, Landmesser U, Haile SR, Corti R, Vanhoutte PM, Lüscher TF, Noll G, Ruschitzka F. Acetaminophen increases blood pressure in patients with coronary artery disease. Circulation. 2010;122(18):1789–96.
10. Cepeda MS, Camargo F, Zea C, Valencia L. Tramadol for osteoarthritis: a systematic review and metaanalysis. J Rheumatol. 2007;34:543–55.
11. Sturmer T, Erb A, Keller F, Gunther KP, Brenner H. Determinants of impaired renal function with use of nonsteroidal anti-inflammatory drugs: the importance of half-life and other medications. Am J Med. 2001;111:521–7.
12. Murray MD, Brater DC, Tierney WM, Hui SL, McDonald CJ. Ibuprofen-associated renal impairment in a large general internal medicine practice. Am J Med Sci. 1990;299:222–9.
13. Stichtenoth DO, Frolich JC. COX-2 and the kidneys. Curr Pharm Des. 2000;6:1737–53.
14. Fosslien E. Cardiovascular complications of non-steroidal anti-inflammatory drugs. Ann Clin Lab Sci. 2005;35:347–85.
15. Richy F, Bruyere O, Ethgen O, Rabenda V, Bouvenot G, Audran M, Herrero-Beaumont G, Moore A, Eliakim R, Haim M, Reginster JY. Time dependent risk of gastrointestinal complications induced by non-steroidal anti-inflammatory drug use: a consensus statement using a meta-analytic approach. Ann Rheum Dis. 2004;63(7):759–66.
16. Moore RA, Derry S, Makinson GT, McQuay HJ. Tolerability and adverse events in clinical trials of celecoxib in osteoarthritis and rheumatoid arthritis: systematic review and meta-analysis of information from company clinical trial reports. Arthritis Res Ther. 2005;7:R644–65.
17. Singh G, Fort JG, Goldstein JL, Levy RA, Hanrahan PS, Bello AE, et al. Celecoxib versus naproxen and diclofenac in osteoarthritis patients: SUCCESS-I Study. Am J Med. 2006;119:255–66.
18. Chan FK, Lanas A, Scheiman J, Berger MF, Nguyen H, Goldstein JL. Celecoxib *versus* omeprazole and diclofenac in patients with osteoarthritis and rheumatoid arthritis (CONDOR): a randomised trial. Lancet. 2010;376:173–9.
19. Schnitzer TJ, Burmester GR, Mysler E, Hochberg MC, Doherty M, Ehrsam E, et al. Comparison of lumiracoxib with naproxen and ibuprofen in the Therapeutic Arthritis Research and Gastrointestinal Event Trial (TARGET), reduction in ulcer complications: randomised controlled trial. Lancet. 2004; 364:665–74.
20. Goldstein JL, Eisen GM, Lewis B, Gralnek IM, Zlotnick S, Fort JG. Investigators. Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole, and placebo. Clin Gastroenterol Hepatol. 2005;3:133–41.
21. Hunt RH, Harper S, Watson DJ, Yu C, Quan H, Lee M, et al. The gastrointestinal safety of the COX-2 selective inhibitor etoricoxib assessed by both endoscopy and analysis of upper gastrointestinal events. Am J Gastroenterol. 2003;98:1725–33.
22. Laine L, Connors LG, Reicin A, et al. Serious lower gastrointestinal clinical events with nonselective NSAID or coxib use. Gastroenterology. 2003; 124:288–92.
23. Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJ, VIGOR Study Group. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Med. 2000;343(21):1520–8.
24. Laine L, Curtis SP, Langman M, et al. Lower gastrointestinal events in a double-blind trial of the cyclooxygenase-2 selective inhibitor etoricoxib and the traditional nonsteroidal anti-inflammatory drug diclofenac. Gastroenterology. 2008;135:1517–25.

25. Goldstein JL, Eisen GM, Lewis B, et al. Small bowel mucosal injury is reduced in healthy subjects treated with celecoxib compared with ibuprofen plus omeprazole, as assessed by video capsule endoscopy. Aliment Pharmacol Ther. 2007;25:1211–22.
26. Hawkey CJ, Ell C, Simon B, et al. Less small-bowel injury with lumiracoxib compared with naproxen plus omeprazole. Clin Gastroenterol Hepatol. 2008; 6:536–44.
27. Maiden L, Thjodleifsson B, Seigal A, et al. Long-term effects of nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 selective agents on the small bowel: a cross-sectional capsule enteroscopy study. Clin Gastroenterol Hepatol. 2007;5:1040–5.
28. Yang M, Wang HT, Zhao M, Meng WB, Ou JQ, He JH, Zou B, Lei PG. Network meta-analysis comparing relatively selective COX-2 inhibitors versus Coxibs for the prevention of NSAID-induced gastrointestinal injury. Medicine (Baltimore). 2015;94(40), e1592.
29. Hunt RH, Lanas A, Stichtenoth DO, Scarpignato C. Myths and facts in the use of anti-inflammatory drugs. Ann Med. 2009;41(6):423–37.
30. Aw TJ, Haas SJ, Liew D, Krum H. Meta-analysis of cyclooxygenase-2 inhibitors and their effects on blood pressure. Arch Intern Med. 2005;165:490–6.
31. Fiorucci S, Distrutti E. COXIBs, CINODs and H_2S-releasing NSAIDs: current perspectives in the development of safer non steroidal anti-inflammatory drugs. Curr Med Chem. 2011;18:3494–505.
32. Wallace J. Cooperative modulation of gastrointestinal mucosal defence by prostaglandins and nitric oxide. Clin Invest Med. 1996;19:346–51.
33. Lohmander LS, McKeith D, Svensson O, Malmenäs M, Bolin L, Kalla A, Genti G, Szechinski J, Ramos-Remus C, STAR Multinational Study Group. A randomised, placebo controlled, comparative trial of the gastrointestinal safety and efficacy of AZD3582 versus naproxen in osteoarthritis. Ann Rheum Dis. 2005;64(3):449–56.
34. Wallace JL. Mechanisms, prevention and clinical implications of nonsteroidal anti-inflammatory drug-enteropathy. World J Gastroenterol. 2013; 19:1861–76.
35. Blackler R, Syer S, Bolla M, Ongini E, Wallace JL. -Gastrointestinal-sparing effects of novel NSAIDs in rats with compromised mucosal defence. PLoS One. 2012;7, e35196.
36. Hernandez-Diaz S, Garcia-Rodriguez LA. Association between nonsteroidal anti-inflammatory drugs and upper gastrointestinal tract bleeding/perforation: an overview of epidemiologic studies published in the 1990s. Arch Intern Med. 2000;160:2093–9.
37. Yuan Y, Tsoi K, Hunt RH. Selective serotonin reuptake inhibitors and risk of upper gastrointestinal bleeding: confusion or confounding? Am J Med. 2006;119:719–27.
38. Meijer WE, Heerdink ER, Nolen WA, Herings RM, Leufkens HG, Egberts AC. Association of risk of abnormal bleeding with degree of serotonin reuptake inhibition by antidepressants. Arch Intern Med. 2004;164:2367–70.
39. de Abajo FJ, Montero D, Garcia-Rodriguez LA, Madurga M. Antidepressants and risk of upper gastrointestinal bleeding. Basic Clin Pharmacol Toxicol. 2006;98:304–10.
40. Lanas A, Hunt R. Prevention of anti-inflammatory drug-induced gastrointestinal damage: Benefits and risks of therapeutic strategies. Ann Med. 2006;38:415–28.
41. Lanas A, Ballina J, Sobreviela E, et al. Recent advances in the field of NSAID-associated side effects are not appropriately translated into clinical practice. Gastroenterology. 2007;132 Suppl 2:A86.
42. Huang JQ, Sridhar S, Hunt RH. Role of *Helicobacter pylori* infection and nonsteroidal anti-inflammatory drugs in peptic-ulcer disease: a meta-analysis. Lancet. 2002;359:14–22.
43. Chan FK, To KF, Wu JC, et al. Eradication of *Helicobacter pylori* and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomised trial. Lancet. 2002;359:9–13.
44. Chan FK, Sung JJ, Chung SC, et al. Randomised trial of eradication of *Helicobacter pylori* before non-steroidal anti-inflammatory drug therapy to prevent peptic ulcers. Lancet. 1997;350:975–9.
45. Hawkey CJ, Tulassay Z, Szczepanski L, et al. Randomised controlled trial of *Helicobacter pylori* eradication in patients on non-steroidal anti-inflammatory drugs: HELP NSAIDs study. Helicobacter Eradication for Lesion Prevention. Lancet. 1998;352:1016–21.
46. de Leest HT, Steen KS, Lems WF, et al. Eradication of *Helicobacter pylori* does not reduce the incidence of gastroduodenal ulcers in patients on long-term NSAID treatment: double-blind, randomized, placebo-controlled trial. Helicobacter. 2007; 12:477–85.
47. Lai KC, Lau CS, Ip WY, et al. Effect of treatment of *Helicobacter pylori* on the prevention of gastroduodenal ulcers in patients receiving long-term NSAIDs: a double-blind, placebo-controlled trial. Aliment Pharmacol Ther. 2003;17:799–805.
48. Vergara M, Catalan M, Gisbert JP, et al. Meta-analysis: role of *Helicobacter pylori* eradication in the prevention of peptic ulcer in NSAID users. Aliment Pharmacol Ther. 2005;21:1411–8.
49. Chan FK, Chung SC, Suen BY, Lee YT, Leung WK, Leung VK, et al. Preventing recurrent upper gastrointestinal bleeding in patients with *Helicobacter pylori* infection who are taking low-dose aspirin or naproxen. N Engl J Med. 2001;344:967–73.
50. Labenz J, Blum AL, Bolten WW, Dragosics B, Rosch W, Stolte M, et al. Primary prevention of diclofenac associated ulcers and dyspepsia by omeprazole or triple therapy in *Helicobacter pylori* positive patients: a randomised, double blind, placebo controlled, clinical trial. Gut. 2002;51:329–35.

51. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, Gisbert JP, Graham DY, Rokkas T, El-Omar EM, Kuipers EJ, European Helicobacter Study Group. Management of *Helicobacter pylori* infection—the Maastricht IV/Florence Consensus Report. Gut. 2012;61(5): 646–64.
52. Scarpignato C, Lanas A, Blandizzi C, Lems WF, Hermann M, RH H, International NSAID Consensus Group. Safe prescribing of non-steroidal anti-inflammatory drugs in patients with osteoarthritis--an expert consensus addressing benefits as well as gastrointestinal and cardiovascular risks. BMC Med. 2015;13:55.
53. Coté GA, Rice JP, Bulsiewicz W, Norvell JP, Christensen K, Bobb A, Postelnick M, Howden CW. Use of physician education and computer alert to improve targeted use of gastroprotection among NSAID users. Am J Gastroenterol. 2008;103: 1097–103.
54. Lanas A, Polo-Tomás M, Roncales P, Gonzalez MA, Zapardiel J. Prescription of and adherence to non-steroidal anti-inflammatory drugs and gastroprotective agents in at-risk gastrointestinal patients. Am J Gastroenterol. 2012;107(5):707–14.
55. Lanas A. Cost stratification of nonsteroidal anti-inflammatory drug-associated gastrointestinal side effects. Med Clin (Barc). 2000;114 Suppl 3:46–53.
56. Scheiman JM, Devereaux PJ, Herlitz J, Katelaris PH, Lanas A, Veldhuyzen van Zanten S, Nauclér E, Svedberg LE. Prevention of peptic ulcers with esomeprazole in patients at risk of ulcer development treated with low-dose acetylsalicylic acid: a randomised, controlled trial (OBERON). Heart. 2011;97(10):797–802.
57. Cullen D, Bardhan KD, Eisner M, Kogut DG, Peacock RA, Thomson JM, et al. Primary gastro duodenal prophylaxis with omeprazole for non-steroidal anti-inflammatory drug users. Aliment Pharmacol Ther. 1998;12:135–40.
58. Ekstrom P, Carling L, Wetterhus S, Wingren PE, Anker-Hansen O, Lundegardh G, et al. Prevention of peptic ulcer and dyspeptic symptoms with omeprazole in patients receiving continuous non- steroidal anti-inflammatory drug therapy. A Nordic multicentre study. Scand J Gastroenterol. 1996;31:753–8.
59. Lin KJ, Hernández-Díaz S, García Rodríguez LA. Acid suppressants reduce risk of gastrointestinal bleeding in patients on antithrombotic or anti-inflammatory therapy. Gastroenterology. 2011;141: 71–9.
60. Leontiadis GI, Sreedharan A, Dorward S, et al. Systematic reviews of the clinical effectiveness and cost-effectiveness of proton pump inhibitors in acute upper gastrointestinal bleeding. Health Technol Assess. 2007;11:iii–iv. 1–164.
61. Rostom A, Dube C, Wells G, Tugwell P, Welch V, Jolicoeur E, et al. Prevention of NSAID-induced gastro-duodenal ulcers. Cochrane Database Syst Rev. 2004;(4)CD002296.
62. Robinson M. Proton pump inhibitors: update on their role in acid-related gastrointestinal diseases. Int J Clin Pract. 2005;59:709–15.
63. Yoda Y, Amagase K, Kato S, et al. Prevention by lansoprazole, a proton pump inhibitor, of indomethacin-induced small intestinal ulceration in rats through induction of heme oxygenase-1. J Physiol Pharmacol. 2010;61:287–94.
64. Ho PM, Maddox TM, Wang L, et al. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. JAMA. 2009;301:937–44.
65. Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. N Engl J Med. 2009;360:363–75.
66. Juurlink DN, Gomes T, Ko DT, et al. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. CMAJ. 2009;180:713–8.
67. Bhatt DL, Cryer BL, Contant CF, Investigators COGENT, et al. Clopidogrel with or without omeprazole in coronary artery disease. N Engl J Med. 2010;363:1909–17.
68. Yeomans ND, Tulassay Z, Juhasz L, Racz I, Howard JM, Van Rensburg CJ, et al. A comparison of omeprazole with ranitidine for ulcers associated with nonsteroidal anti-inflammatory drugs. Acid Suppression Trial: Ranitidine versus Omeprazole for NSAID-associated Ulcer Treatment (ASTRONAUT) Study Group. N Engl J Med. 1998;338:719–26.
69. Fujimori S, Seo T, Gudis K, et al. Prevention of nonsteroidal anti-inflammatory drug-induced small-intestinal injury by prostaglandin: a pilot randomized controlled trial evaluated by capsule endoscopy. Gastrointest Endosc. 2009;69:1339–46.
70. Davies GR, Wilkie ME, Rampton DS. Effects of metronidazole and misoprostol on indomethacin-induced changes in intestinal permeability. Dig Dis Sci. 1993;38:417–25.
71. Hawkey CJ, Karrasch JA, Szczepanski L, Walker DG, Barkun A, Swannell AJ, et al. Omeprazole compared with misoprostol for ulcers associated with nonsteroidal anti-inflammatory drugs. Omeprazole versus Misoprostol for NSAID- induced Ulcer Management (OMNIUM) Study Group. N Engl J Med. 1998;338:727–34.
72. Graham DY, Agrawal NM, Campbell DR, Haber MM, Collis C, Lukasik NL, et al. Ulcer prevention in long-term users of nonsteroidal anti-inflammatory drugs: results of a double-blind, randomized, multicenter, active- and placebo- controlled study of misoprostol vs. lansoprazole. Arch Intern Med. 2002; 162:169–75.
73. Niwa Y, Nakamura M, Ohmiya N, et al. Efficacy of rebamipide for diclofenac-induced small-intestinal mucosal injuries in healthy subjects: a prospective, randomized, double-blinded, placebo-controlled, cross-over study. J Gastroenterol. 2008; 43:270–6.

74. Kim DH, Na HK, Oh TY, Kim WB, Surh YJ. Eupatilin, a pharmacologically active flavone derived from Artemisia plants, induces cell cycle arrest in ras-transformed human mammary epithelial cells. Biochem Pharmacol. 2004;68:1081–7.
75. Lee KN, Lee OY, Choi MG, Choi SR, Lee DH, Lee YC, Kim TN, Choi SC, Rew JS, Seol SY. Prevention of NSAID-associated gastroduodenal injury in healthy volunteers-a randomized, double-blind, multicenter study comparing DA- 9601 with misoprostol. J Korean Med Sci. 2011;26:1074–80.
76. Lee OY, Kang DH, Lee DH, Chung IK, Jang JY, Kim JI, Cho JW, Rew JS, Lee KM, Kim KO, Choi MG, Lee SW, Lee ST, Kim TO, Shin YW, Seol SY. A comparative study of DA-9601 and misoprostol for prevention of NSAID-associated gastroduodenal injury in patients undergoing chronic NSAID treatment. Arch Pharm Res. 2014;37(10):1308–16.
77. Saitta KS, Zhang C, Lee KK, Fujimoto K, Redinbo MR, Boelsterli UA. Bacterial b-glucuronidase inhibition protects mice against enteropathy induced by indomethacin, ketoprofen or diclofenac: mode of action and pharmacokinetics. Xenobiotica. 2014;44:28–35.
78. Bhatt DL, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD, Johnson DA, Mahaffey KW, Quigley EM, Harrington RA, Bates ER, Bridges CR, Eisenberg MJ, Ferrari VA, Hlatky MA, Kaul S, Lindner JR, Moliterno DJ, Mukherjee D, Schofield RS, Rosenson RS, Stein JH, Weitz HH, Wesley DJ, American College of Cardiology Foundation, American College of Gastroenterology, American Heart Association. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use. Am J Gastroenterol. 2008;103(11):2890–907.

Part III

Aspirin

NSAIDS and Aspirin: Recent Advances and Implications for Clinical Management

7

Karsten Schrör and Michael Voelker

Aspirin Pharmacodynamics

General Aspects

Aspirin (acetylsalicylic acid or ASA) is in clinical use for over 100 years. As a salicylate derivative, it possesses the three properties of nonsteroidal anti-inflammatory drugs (NSAIDs): analgesic, antipyretic, and anti-inflammatory. Regarding the mode of action, it was originally believed that aspirin was only the inactive prodrug of the active metabolite salicylate [1]. At the high doses which were used at the time, i.e., 3–4 g per day and more, there was clearly a significant contribution of salicylate to the overall anti-inflammatory action of the compound. However, after the detection by John Vane that aspirin inhibits prostaglandin biosynthesis, being much more potent than salicylate [2], this view has changed in favor of a primary role of aspirin itself. Another important discovery was that aspirin unlike salicylate also inhibits platelet function and the demonstration that acetylation of platelet cyclooxygenase (COX) is required for this effect [3]. This finding not only has broadened the spectrum of clinically relevant pharmacological actions of aspirin but also introduced a new target: the blood platelet. Today, different and at least partially independent pharmacodynamic actions of aspirin and its primary metabolite salicylate are generally appreciated and will act in concert by improving the acetylation reaction and adding the direct (anti)metabolic actions of salicylate (Fig. 7.1) (for details see Schrör, Wiley 2016).

After recognition of acetylation as a primary mode of action of aspirin, the question arose whether the high doses of the compound, used initially by analogy with the "active metabolite" concept of salicylate, are really necessary. It became increasingly clear that clinically relevant aspirin actions, i.e., antithrombotic, analgesic, antipyretic, and partially the anti-inflammatory effects of aspirin, can be obtained at much lower doses, i.e., 1–2 g per day or even less and are mainly due to target-specific acetylation. A recent proteomic analysis of living (tumor) cells has identified 120 acetylated proteins, most of them not previously reported to be acetylated by aspirin [4]. Currently, more than 500 target proteins of aspirin-induced acetylation have been identified [5]. The biological consequences of these transacetylations are only incompletely understood but might be considerable. Figure 7.2 provides an overview of major pharmacological actions of aspirin.

K. Schrör, M.D.
Institut für Pharmakologie und Klinische Pharmakologie, Universitäts Klinikum, Heinrich-Heine Universität Düsseldorf, Düsseldorf, Germany

M. Voelker, Ph.D. (✉)
Bayer AG, Consumer Health Division, Global Medical Affairs, Leverkusen, Germany
e-mail: michael.voelker@bayer.com

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_7

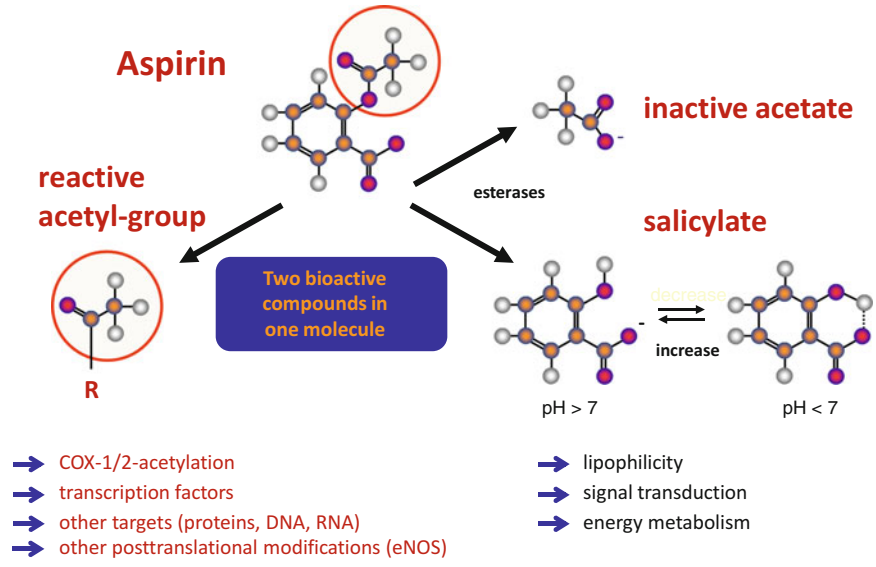


Fig. 7.1 The two active principles of aspirin and their cellular targets

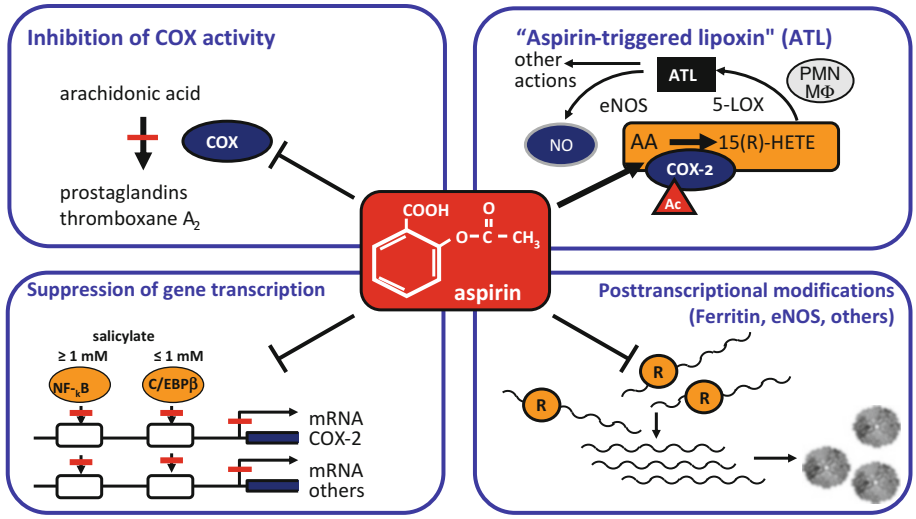


Fig. 7.2 The multiple pharmacological actions of aspirin

Reactive Acetyl Group and Salicylate: Two Synergistically Acting Components of Aspirin

The different pharmacodynamic actions of aspirin require different doses. Antiplatelet doses of aspirin (75–325 mg) result in peak acetylsalicylate levels of 1–3 μg/mL, i.e., about 6–20 μM [6]. There is a clear dose dependency for plasma levels of both, unmetabolized aspirin and salicylate, and an expected doubling of these levels to about 10–12 μg/mL (60–70 μM) at a 1 g aspirin analgesic dose. Because of the longer half-life, concentrations of the salicylate metabolite in plasma are about four- to eightfold higher and will further increase at repeated dosing because of saturation of several capacity-limited phase-II metabolic pathways. This results in a markedly prolonged half-life [7]. In the low millimolar range, i.e., concentrations of 2–5 mM, salicylate will uncouple oxidative phosphorylation with numerous follow-up effects, most importantly nonspecific kinase inhibition [8]. At therapeutic doses, i.e., the low-to-medium μmolar range, the pharmacodynamic effects of aspirin on COX-1 and COX-2, and their biological consequences for pain, fever, and inflammation are primarily acetylation mediated [9]. However, salicylate will contribute at higher doses as evidenced for example by "sweating," i.e., production of extra heat, a typical side effect of anti-inflammatory and antipyretic treatment.

Aspirin and COX-1

The molecular mode of antiplatelet action of aspirin is acetylation of platelet COX-1, resulting in reduced generation of PG-endoperoxides and thromboxane A_2. Salicylate may contribute to these effects by putting the acetyl group into the right position for binding to and acetylation of $serine_{530}$ in the substrate channel of COX-1. Inhibition of thromboxane formation will also eliminate any further autocrine and paracrine actions of thromboxane on cells in the neighborhood. This also includes inhibition of multiple inflammatory actions exerted by non-lipid mediators as outlined in detail elsewhere [10, 11].

COX-2 acetylation at the functionally identical serine ($serine_{512}$) follows a similar pattern. Transacetylation of cyclooxygenases by aspirin is nonselective and is seen in vitro for both enzymes at comparable potency, being complete at about 100 μM. The somewhat higher in vivo concentrations for inhibition of COX-2 are probably due to the enhanced turnover rate of the COX-2 protein—the half-life being a few hours—as opposed, for example, to the COX-1 of blood platelets where the enzyme is apparently stable throughout the lifetime of the platelet. The acetylation of platelet COX-1 by aspirin does not require metabolic conversion and depends on the half-life of unmetabolized aspirin in blood, amounting to 20–30 min. However, the duration of action of aspirin is not determined by the plasma half-life but by the biological half-life of the acetylated target, i.e., a few hours for endothelial cells, a few days for platelets, but about 20 days for albumin. This long protein survival will probably allow for cumulative acetylation with repeated dosing as originally demonstrated for the platelet COX-1 more than 30 years ago [12].

Acetylation by aspirin of the platelet COX-1 has to reduce its enzymatic activity by >95 % in order to inhibit platelet function. The reason is the nonlinearity of the concentration-response curve [13], resembling an all-or-none type of response. Compounds, such as some NSAIDs (ibuprofen, indomethacin) and dipyrone (metamizole), which interact with aspirin (salicylate) binding inside the channel of COX-1 might, therefore, prevent the antiplatelet effect of aspirin because of their about three orders of magnitude higher affinity to the hydrophobic binding sites of arachidonic acid inside the COX-1 channel [14, 15]. Interactions between aspirin and NSAIDs have been shown to have a clinical impact in subjects taking regular ibuprofen or related NSAIDs because of chronic pain who also need to take regular aspirin for prevention of myocardial infarction [16]. Thus, several NSAIDs such as ibuprofen and diclofenac in contrast to aspirin not only bear an increased vascular risk by themselves [17], but might also negatively interact with aspirin.

Aspirin and COX-2: "Aspirin-Triggered Lipoxin"

Acetylation of COX-2 by aspirin reduces COX-2-dependent prostaglandin production. This inhibition in clinical conditions is mostly incomplete [18], but nevertheless associated with biological responses, such as reduced inflammation [19], analgesic [20] and antipyretic effects [21]. Thus, there is no all-or-none reaction type as with the platelet but rather a graded response. Possible explanation for this might be the more rapid turnover rate of the enzyme in nucleated cells as well as the lower peroxide tone [22]. In addition, there might be accumulation of salicylate and an increased proportion of the nonionized form at the acid pH of an inflamed area. This will enhance the anti-inflammatory action of aspirin.

Acetylation of COX-2 by aspirin not only inhibits prostaglandin production but also changes the steric structure of the enzyme and its functionality in direction of a 15-lipoxygenase. This enzyme generates a new product, 15-(R)-HETE, at least tenfold higher amounts than the COX-metabolite PGE_2 [23, 24]. 15-(R)-HETE is the precursor of 15-epi-lipoxin A_4 or "aspirin-triggered lipoxin" (ATL), resulting from synergistic interaction of acetylated COX-2 (15-(R)-HETE) with lipoxygenases from white cells [25]. Lipoxins operate during self-limited acute inflammatory responses that enable the return to homeostasis by resolution of the inflammatory reaction [26]. Interestingly, ATL formation subsequent to aspirin has been shown already at antiplatelet doses of 75 mg/day in man. The result was inhibition of leukocyte accumulation at a local inflammatory site (skin blister). This involved both stimulation of ATL-production as well as local upregulation of lipoxin receptors [27]. Thus, low-dose aspirin is able to interact with the lipoxin system, and this possibly involves both COX-1 (inhibition of thromboxane) and COX-2 (generation of 15-(R)-HETE). These actions differ qualitatively from those of traditional NSAIDs and selective COX-2 inhibitors: Both classes of compounds only competitively, i.e., reversibly, inhibit COX-2 and COX-1 activities and are unable to synthesize lipoxins.

Aspirin, eNOS, Hemeoxygenase-1, and Oxidative Stress

Finally, aspirin has been shown to protect from low-grade inflammation-related oxidative stress via enhanced endothelial NO synthase activity (eNOS) and subsequently enhanced NO production [28]. Mechanistically, this is can be explained by posttranslational lysine acetylation in endothelial cells and platelets [29]. The required concentrations of aspirin are in the low μmolar range, in the in vitro study of Taubert and colleagues between 0.01 and 1 μM, the EC_{50} being 50 nM [28]. Interestingly, the aspirin analog 2-(acetoxyphenyl)hept-2-ynyl sulfide (APHS), a 60-fold more potent and 100-fold more selective COX-2 inhibitor than inhibitor of COX-1 [30], was found to be at least as potent as aspirin. NO stimulates the expression of downstream enzymes, among them hemoxygenase-1 (HO-1), thus improving oxygen defense and suggesting a connection between antithrombotic and anti-inflammatory actions of aspirin [31–33]. Recently, two randomized clinical trials have demonstrated that aspirin (81–1300 mg/day) significantly increased hemoxygenase-1 (HO-1) activity by about 50 % and at the same time reduced asymmetrical dimethylarginine, an inhibitor of NO synthase, by 30 %. Both changes were highly significant and independent of the aspirin doses, suggesting HO-1 as another downstream target of aspirin [34, 35].

Translation of Pharmacodynamics Actions of Aspirin into Clinical Effects

These multiple pharmacodynamic actions of aspirin translate into clinical effects. Most of them are caused by acetylation of COX-1 and COX-2, respectively. At high doses, salicylate will contribute to these actions as well. In addition, aspirin might also affect other mediator systems, for example, in pain control and

inflammation. The following are few examples for this interplay.

Anti-inflammatory Actions

The anti-inflammatory actions of aspirin are mechanistically more complex. They involve acetylation of COX-1 but also COX-2 and inhibition of prostaglandin production, most importantly PGE_2 as a key proinflammatory and pain receptor-sensitizing mediator. Generation of "aspirin-triggered lipoxin" (ATL) will contribute to the anti-inflammatory and inflammation resolving action of aspirin [26]. In this context, enhanced endothelial NO production and improved oxygen defense by upregulation of hemeoxygenase-1 will be important additive effects to preserve endothelial function [36]. Salicylate will contribute to these actions by uncoupling of oxidative phosphorylation, by retarding the inflammatory process, as well as by enhancing the local levels of adenosine, another anti-inflammatory mediator, specifically for inhibition of white cell functions [37, 38]. There is a wide range of effective doses, dependent on the kind of inflammatory response and the affected tissue. Antiplatelet doses were found to be effective in control of ATL formation and other platelet-related inflammatory reactions, including innate immune signaling via inhibition of lipoxin-mediated inhibition of proinflammatory cytokine production [39, 40].

Analgesic Actions

The modes of analgesic actions of aspirin are also complex and involve both peripheral and central mechanisms of pain control. Interestingly, they appear to be less dependent on the nature of the painful stimulus [41]. Nevertheless, inflammatory pain, the most common peripheral pain, is an integrative event because of the multiple pain mediators, present in the "inflammatory soup" [42]. Inhibition of prostaglandin biosynthesis is the key mechanism in the analgesic action of aspirin on inflammatory—and ischemic—pain. Inhibition of both COX-1 and COX-2 appear to be involved as well as ATL formation, at least in inflammatory pain [43]. Inhibition of prostaglandin formation at a site of injury subsequently reduces sensitization of nociceptive nerve terminals and afferent pain signaling. Inhibition of neuronal COX-2 which is upregulated in situations of inflammatory pain [44] might also contribute to reduced nociception.

Central analgesic actions of aspirin involve additional classes of pain mediators. Most interesting are endocannabinoids, such as anandamide, which are high-affinity substrates for COX-2. Interestingly, desensitization of cannabinoid receptors by long-term treatment with cannabis abolished the analgesic action of aspirin in an experimental model of visceral pain, suggesting a tight interaction of aspirin with this endogenous system of pain control [45]. Enhanced serotonin formation in selected areas of the CNS by aspirin with subsequent downregulation of serotonin receptors might also mediate central antinociceptive actions of aspirin, possibly acting in concert with endocannabinoids [46]. These central actions of aspirin are probably of major significance in treatment of headache, i.e., tension-type headache [47] and migraine [48].

Antipyretic Actions

The antipyretic action of aspirin involves several components, for example, inhibition of COX-2 and COX-2-dependent PGE_2 production which are both upregulated by pyrogenic cytokines [49]. In addition, salicylate itself also contributes to the antipyretic action, by uncoupling of oxidative phosphorylation, i.e., heat loss by sweating [50].

Antithrombotic Actions

Inhibition of platelet-derived thromboxane formation is the key explanation for the antithrombotic effects of aspirin in prevention of arterial thromboembolism, such as myocardial infarction and (ischemic) stroke. These actions are most likely due to COX acetylation and do not require any direct effects of salicylate. Therefore, optimum doses are antiplatelet doses of around 75–100 mg/day. As mentioned before, several NSAIDs, most notably ibuprofen, will interact with aspirin binding inside the COX-1 channel and might prevent the antiplatelet effect

of the compound [14] as well as its cardioprotective action [16, 51]. Interestingly, no such effect is seen with diclofenac [52].

Aspirin Pharmacokinetics

Absorption

Stomach

Dissolution of a plain standard aspirin tablet by 50 % in 0.1 N HCl in vitro is quite slow and requires 30–60 min. Thus, the acidic pH in the stomach favors the stability of aspirin and prevents hydrolytic cleavage to the gastric irritant salicylate. Standard doses of 500 mg plain aspirin will result in millimolar concentrations of the compound in 50–100 mL gastric juice. Only about 10 % of a pre-dissolved aspirin is absorbed from/in the stomach. In addition to the poor solubility of the compound at strong acidic pH, another reason is also the small absorption surface of the stomach mucosa amounting to only 0.2–0.3 m^2 or 0.1 % of the resorptive surface of the small intestine. The use of pre-dissolved preparations or water-soluble sodium-salts will improve absorption and increases systemic bioavailability [53]. Similar results are seen with disintegrating preparations [54].

The penetration of plain aspirin into and out of epithelial cells of the stomach mucosa is strongly controlled by luminal pH. As a consequence of the different pH between stomach juice and cytosol of the mucosa cells, there is significant intracellular accumulation with subsequent erosive actions on the mucosa epithelial cells [55, 56]. -pH-dependent distribution kinetics for aspirin between the extra- and intracellular space is not only relevant for the stomach—though it is here most impressive—but is also true for all other compartments of the body, thus as kidney (tubular) epithelial cells but also for local accumulation of salicylates at sites of inflammation with a more acidic pH.

Intestine

Like most other drugs, aspirin is mainly absorbed in the upper intestine by passive diffusion of the nonionized form. The pH in the duodenum is about 2–4 and then increases gradually toward 7–8 in the distal small intestine and colon. The large surface of the (small) intestine, amounting to 100–200 m^2, as well as the steadily and markedly increasing solubility of aspirin with increasing pH finally results in a complete intestinal absorption of the compound, despite of a higher proportion of the dissociated, ionized fraction.

Bioavailability

There is a significant "first-pass" metabolism of aspirin to salicylate during intestinal uptake and subsequent passage to the liver [57, 58]. Thus, the duration of passage through the intestine, i.e., the duration of exposition of aspirin to esterases of the intestinal wall and presystemic circulation, is critical for systemic bioavailability of the uncleaved compound. These factors are less important for the bioavailability of the primary metabolite salicylic acid.

Distribution Volume

The apparent distribution volume of salicylates is dose dependent. At antiplatelet and analgesic doses, it amounts to about 0.2 L/kg. This is equivalent to a predominant distribution in the extracellular space, probably because of the 80–95 % high-affinity (k_D: 25 μM) binding to plasma albumin This does not affect the pharmacodynamic potency of aspirin-induced acetylation as assessed from COX inhibition, but reduces that of salicylate by about one order of magnitude [59]. At high aspirin doses or salicylate poisoning, the apparent distribution volume is increased to about 0.5 L/kg. This is due to the saturation of salicylate-binding sites to plasma albumin, subsequent diffusion of salicylate into the intracellular space, and increasing binding to tissue proteins with falling tissue pH. These events are additionally enhanced by saturation of phase-II metabolic pathways of salicylate.

Biotransformation

Aspirin hydrolysis after oral intake starts already in the stomach mucosa and continues in the

intestinal mucosa, portal vein blood, and liver. The deacetylation process follows a dose-independent, zero-order kinetics and reduces the systemic bioavailability of standard plain aspirin to about 50 % at single doses between 40-1300 mg [57, 58, 60, 61]. This applies for standard preparations of plain aspirin but not for formulations with delayed release, e.g., enteric-coated formulations.

Aspirin Esterases

There are (at least) three "aspirin" esterases in the systemic circulation that hydrolyze aspirin to acetate and salicylic acid: the aspirin esterase of red cells and the two aspirin esterases of plasma [62]. The red cell enzyme activity explains the much faster aspirin hydrolysis in vitro in whole blood as opposed to plasma. The aspirin esterase activity in plasma is due to two different enzymes: butyrylcholinesterase (pseudo-cholinesterase) [63] and a recently detected homomeric platelet activating factor acetylhydrolase (PAFAH1b2). Overall, changes in aspirin esterase activity appear not to have a major impact for the analgesic/anti-inflammatory actions of standard aspirin tablets. This is not surprising, since in these indications, aspirin and salicylate act synergistically and both inhibit COX-2, though at different pharmacological potencies in vivo.

Salicylate is the primary metabolite of aspirin. Its biotransformations involve the generation of multiple products. The spectrum of metabolites is dose dependent as is the excretion rate. The speed of renal excretion ultimately determines the plasma level and half-life of salicylate. This amounts to about 3 h at analgesic doses [64]. The major product formed from salicylic acid via conjugation with glycine is salicyluric acid. Salicylic acid can also be conjugated with glucuronic acid to form acyl and phenolic glucuronides, respectively. Glucuronidation of salicylate occurs via polymorphic UDP-glucuronosyltransferases (UGT) [65]. The variant UGT1A6*2 may confer more rapid glucuronidation of salicylic acid than the wild-type UGT1A6 *1/*1, allowing for faster excretion [66]. These genomic variations in enzyme expression have been brought into connection with the chemopreventive effect of aspirin in colorectal cancer since the UGT1A6 genotype was found to strongly increase the risk of colorectal cancer [67]. The formation of the main metabolite salicyluric acid by conjugation with glycine is capacity limited and becomes saturated already at doses of >300 mg [68]. Higher doses lead to accumulation of salicylate because of marked prolongation of the half-life which may increase up to 20 h and more in case of salicylate poisoning. Possible reason are depletion of the glycine pool in the liver and depletion of ATP-levels, i.e., reduced formation of "activated" salicylic acid because of uncoupling of oxidative phosphorylation [69, 70]. There is also an increased proportion of free salicylic acid because of reduced albumin binding at higher plasma level. Thus, salicylate can easily penetrate into tissues, in particular at the acidic pH during inflammation and/or intoxication with metabolic acidosis.

Elimination

The elimination of aspirin occurs completely (>98 %) as salicylic acid and salicylic acid metabolites in urine. Similar to biotransformations, the excretion of salicylates is also largely dose dependent. After saturation of the major metabolic routes, unchanged salicylate becomes also the main metabolite in urine. At a single dose of 0.5–1 g aspirin, the approximate recovery rates of salicylate and its metabolites in urine are as follows: 70–75 % salicyluric acid, including glucuron-conjugated products, 10 % salicylic acid, 1–2 % gentisic acid, and <1 % gentisuric acid [70–72]. The interindividual pattern of salicylate metabolites is highly variable whereas the intraindividual variation is low and is probably genetically defined [73].

Aspirin Formulations for Inflammation, Pain, and Fever

General Aspects

Aspirin was first synthesized in 1897 and sold as a tablet for many decades for conditions of inflammation, pain, and fever. At single doses of 500–1000 mg and daily doses of up to 4000 mg, it is used in self-medication to symptomatically treat acute mild to moderate pain, such as tension-type headache [74–76], migraine headache [48, 77–82], sore throat [83], primary dysmenorrhea [84], and dental pain [85–88], as well as fever [89]. Over the years, various pharmaceutical formulations have been developed. These formulations include plain tablets, disintegrating tablets, chewable tablets, effervescent tablets, granules, and granules in suspension. All these different pharmaceutical forms have particular pharmacokinetic characteristics and provide choices for patients. These galenic preparations impact the pharmacokinetic and pharmacodynamic actions of aspirin for the given indication and therefore have an impact on efficacy and safety. For instance, modifications to a plain tablet by increasing the rate of tablet dissolution can reduce the time of absorption and therefore shorten the time to onset of a clinical effect [54, 90].

Whereas published product-specific pharmacokinetic information on aspirin products for pain and fever is limited, the originator of aspirin-containing products, Bayer AG, Germany, owns a clinical trial database containing numerous reports of the pharmacokinetics of various aspirin formulations. Although in a few countries aspirin products with a dose of 300–325 mg are available, globally the dose of 500 mg is the most important and mostly investigated aspirin strength used for the treatment pain and fever.

Specific Bioavailability for Pain and Fever Formulations

Important parameters for the treatment of pain and fever typically include bioavailability (total exposure of drug measured as area under the plasma concentration time curve [AUC]), maximum plasma concentration (C_{max}), and time to reach maximum plasma concentration (T_{max}). Tables 7.1 and 7.2 summarize the

Table 7.1 Summary pharmacokinetic data of acetylsalicylic acid for 500 mg dose-strength aspirin formulations [90]

Formulation	No. of studies	C_{max} (mg/L)	AUC (mg × h/L)	T_{max} (h)
Aspirin tablet	10	Mean: 5.43 ± 1.38	Mean: 6.21 ± 1.24	Median: 0.50 ± 0.16
		95 % CI: 4.66–6.21	95 % CI: 5.51–6.91	95 % CI: 0.40–0.60
		Median: 5.69 ± 1.38	Median: 6.22 ± 1.24	
Aspirin effervescent tablet	3	Mean: 10.45 ± 1.18	Mean: 5.27 ± 0.51	Median: 0.33 ± 0.02
		95 % CI: 9.12–11.78	95 % CI: 4.69–5.85	95 % CI: 0.30–0.36
		Median: 11.08 ± 1.18	Median: 5.31 ± 0.51	
Aspirin granules	6	Mean: 5.42 ± 1.03	Mean: 6.18 ± 1.36	Median: 0.46 ± 0.13
		95 % CI: 4.59–6.25	95 % CI: 5.09–7.27	95 % CI: 0.36–0.56
		Median: 5.48 ± 1.03	Median: 5.97 ± 1.36	
Aspirin granules in suspension	6	Mean: 12.77 ± 1.94	Mean: 6.77 ± 1.63	Median: 0.25 ± 0.04
		95 % CI: 11.43–14.11	95 % CI: 5.64–7.90	95 % CI: 0.22–0.28
		Median: 12.69 ± 1.94	Median: 6.02 ± 1.63	
Aspirin disintegrating tablet	6	Mean: 13.89 ± 1.08	Mean: 6.95 ± 0.67	Median: 0.30 ± 0.02
		95 % CI: 13.03–14.77	95 % CI: 6.42–7.48	95 % CI: 0.28–0.32
		Median: 13.75 ± 1.08	Median: 6.84 ± 0.66	
Aspirin chewable tablet	2	Mean: 6.25 ± 0.24	Mean: 4.67 ± 0.03	Median: 0.33 ± 0
		95 % CI: 5.92–6.58	95 % CI: 4.63–4.71	95 % CI: n/a
		Median: 6.25 ± 0.24	Median: 4.67 ± 0.03	

Table 7.2 Summary pharmacokinetic data of salicylic acid for 500 mg dose-strength aspirin formulations [90]

Formulation	No. of studies	C_{max} (mg/L)	AUC (mg × h/L)	T_{max} (h)
Aspirin tablet	10	Mean: 25.45 ± 3.64	Mean: 145.67 ± 35.24	Median: 2.00 ± 0.54
		95 % CI: 23.19–27.71	95 % CI:123.82–167.50	95 % CI: 1.66–2.34
		Median: 25.56 ± 3.64	Median: 125.76 ± 35.24	
Aspirin effervescent tablet	3	Mean: 27.54 ± 1.18	Mean: 138.07 ± 8.89	Median: 0.75 ± 0.05
		95 % CI: 26.20–28.88	95 % CI: 128.00–148.13	95 % CI: 0.70–0.80
		Median: 27.31 ± 1.18	Median: 141.90 ± 8.89	
Aspirin granules	6	Mean: 25.51 ± 4.59	Mean: 158.4 ± 50.50	Median: 2.00 ± 0.54
		95 % CI: 21.84–29.18	95 % CI: 118.00–198.80	95 % CI: 1.56–2.44
		Median: 25.50 ± 4.59	Median: 156.00 ± 50.50	
Aspirin granules in suspension	6	Mean: 29.08 ± 2.66	Mean: 132.54 ± 16.22	Median: 0.83 ± 0.15
		95 % CI: 27.31–31.95	95 % CI: 121.30–143.78	95 % CI: 0.72–0.94
		Median: 29.64 ± 2.66	Median: 133.51 ± 16.22	
Aspirin disintegrating tablet	6	Mean: 31.80 ± 1.81	Mean: 179.07 ± 15.27	Median: 0.75 ± 0.05
		95 % CI: 30.35–33.25	95 % CI: 166.85–191.29	95 % CI: 0.71–0.79
		Median: 31.00 ± 1.81	Median: 182.15 ± 15.27	
Aspirin chewable tablet	2	Mean: 23.24 ± 1.17	Mean: 123.18 ± 0.24	Median: 1.25 ± 0
		95 % CI: 21.62–24.86	95 % CI: 122.85–123.51	95 % CI: n/a
		Median: 23.24 ± 1.17	Median: 123.18 ± 0.24	

pharmacokinetics of acetylsalicylic acid and salicylic acid of currently available aspirin 500 mg formulations manufactured by Bayer AG, Germany [91]. The data were extracted from the individual study reports for each study and cover a range of over a decade.

Overall the various aspirin 500 mg formulations display are relatively narrow range of AUC lying generally between 5 and 7 mg × h/L for acetylsalicylic acid and between 125 and 180 mg × h/L for salicylic acid (Fig. 7.3). Consequently the AUC can be considered as being not dependent on the formulation. This is reflecting the high bioavailability of aspirin.

Time to maximum plasma concentration (T_{max}) provides the information when the concentration of the active ingredient reaches its peak. Figure 7.4 reveals a substantial effect of the formulation on T_{max}. Formulations with pre-dissolved aspirin (effervescent tablet and granules in suspension) and disintegrating tablets have a much lower T_{max} than solid formulation tablet and dry granules (overall approximately 0.25–0.35 h versus 0.45–0.50 h for acetylsalicylic acid and approximately 0.75– 1;0.85 h versus 2.0 h). Pre-dissolved aspirin formulations or formulations which are quickly provided in a soluble form in the stomach may shorten the pharmaceutical phase and consequently reduce T_{max}.

The pattern seen for T_{max} is inversely correlated with the maximum plasma concentration (C_{max}). Formulations with lower T_{max} show higher C_{max} than formulations with longer T_{max}. This effect is more pronounced for acetylsalicylic acid (tablet and granules approximately 5.4 μg/mL versus >10 μg/mL for soluble and disintegrating formulations) than for salicylic acid (tablets and granules approximately 25 mg/mL versus 27.5–32.0 μg/mL for soluble and disintegrating formulations) (Fig. 7.5).

Generally, it can be concluded that aspirin formulations at doses used for treatment of pain and fever have a major impact on T_{max} and $C_{max.}$ A decrease in T_{max} is correlated with an increase in C_{max}. These parameters will have clinical consequences since they determine the efficacy and safety of aspirin formulations. Rapidly bioavailable formulations such as disintegrating and effervescent tablets might be the formulation of choice to manage acute painful conditions, where

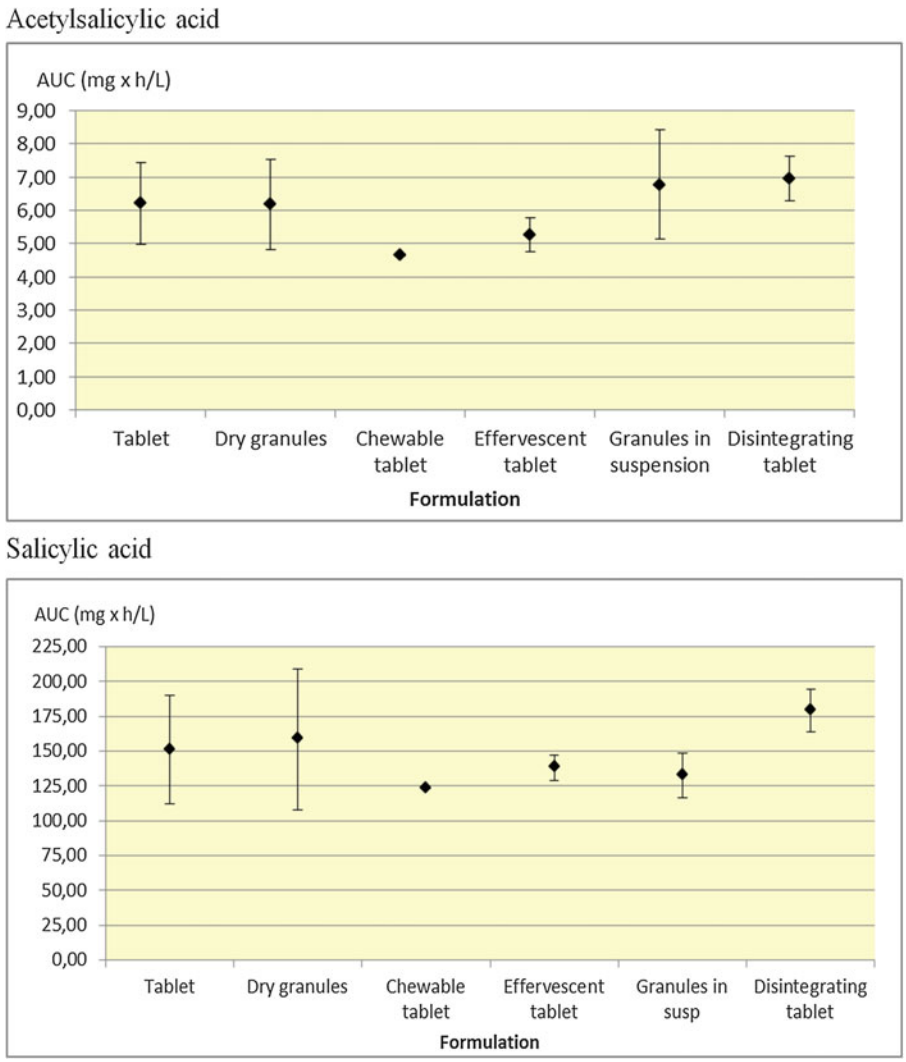


Fig. 7.3 Summary of acetylsalicylic acid and salicylic acid AUC for aspirin 500 mg formulations (Mean ± SD) [90]

rapid onset of action is desirable. For example, for a disintegrating aspirin, 500 mg tablet has been shown that the reduction of the time to maximum plasma concentration leads to a two-fold faster onset of action in a model of acute pain [54, 88]. The pharmacokinetics and the use of this formulation for primary headaches has been reviewed in an expert opinion by Lecchi et al. and recommended as first-line therapy [91].

Muir et al. [92] found similar pharmacokinetic differences when he compared 600 mg aspirin plain tablet with 600 mg aspirin soluble formulation. The C_{max} was 5.23 μg/mL and 10.79 μg/mL, respectively, with a T_{max} of 0.68 h and 0.26 h, respectively. The AUC was similar for both dosage forms (6.49 and 6.83 μg × h/mL). This was also seen in another study by Muir et al. [93] compared 600 mg of soluble aspirin with 600 mg of mouth-dispersible aspirin and 650 mg of plain aspirin. T_{max} increased from 20.5 min to 28.3 min and 60.4 min, whereas C_{max} decreased from 13.82 to 5.66 μg/mL and 5.51 μg/mL. Pharmacokinetic differences for various formulations with a dose-strength of 600 and 650 mg have also been described by Sagar et al. favoring the soluble formulation

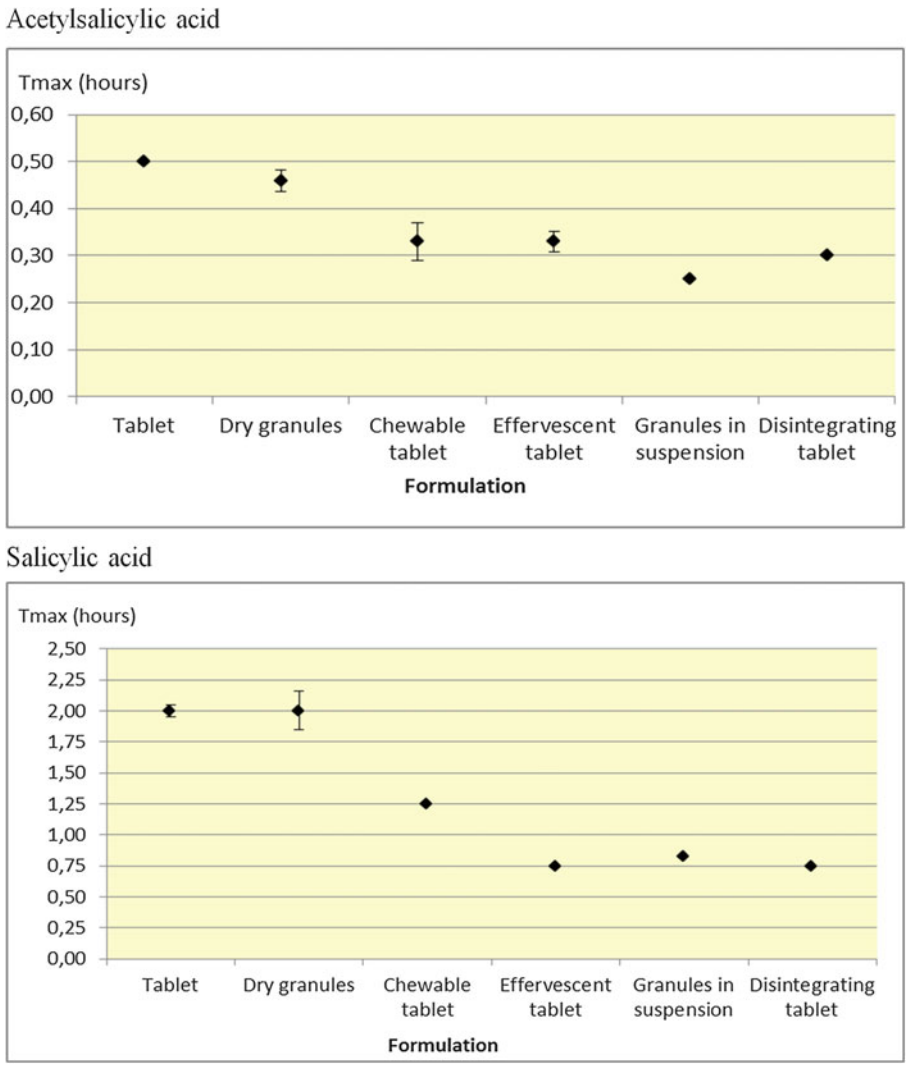


Fig. 7.4 Summary of acetylsalicylic acid and salicylic acid T_{max} for aspirin 500 mg formulations (Median ± SD) [90]

[94]. The absolute pharmacokinetic data of the two Muir studies, the Sagar study and additionally the pharmacokinetic data for a 600 mg dose of aspirin investigated by Brandon et al. [95], are consistent with the data presented above for the 500 mg dose considering the 20 % dose increase. Brandon found a C_{max} of 7.6 mg/L for acetylsalicylic acid and 40.2 mg/L for salicylic acid. The T_{max} was 0.59 h and 1.75 h, respectively, and the AUC 8.53 mg × h/L and 293 mg × h/L, respectively. These findings further illustrate the clinical importance of availability of different aspirin formulations.

Pharmacokinetic studies are usually done in the fasted state. However, another important factor for aspirin's pharmacokinetic is the impact of the fed state. Stillings et al. [96] investigated 900 mg of soluble aspirin in the fed and the fasted state. The overall bioavailability of soluble aspirin was unaffected by food, whereas for maximum concentration, a decrease was observed. AUC was 7.99 µg × h/mL in the fasted state and 8.47 µg × h/mL in the fed state; C_{max} was 16.8 µg/mL in fasted state and 13.7 µg/mL in the fed state. The 18 % decrease in C_{max} corresponds with a rate of absorption affected by food as

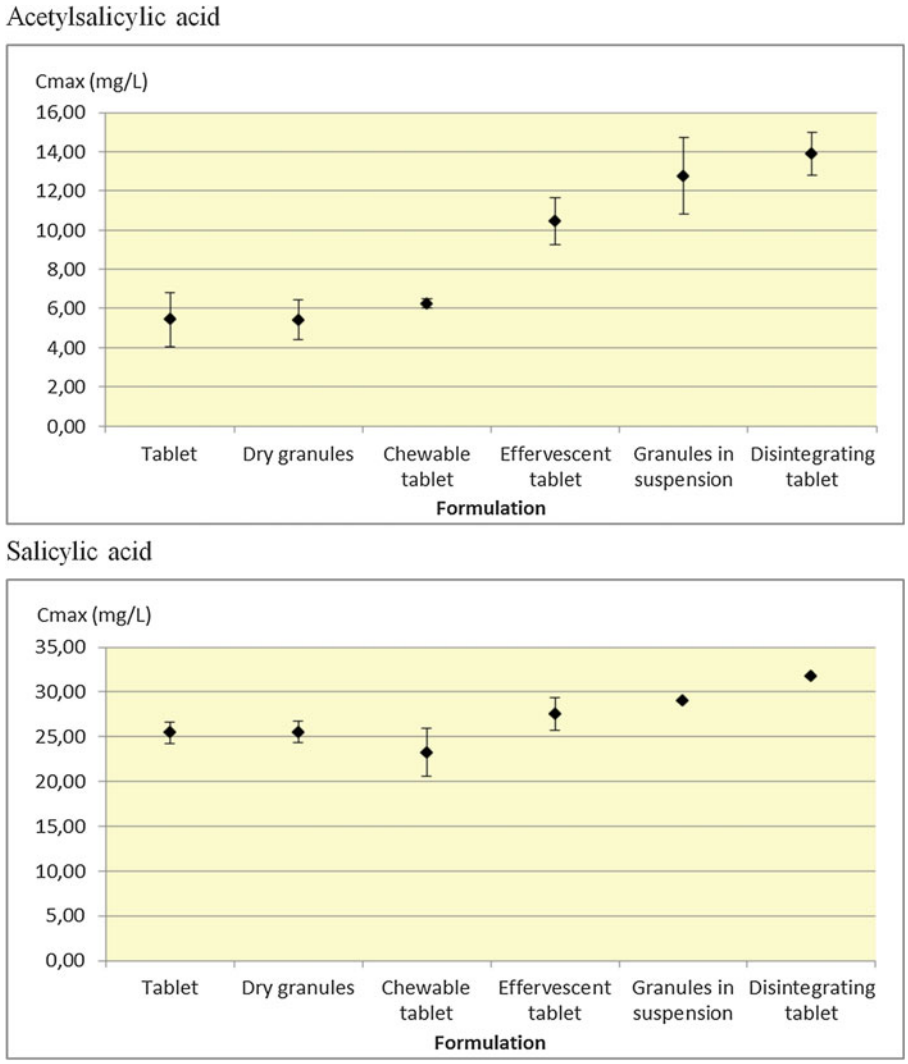


Fig. 7.5 Summary of acetylsalicylic acid and salicylic acid C_{max} for aspirin 500 mg formulations (Mean ± SD) [90]

shown by the higher T_{max} for the fed state (0.50 h) compared to the fasted state (0.33 h). Unchanged overall bioavailability and decrease in C_{max} was also observed in an unpublished study investigating the pharmacokinetics of 500 mg disintegrating aspirin administered in the fasted and the fed state [97]. AUC was 7.25 µg × h/mL and 6.73 µg × h/mL in the fasted and fed state, respectively; C_{max} was 14.76 and 6.33 µg/mL. However, the change in T_{max} was less affected by food in this study (T_{max} fasted versus fed = 0.340 h versus 0.385 h). To further support that food does not affect the extent of absorption, both studies calculated the 90 % confidence interval of the point estimate for the fed-fasting ratio for AUC. This was 0.96–1.17 (fed-fasting) for the study of Stillings and colleagues [96] and 1.04–1.13 (fasting-fed) for the Bayer study and fell entirely within the conventional bioequivalence interval (range 0.8–1.25).

In a few countries, an intravenously administered 1000 mg D,L-lysine-aspirin glycine equivalent to 500 mg acetylsalicylic acid is available to treat acute moderate to severe pain and acute headache of migraine attacks and fever. The product is a white powder to be reconstituted

with water for injection prior to intravenous administration releasing aspirin directly into the systemic circulation and bypass the liver and other sites of aspirin esterases. Nagelschmitz et al. compared the pharmacokinetics and pharmacodynamics of D,L-lysine acetylsalicylate glycine to aspirin 500 mg oral tablets [6]. For acetylsalicylic acid, AUC and C_{max} was much higher for the intravenous formulation (AUC: 10.3 versus 5.12 mg × h/L; C_{max}: 54.2 versus 4.8 mg/L). The median T_{max} was 0.017 h versus 0.5 h. The corresponding data for salicylic acid are AUC 98.5 versus 126 mg × h/L; C_{max} 21.58 versus 22.85 mg/L; T_{max} 0.667 versus 1.5 h. It is obvious that intravenous aspirin leads to higher acetylsalicylic acid AUC and C_{max} and to lower T_{max} than orally administered formulations, but that for salicylic acid AUC and C_{max} differences are diminishing, whereas for T_{max} a difference is still present. This formulation is of particular interest in clinical situations where immediate pharmacodynamic action is required, e.g., acute coronary syndrome [6, 98]; oral administration is not indicated, e.g., because of unconsciousness, difficulties in swallowing, and impaired absorption in acute myocardial infarction [6] and because of vomiting in migraine [99–102] or in severe migraine attacks to be treated in the emergency setting [99–102].

References

1. Dreser H. Pharmakologisches über Aspirin (Acetylsalizylsäure). Pflügers Arch Physiol. 1899;76:306–18.
2. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol. 1971;231(25):232–5.
3. Roth GJ, Stanford N, Majerus PW. Acetylation of prostaglandin synthase by aspirin. Proc Natl Acad Sci U S A. 1975;72(8):3073–6.
4. Bateman LA, et al. An alkyne-aspirin chemical reporter for the detection of aspirin-dependent protein modification in living cells. J Am Chem Soc. 2013;135(39):14568–73.
5. Wang J, et al. Mapping sites of aspirin-induced acetylations in live cells by quantitative acid-cleavable activity-based protein profiling (QA-ABPP). Scientific Reports. 2015;5.
6. Nagelschmitz J, Blunk M, Krätschmar J, et al. Pharmacokinetics and pharmacodynamics of acetylsalicylic acid after intravenous and oral administration to healthy volunteers. Clin Pharmacol. 2013;5:1–9.
7. Shen J, et al. Model representation of salicylate pharmacokinetics using unbound plasma salicylate concentrations and metabolite urinary excretion rates following a single oral dose. J Pharmacokinet Biopharm. 1991;19(5):575–95.
8. Frantz B, O'Neill EA. The effect of sodium salicylate and aspirin on NF-kappa B. Science. 1995;270 (5244):2017–9.
9. Schrör K, Rauch BH. Aspirin and lipid mediators in the cardiovascular system. Prostaglandins Other Lipid Mediat. 2015;121(Pt A):17–23.
10. Weyrich AS, Lindemann S, Zimmerman GA. The evolving role of platelets in inflammation. J Thromb Haemost. 2003;1(9):1897–905.
11. Hohlfeld T, Schrör K. Antiinflammatory effects of aspirin in ACS: relevant to its cardiocoronary actions? Thrombosis Haemost. 2015;114:469–77.
12. Patrignani P, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J Clin Invest. 1982;69(6):1366–72.
13. Reilly IA, FitzGerald GA. Inhibition of thromboxane formation in vivo and ex vivo: implications for therapy with platelet inhibitory drugs. Blood. 1987;69 (1):180–6.
14. Catella-Lawson F, et al. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. N Engl J Med. 2001;345(25):1809–17.
15. Saxena A, et al. Drug/drug interaction of common NSAIDs with antiplatelet effect of aspirin in human platelets. Eur J Pharmacol. 2013;721 (1-3):215–24.
16. Hennekens CH, et al. Hypothesis formulation from subgroup analyses: nonadherence or nonsteroidal anti-inflammatory drug use explains the lack of clinical benefit of aspirin on first myocardial infarction attributed to "aspirin resistance". Am Heart J. 2010;159(5):744–8.
17. Bhala N, et al. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. Lancet. 2013;382 (9894):769–79.
18. FitzGerald GA, et al. Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. J Clin Invest. 1983;71(3):676–88.
19. Higgs GA, et al. Pharmacokinetics of aspirin and salicylate in relation to inhibition of arachidonate cyclooxygenase and antiinflammatory activity. Proc Natl Acad Sci U S A. 1987;84(5):1417–20.
20. Hersh EV, Moore PA, Ross GL. Over-the-counter analgesics and antipyretics: a critical assessment. Clin Ther. 2000;22(5):500–48.

21. Schwartz JI, et al. Cyclooxygenase-2 inhibition by rofecoxib reverses naturally occurring fever in humans. Clin Pharmacol Ther. 1999;65(6):653–60.
22. Aronoff DM, et al. Inhibition of prostaglandin H2 synthases by salicylate is dependent on the oxidative state of the enzymes. J Pharmacol Exp Ther. 2003;304(2):589–95.
23. Mancini JA, et al. Mutation of serine-516 in human prostaglandin G/H synthase-2 to methionine or aspirin acetylation of this residue stimulates 15-R-HETE synthesis. FEBS Lett. 1994;342 (1):33–7.
24. Lecomte M, et al. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. J Biol Chem. 1994;269(18):13207–15.
25. Claria J, Serhan CN. Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. Proc Natl Acad Sci U S A. 1995;92(21):9475–9.
26. Spite M, Serhan CN. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. Circ Res. 2010;107(10):1170–84.
27. Morris T, et al. Effects of low-dose aspirin on acute inflammatory responses in humans. J Immunol. 2009;183(3):2089–96.
28. Taubert D, et al. Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action. Br J Pharmacol. 2004;143(1):159–65.
29. Jung SB, et al. Histone deacetylase 3 antagonizes aspirin-stimulated endothelial nitric oxide production by reversing aspirin-induced lysine acetylation of endothelial nitric oxide synthase. Circ Res. 2010;107(7):877–87.
30. Kalgutkar AS, et al. Aspirin-like molecules that covalently inactivate cyclooxygenase-2. Science. 1998;280(5367):1268–70.
31. Grosser N, et al. Heme oxygenase-1 induction may explain the antioxidant profile of aspirin. Biochem Biophys Res Commun. 2003;308(4):956–60.
32. Grosser N, Schröder H. Aspirin protects endothelial cells from oxidant damage via the nitric oxide-cGMP pathway. Arterioscler Thromb Vasc Biol. 2003;23(8):1345–51.
33. Nascimento-Silva V, et al. Novel lipid mediator aspirin-triggered lipoxin A4 induces heme oxygenase-1 in endothelial cells. Am J Physiol Cell Physiol. 2005;289(3):C557–63.
34. Hennekens CH, et al. A randomized trial of aspirin at clinically relevant doses and nitric oxide formation in humans. J Cardiovasc Pharmacol Ther. 2010;15 (4):344–8.
35. Hetzel S, et al. Aspirin increases nitric oxide formation in chronic stable coronary disease. J Cardiovasc Pharmacol Ther. 2013;18(3):217–21.
36. Hennekens CH. A randomized trial of aspirin at clinically relevant doses and nitric oxide formation in humans. J Cardiovasc Pharmacol. 2010;15.
37. Cronstein BN, Montesinos MC, Weissmann G. Salicylates and sulfasalazine, but not glucocorticoids, inhibit leukocyte accumulation by an adenosine-dependent mechanism that is independent of inhibition of prostaglandin synthesis and p105 of NFkappaB. Proc Natl Acad Sci U S A. 1999;96 (11):6377–81.
38. Hasko G, et al. Adenosine receptors: therapeutic aspects for inflammatory and immune diseases. Nat Rev Drug Discov. 2008;7(9):759–70.
39. O'Brien M, et al. Aspirin attenuates platelet activation and immune activation in HIV-1-infected subjects on antiretroviral therapy: a pilot study. J Acquir Immune Defic Syndr. 2013;63(3):280–8.
40. Chiang N, et al. Aspirin triggers antiinflammatory 15-epi-lipoxin A4 and inhibits thromboxane in a randomized human trial. Proc Natl Acad Sci U S A. 2004;101(42):15178–83.
41. Edwards JE, et al. Oral aspirin in postoperative pain: a quantitative systematic review. Pain. 1999;81 (3):289–97.
42. Scholz J, Woolf CJ. Can we conquer pain? Nat Neurosci. 2002;5(Suppl):1062–7.
43. Svensson CI, Zattoni M, Serhan CN. Lipoxins and aspirin-triggered lipoxin inhibit inflammatory pain processing. J Exp Med. 2007;204(2):245–52.
44. Vardeh D, et al. COX2 in CNS neural cells mediates mechanical inflammatory pain hypersensitivity in mice. J Clin Invest. 2009;119(2):287–94.
45. Anikwue R, et al. Decrease in efficacy and potency of nonsteroidal anti-inflammatory drugs by chronic delta(9)-tetrahydrocannabinol administration. J Pharmacol Exp Ther. 2002;303(1):340–6.
46. Ruggieri V, et al. The antinociceptive effect of acetylsalicylic acid is differently affected by a CB1 agonist or antagonist and involves the serotonergic system in rats. Life Sci. 2010;86(13-14):510–7.
47. Göbel H, et al. Acetylsalicylic acid activates antinociceptive brain-stem reflex activity in headache patients and in healthy subjects. Pain. 1992;48 (2):187–95.
48. Diener HC, et al. Aspirin in the treatment of acute migraine attacks. Expert Rev Neurother. 2006;6 (4):563–73.
49. Adler RD, et al. The effect of salicylate on pyrogen-induced fever in man. Clin Sci. 1969;37(1):91–7.
50. Rosendorff C, Cranston WI. Effects of salicylate on human temperature regulation. Clin Sci. 1968;35 (1):81–91.
51. MacDonald TM, Wei L. Effect of ibuprofen on cardioprotective effect of aspirin. Lancet. 2003;361 (9357):573–4.
52. Hohlfeld T, Saxena A, Schror K. High on treatment platelet reactivity against aspirin by non-steroidal anti-inflammatory drugs--pharmacological mechanisms and clinical relevance. Thromb Haemost. 2013;109(5):825–33.

53. Mason WD, Winer N. Influence of food on aspirin absorption from tablets and buffered solutions. J Pharm Sci. 1983;72(7):819–21.
54. Voelker M, Hammer M. Dissolution and pharmacokinetics of a novel micronized aspirin formulation. Inflammopharmacology. 2012;20(4):225–31.
55. Graham DY, Smith JL. Aspirin and the stomach. Ann Intern Med. 1986;104(3):390–8.
56. Cryer B, Feldman M. Effects of very low dose daily, long-term aspirin therapy on gastric, duodenal, and rectal prostaglandin levels and on mucosal injury in healthy humans. Gastroenterology. 1999;117 (1):17–25.
57. Rowland M, et al. Absorption kinetics of aspirin in man following oral administration of an aqueous solution. J Pharm Sci. 1972;61(3):379–85.
58. Rowland M, et al. Kinetics of acetylsalicylic acid disposition in man. Nature. 1967;215(5099):413–4.
59. Warner TD, et al. Influence of plasma protein on the potencies of inhibitors of cyclooxygenase-1 and -2. FASEB J. 2006;20(3):542–4.
60. Harris PA, Riegelman S. Influence of the route of administration on the area under the plasma concentration-time curve. J Pharm Sci. 1969;58 (1):71–5.
61. Pedersen AK, FitzGerald GA. Dose-related kinetics of aspirin. Presystemic acetylation of platelet cyclooxygenase. N Engl J Med. 1984;311 (19):1206–11.
62. Zhou Y, Boudreau DM, Freedman AN. Trends in the use of aspirin and nonsteroidal anti-inflammatory drugs in the general U.S. population. Pharmacoepidemiol Drug Saf. 2013;23(1):43–50.
63. Adebayo GI, Williams J, Healy S. Aspirin esterase activity—evidence for skewed distribution in healthy volunteers. Eur J Intern Med. 2007;18 (4):299–303.
64. Rowland M, Riegelman S. Pharmacokinetics of acetylsalicylic acid and salicylic acid after intravenous administration in man. J Pharm Sci. 1968;57:1313–9.
65. Kuehl GE, et al. Glucuronidation of the aspirin metabolite salicylic acid by expressed UDP-glucuronosyltransferases and human liver microsomes. Drug Metab Dispos. 2006;34 (2):199–202.
66. Chen Y, et al. UGT1A6 polymorphism and salicylic acid glucuronidation following aspirin. Pharmacogenet Genomics. 2007;17(8):571–9.
67. Osawa K, et al. Association between polymorphisms in UDP-glucuronosyltransferase 1A6 and 1A7 and colorectal cancer risk. Asian Pac J Cancer Prev. 2012;13(5):2311–4.
68. Bedford C, Cummings AJ, Martin BK. A kinetic study of the elimination of salicylate in man. Br J Pharmacol Chemother. 1965;24:418–31.
69. Notarianni LJ, Ogunbona FA, Oldham HG. Glycine conjugation of salicylic acid after aspirin overdose. Br J Clin Pharmacol. 1983;15.
70. Forman WB, Davidson ED, Webster Jr LT. Enzymatic conversion of salicylate to salicylurate. Mol Pharmacol. 1971;7(3):247–59.
71. Wilson JT, et al. Gentisuric acid: metabolic formation in animals and identification as a metabolite of aspirin in man. Clin Pharmacol Ther. 1978;23 (6):635–43.
72. Cham BE, et al. Simultaneous liquid-chromatographic quantitation of salicylic acid, salicyluric acid, and gentisic acid in urine. Clin Chem. 1980;26(1):111–4.
73. Caldwell J, Gorman JO', Smith RL. Inter-individual differences in the glycine conjugation of salicylic acid [proceedings]. Br J Clin Pharmacol, 1980. 9 (1): 114p.
74. Steiner TJ, Lange R, Voelker M. Aspirin in episodic tension-type headache: placebo-controlled dose-ranging comparison with paracetamol. Cephalalgia. 2003;23(1):59–66.
75. Martinez-Martin P, et al. Efficacy and safety of metamizol vs. acetylsalicylic acid in patients with moderate episodic tension-type headache: a randomized, double-blind, placebo- and active-controlled, multicentre study. Cephalalgia. 2001;21 (5):604–10.
76. Gatoulis SC, Voelker M, Fisher M. Assessment of the efficacy and safety profiles of aspirin and acetaminophen with codeine: results from 2 randomized, controlled trials in individuals with tension-type headache and postoperative dental pain. Clin Ther. 2012;34(1):138–48.
77. MacGregor EA, Dowson A, Davies PT. Mouth-dispersible aspirin in the treatment of migraine: a placebo-controlled study. Headache. 2002;42 (4):249–55.
78. Lange R, Schwarz JA, Hohn M. Acetylsalicylic acid effervescent 1000 mg (Aspirin) in acute migraine attacks; a multicentre, randomized, double-blind, single-dose, placebo-controlled parallel group study. Cephalalgia. 2000;20(7):663–7.
79. Diener HC, et al. Placebo-controlled comparison of effervescent acetylsalicylic acid, sumatriptan and ibuprofen in the treatment of migraine attacks. Cephalalgia. 2004;24(11):947–54.
80. Diener HC, et al. Efficacy of 1,000 mg effervescent acetylsalicylic acid and sumatriptan in treating associated migraine symptoms. Eur Neurol. 2004;52(1):50–6.
81. Lipton RB, et al. Aspirin is efficacious for the treatment of acute migraine. Headache. 2005;45(4):283–92.
82. Lampl C, Voelker M, Diener HC. Efficacy and safety of 1,000 mg effervescent aspirin: individual patient data meta-analysis of three trials in migraine headache and migraine accompanying symptoms. J Neurol. 2007;254(6):705–12.
83. Eccles R, et al. Effects of acetylsalicylic acid on sore throat pain and other pain symptoms associated with acute upper respiratory tract infection. Pain Med. 2003;4(2):118–24.

84. Zhang WY, Li Wan A. Po, Efficacy of minor analgesics in primary dysmenorrhoea: a systematic review. Br J Obstet Gynaecol. 1998;105(7):780–9.
85. Forbes JA, et al. Evaluation of an ibuprofen controlled-release tablet and placebo in postoperative oral surgery pain. Pharmacotherapy. 1991;11(3):242–8.
86. Forbes JA, et al. Analgesic efficacy of bromfenac, ibuprofen, and aspirin in postoperative oral surgery pain. Clin Pharmacol Ther. 1992;51(3):343–52.
87. Cooper SA. Comparative analgesic efficacies of aspirin and acetaminophen. Arch Intern Med. 1981;141(3):282–5.
88. Cooper SA, Voelker M. Evaluation of onset of pain relief from micronized aspirin in a dental pain model. Inflammopharmacology. 2012;20(4):233–42.
89. Bachert C, et al. Aspirin compared with acetaminophen in the treatment of fever and other symptoms of upper respiratory tract infection in adults: a multicenter, randomized, double-blind, double-dummy, placebo-controlled, parallel-group, single-dose, 6-hour dose-ranging study. Clin Ther. 2005;27 (7):993–1003.
90. Kanani K, Gatoulis SC, Voelker M. Influence of differing analgesic formulations of aspirin on pharmacokinetic parameters. Pharmaceutics. 2015;7 (3):188–98.
91. Lecchi M, et al. Pharmacokinetics and safety of a new aspirin formulation for the acute treatment of primary headaches. Expert Opin Drug Metab Toxicol. 2014;10(10):1381–95.
92. Muir N, et al. Comparative bioavailability of aspirin and paracetamol following single dose administration of soluble and plain tablets. Curr Med Res Opin. 1997;13(9):491–500.
93. Muir N, et al. The influence of dosage form on aspirin kinetics: implications for acute cardiovascular use. Curr Med Res Opin. 1997;13 (10):547–53.
94. Sagar KA, Smyth MR. A comparative bioavailability study of different aspirin formulations using on-line multidimensional chromatography. J Pharm Biomed Anal. 1999;21(2):383–92.
95. Brandon RA, et al. A new formulation of aspirin: bioavailability and analgesic efficacy in migraine attacks. Cephalalgia. 1986;6(1):19–27.
96. Stillings M, et al. Comparison of the pharmacokinetic profiles of soluble aspirin and solid paracetamol tablets in fed and fasted volunteers. Curr Med Res Opin. 2000;16(2):115–24.
97. Voelker M, Centofani R. A bioavailability study of fast release aspirin under fasting and fed conditions and regular aspirin under fed conditions. Bayer Study Report. PH-36651, 2011-11-24, 2011.
98. Zeymer U, Gebert I, Roitenberg A, Hohlfeld T. Prospective, randomized comparison of 500 mg and 250 mg acetylsalicylic acid i.v. and 300 mg p.o. in patients with acute coronary syndrome, measured by time-dependent thromboxane inhibition (ACUTE). Abstr. AHA Scientific Sessions, Nov 7–11, Orlando, FL, USA, 2015.
99. Limmroth V, May A, Diener H. Lysine-acetylsalicylic acid in acute migraine attacks. Eur Neurol. 1999;41(2):88–93.
100. Leniger T, et al. Comparison of intravenous valproate with intravenous lysine-acetylsalicylic acid in acute migraine attacks. Headache. 2005;45 (1):42–6.
101. Weatherall MW, et al. Intravenous aspirin (lysine acetylsalicylate) in the inpatient management of headache. Neurology. 2010;75(12):1098–103.
102. Neurologie, D.G.f., Therapie der Migräne. 2012.

Nonprescription Analgesic Anti-inflammatory Drugs: Efficacy and Safety

8

Denis M. McCarthy

Introduction

In the face of rising costs of patented, prescription medications and doctor visits, more and more of the world's population are turning to over-the-counter (OTC) nonprescription drugs, for the treatment of common conditions that are either painful or inflammatory, or accompanied by fever, or flu-like, often musculoskeletal symptoms. Since 2008 the volume of drugs sold OTC has risen at a faster rate than that of drugs sold on prescription. The reasons are not hard to find: in general, these drugs are inexpensive, easily and widely available, and effective in most mild or moderate conditions, and are very safe when used short term or in low dosage. The dose legally approved for OTC use varies from country to country, but generally is half of the minimum prescription dose of the same compound approved by the relevant Regulatory Agency, after safety and efficacy have been established in that population. Data from five European countries estimate that, in most cases, the duration for which these drugs are used on average is 2.2 ± 4.4 days and that the average number of tablets used each day is 2.2 ± 1.8. Users are told to limit use to a maximum of 4 days per episode and, if the illness lasts more than this, despite using OTC medications, that the advice of a health-care provider should be sought. The major compounds for discussion are aspirin, diclofenac, ibuprofen, paracetamol (acetaminophen), and naproxen: among these, diclofenac appears to enjoy major worldwide sales, but, because of some concerns about safety and regulatory costs, some of the drugs are not approved in all countries. Not discussed here are less widely used compounds like dipyrone (metamizole), or a considerable number of mixed OTC compounds, for example, aspirin-phenacetin-codeine or ibuprofen-paracetamol, that often improve efficacy with little or no added risk [1].

Efficacy

These drugs are principally used for the relief of pain but in addition have anti-inflammatory, antipyretic, analgesic, and platelet inhibitory actions: as a broad generalization, all of these OTC compounds are similarly effective, except perhaps in treating inflammatory pain where the nonsteroidal anti-inflammatory drugs (NSAIDs) are superior to paracetamol (acetaminophen). Diclofenac, ibuprofen, naproxen, and aspirin are NSAIDs. Paracetamol, while classified as an NSAID by some regulatory agencies, and possessing weak cyclooxygenase-2 inhibitory actions [2], is generally not considered by

D.M. McCarthy, M.D, Ph.D., F.A.C.P., F.R.C.P.I. (✉)
University of New Mexico School of Medicine,
& Veteran's Administration Medical Center-111,
Albuquerque, USA
e-mail: denis.mccarthy2@va.gov

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_8

clinicians to be an NSAID and possesses significantly weaker anti-inflammatory action. Results from a recent careful study from Australia, where quality of evidence was critically assessed, showed that for spinal (acute lower back) pain, paracetamol was no better than placebo, and while there was a statistically significant benefit from using it to treat the pain of osteoarthritis of the knee or hip, it was not of a clinically useful magnitude [3]. Paracetamol, however, is quite effective and widely used to relieve most-mild-to-moderate pains, reduce fevers, relieve flu-like symptoms, and treat tension-type headaches, dysmenorrhea, and milder osteoarthritis.

After use in over a billion subjects, the efficacy of diclofenac in OTC doses has been established in relieving post-extraction dental pain, tension-type headache, and menstrual pain and in reduction of the severity of the symptom complex of fever, sore throat, and flu-like malaise, including muscle/joint aches and pains [4]. The efficacies of ibuprofen [5] and aspirin [6] in OTC doses are also well documented. Other OTC compounds enjoy broadly similar indications approved for their use. Nevertheless, actual patterns of their use may vary slightly. For instance, naproxen, a potent drug, tends to be used mainly for inflammatory pain, particularly the pain of mild-to-moderate arthritis, including rheumatoid arthritis, ankylosing spondylitis and gout, and also dysmenorrhea. Aspirin, ibuprofen, naproxen, and paracetamol, alone or combined with other agents, are all OTC drugs commonly used for migraine, tension-type headaches, minor musculoskeletal injuries, milder arthritis, fever, cold, sore throat, and muscle and joint aches and pains. They are also effective in dysmenorrhea and toothache. In migraine, aspirin and ibuprofen are similarly effective [7] and both are superior to paracetamol, but all three are similarly effective in tension-type headache [8]. Naproxen, while superior to placebo, is of limited clinical use in migraine [9]. In general, naproxen or ibuprofen is preferable to aspirin or paracetamol for treating chronic lower back pain, sciatica, or osteoarthritis, though the doses required for relief of more severe pains often exceed those approved in OTC guidelines.

Diclofenac is usually marketed as the potassium salt in doses of 12.5 or 25 mg of drug [4]. These doses are superior to placebo for 6 h in most trials, with onset within 30 min and with the higher dose being generally more effective. With flexible dosing the initial dose of 25 mg may be followed by either a 12.5 or 25 mg dose as needed, up to a maximum OTC dose of 75 mg/day. Although a one-time 50 mg dose is effective in migraine in providing relief from pain and other symptoms, only a minority of patients achieve total relief of pain [4]. The therapeutic gain above placebo is least in the treatment of low back pain [4]. In contrast, the lower dose of 12.5 mg PRN may be sufficiently effective in the treatment of dysmenorrhea or of sore throat-fever-flu-like symptoms. Approved maximal daily doses of other OTC drugs that are comparable to 75 mg/day of diclofenac are 1200 mg/day of ibuprofen (starting dose 400 mg), 3000–4000 mg of paracetamol (acetaminophen) with a starting dose 650 or 1000 mg, 3000–4000 mg of aspirin (starting dose 550–1000 mg), or 660–825 mg of naproxen (starting dose 220–275 mg): the duration of action of naproxen is close to 12 h, allowing less frequent administration than may be required with the other compounds. Before using the drug, users need to check the approved OTC dose for their country of residence. All of the OTC drugs can often be used in doses lower than these maximum recommended doses. All should be used in the lowest effective dose for the least possible time.

Despite the fact that sale of diclofenac is not approved in several countries, its prominent place in world sales of OTC anti-inflammatory drugs argues strongly in favor of its effectiveness in the clinical conditions for which it is generally used. In North America, ibuprofen and naproxen dominate the market, regardless of indication, with aspirin and paracetamol falling well behind. However, recognized by pharmacists but poorly studied, the efficacies of these drugs vary between subjects: an individual patient often chooses to use a particular drug for a specific clinical indication, based on their previous experience with using various members of this class

of compounds. For instance, from 16 systematic reviews of published papers on the efficacy of ibuprofen and paracetamol in achieving ≥50 %, reductions in pain intensity across most acute and chronic pain conditions, it was concluded that "neither of the drugs will be effective for everyone and both are needed" [7]. Some of the differences in efficacy may hinge on differences in bioavailability in the individual. Furthermore, some pharmacists advise patients to take these drugs "with food," a vague term, because this may "lessen gastric irritation." To my knowledge, this has never been formally studied in humans, but animal studies suggest that the advice is poor and that taking the drugs with food is likely to increase small intestinal injury and to delay absorption of the drug [10]. For rapid onset, an important aspect of their effects, these drugs are probably best taken while fasting.

Rapidity of absorption is an important determinant of the speed of onset of the effects of any OTC drug. Various manufacturers have developed fast-acting forms of these drugs, e.g., micronized or effervescent aspirin, lysinate and sodium dehydrate salts, or liquid gelcaps of ibuprofen, and these new forms of drug are now available. The effects of most widely available forms of the drugs are not usually appreciated in less than 20–30 min. Because of some delay in onset, OTC drugs may not be sufficiently effective, for example, in severe migraine, and parenteral treatment with prescription doses of drugs such as intravenous aspirin or subcutaneous sumatriptan may be required. However, many migraine attacks are successfully managed with OTC drugs, including aspirin, ibuprofen, and paracetamol; naproxen may be less effective [9]. There were clear significant differences over paracetamol, favoring NSAIDS as a class (notably ibuprofen, for which there is the largest number of comparisons) in relieving migraine, dental pain, postoperative pain, dysmenorrhea, musculoskeletal pain, and other common conditions, despite any statistically significant differences favoring paracetamol over placebo [1]. Beyond choice of drug, a common reason for lack of efficacy of any OTC analgesic is failure to take the prescribed dose.

Safety

Given the absence of supervision of how the purchaser will use the drug, there is great responsibility faced by regulatory authorities to ensure that any compound licensed for sale OTC without a prescription will be very safe. This creates a need for the accompanying instructions for use to provide a wide margin of safety, to allow for risks that arise, not only when the drug is used as directed but also when directions are not followed. The OTC analgesics discussed here, used as directed, are all very safe, and no serious adverse events have attended their use. However, because OTC compounds, despite the recommendations and warnings posted in the label, are often used in higher doses and for longer durations than stipulated, some inferences about safety issues that arise because the drugs are so readily available must also be considered. In this context, it must be recognized that the safety of a particular OTC product is sometimes inferred partly from the results of its use in long-term trials that employed higher doses of drug for longer periods: applying these data to the outcomes of short-term use of the drug in OTC doses is scientifically invalid. However, drugs approved for OTC sale are often used in ways not in accordance with OTC guidelines. Safety of OTC analgesics as discussed here applies to use of the drug when no other risk factor is present. Such risk factors include co-use of other drugs, or presence of a condition likely to be complicated by the therapy, for example, using naproxen in the presence of an active duodenal ulcer, or actively drinking >3 drinks/day when using acetaminophen. Finally, the apparent magnitude of the overall public health risk of the sale of these compounds is affected by the assumption that all pharmacists or druggists are acting as gatekeepers in ensuring compliance with OTC guidelines: this reliance may be misplaced. A recent study in the Netherlands revealed that only 16.7 % of pharmacists, in 228 stores tested, followed the correct guidelines in dispensing OTC drugs [11].

Paracetamol (Acetaminophen)

Paracetamol is the world's most widely used analgesic drug, but there is increasing concern about the danger to the liver [12, 13], kidney [14], and lung [15] and about an increase in all-cause mortality attending the use of doses higher than 4000 mg/day, especially over longer periods [16]. In contrast, in extremely widespread use, either on its own or as a component of over 200 other OTC compounds, there have been no serious adverse events unequivocally attributable to the short-term use of OTC paracetamol. The incidences of nausea, vomiting, or dyspepsia are not different from placebo. Use does not damage the gastric mucosa and is not accompanied by gastric erosions, despite rare reports of an association of paracetamol use with GI bleeding, an association now thought to be due to confounding. In the investigation by Lewis and colleagues, of the contributions of nonaspirin NSAIDS, obtained OTC, to the occurrence of serious GI bleeding in the community, no use of paracetamol could be identified [17] (see Fig. 8.1). Use of paracetamol is generally regarded as very safe, as long as OTC guidelines are followed.

Ibuprofen

Following on from paracetamol, which is regarded as a very safe OTC drug, a large number of clinical trials show no clear differences in gastrointestinal safety between ibuprofen and paracetamol, when used in OTC doses for the recommended durations [18, 19]. These data are supported by similar findings in one large 7-day clinical trial involving 8633 patients [20] and in a large independent meta-analysis of all randomized controlled clinical trials of aspirin use [21]. This lack of difference from paracetamol is supported by the results of two Cochrane analyses that looked at the effects of single doses of ibuprofen and found no differences from placebo [22, 23].

The only other possible risk attending the use of ibuprofen is that of an increase in cardiovascular risk, including precipitation of heart failure: this has not been seen with OTC use. In two large studies, baseline NSAID use was associated with reductions in all-cause mortality and CV mortality [24, 25]. At prescription doses of ibuprofen, the drug may reduce the cardioprotective inhibition of platelets by daily low-dose aspirin, increasing the risk of myocardial infarction and other thrombotic events, but there is no evidence of any increased risk due to this drug interaction in OTC doses are used for short periods [18]. Given that the platelet life exceeds 5 days and that the action of ibuprofen is only about 8 h, occasional use of the drug is unlikely to have any effect. OTC ibuprofen is very safe.

Diclofenac

The type and frequency of adverse events in almost 2400 patients given low-dose diclofenac potassium 12.5 mg in single or multiple doses for up to 7 days were similar to those of ibuprofen 200 mg and placebo [4]. In short-term low-dose trials using up to 75 mg/day, there were no serious GI, hepatic, neurologic, or cardiac adverse events reported [4]. It is available OTC in 75 countries. Based on the available data, it was approved for OTC use in the UK in 2008. However, in a recent controversial action by the UK's Medicines and Healthcare products Regulatory Agency (MHRA) and the UK's Commission on Human Medicines (CHM), the OTC status was canceled in the UK, and the drug returned to being a "prescription only medicine" (POM), as it is in the USA, Japan, and Ireland. This decision, based on "the occurrence of 3 more major vascular events compared to placebo" in a cohort of 1000 patients in an ongoing trial [26], is being opposed by the manufacturer Novartis and by UK pharmaceutical trade groups.

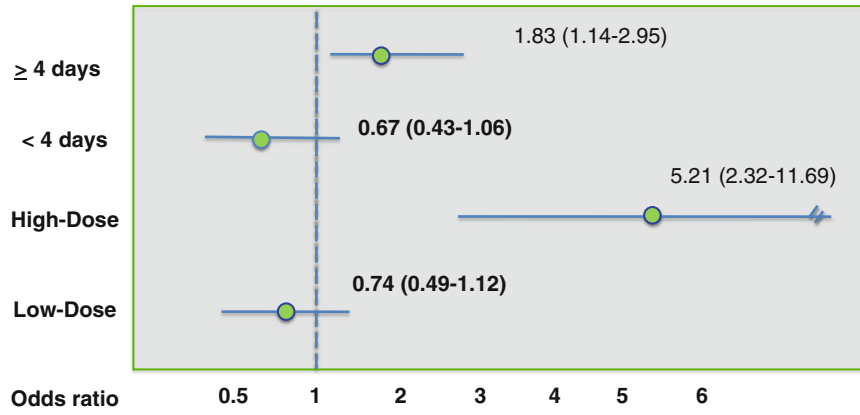


Fig. 8.1 Risk of complications of OTC compounds is affected by frequency of use and dose of drug. Use of OTC aspirin or NSAIDS <4 times during the index week (adjusted OR, 0.67: top comparison) OR use of very low doses of prescription or OTC non-aspirin NSAIDs during the index week (adjusted OR, 0.74: bottom comparison) were not significantly associated with an increased risk of serious gastrointestinal toxicity [17]

Aspirin (Acetylsalicylic Acid)

Aspirin OTC, either as a tablet, an effervescent compound or solution, or a micronized tablet, is a very safe drug in ordinary settings. Bearing in mind an average usage of 2.2 ± 4.4 tablets daily, and an average number of tablets used each day of 2.2 ± 1.8, the amounts of OTC drugs commonly used by ordinary people, the danger of aspirin OTC seems to be overestimated. It is true that because the adverse effects of aspirin depend on the dose and duration of use, used long-term in anti-inflammatory doses, it is more likely to cause serious side effects than some common comparator NSAIDS. Except for prophylactic use of low daily doses for cardiovascular prophylaxis, long-term, daily use of aspirin has greatly declined. Historically, over the period 1975 to 2000, it was largely replaced by nonaspirin NSAIDs. However, prescription doses of these are also recognized as hazardous, and their use is rapidly shrinking, particularly in treating rheumatoid arthritis.

One of the world's leading OTC drugs, the safety of aspirin OTC has in recent years received much scrutiny in two major studies. A meta-analysis of all 67 randomized controlled trials of OTC aspirin performed by the Bayer HealthCare company was performed in 6181 subjects compared with 3515 placebo cases [21]. Only minor gastrointestinal (GI) adverse events (AEs) were noted: none were serious, i.e., no GI ulcer, hemorrhage, perforation, or obstruction was attributed to aspirin and there was no case of cerebral hemorrhage. These data showed that the only significant Gl AE was "dyspepsia," occurring in 9.9 % of aspirin users versus 9.0 % in placebo, OR 1.3 % [95 % CI: 1.1, 1.5]. When compared to ibuprofen or paracetamol, there were no significant differences. The main limitation of the study was that when the drug was used only as needed, the vast majority of the patients ingested only very small doses in 1 day.

This was followed by an extensive literature search, which identified 119,310 articles that included data on side effects of aspirin [27]. Following a complicated methodology, 19,829 evaluable patients were abstracted from the 3893 highest-scoring articles and reviewed individually for data that could be included in the analysis. Among the patients included, 34 % were treated with aspirin, 17 % with placebo, and 49 % an active comparator: 50 % of aspirin users took more than a single dose and dosage in the comparators was similar. Aspirin was associated with an increased risk of minor GI side effects compared to placebo [OR 1.46, 95 % CI 1.15, 1.86] or comparators [OR 1.81,

95%CI 1.61, 2.04]. The relevant GI AEs were all minor (dyspepsia, nausea/vomiting, or abdominal pain). Serious GI events were very rare in any analyzed group. Although minor GI side effects were uncommon, aspirin was associated with higher risks of most GI AEs than were placebo, ibuprofen, diclofenac, or naproxen, but many of the differences failed to reach statistical significance. Significant exceptions were that lower-dose aspirin had more GI AEs than lower doses of either ibuprofen [or 2.67, 95 % CI: 1.22, 5.84] or naproxen [OR 3.52, 95 % CI: 1.01, 12.25].

Because of the large numbers and diverse sources, these analyses are believed to have a high precision, with the reservation that the data for paracetamol and ibuprofen are dominated by a single study [20] and, when this study was removed, the numbers of subjects using either of these two drugs were reduced by 90 %. In the study which was removed [20], when examined separately, the incidences of minor AEs were aspirin, 18.7 %; ibuprofen, 13.7 %; and paracetamol, 14.5 %: placebo rates were not measured but should likely be similar to paracetamol or ibuprofen. Nevertheless, from these studies, it is apparent that the use of OTC aspirin, when compared to ibuprofen or paracetamol, is accompanied by a small but statistically significant increase in the risk of experiencing some minor GI discomfort, mostly mild abdominal pain or transient dyspepsia: the meaning of "dyspepsia" is discussed in depth in both papers [21, 27].

Serious adverse effects were very rarely associated with aspirin, or with any OTC analgesic when used as directed, and were not attributable to the OTC drug. The small increase in minor GI side effects of aspirin is not to be dismissed, but the impact on consumers must be small, as reflected by the popularity of the drug. The uses of the various OTC compounds vary widely from country to country, depending on availability, cost, and other demographic factors [28]. Accurate information, based on large population surveys, is hard to obtain, but in Germany, where OTC analgesic use is rising and where all five drugs are available OTC, a rank order of the percentage of the population using each drug per week was recently estimated as ibuprofen, 8.0 %; aspirin, 5.8 %; paracetamol, 5.2 %; diclofenac, 4.4 %; and naproxen, 0.2 % [28]. This would suggest that the minor GI side effects of OTC aspirin are largely tolerable and not a major deterrent to its OTC use.

Naproxen

Naproxen sodium is slightly different from the other NSAIDs in being the only non-racemic NSAID to have received approval for OTC use. Because of a related longer duration of action, it can be taken at intervals of 8–12 h, but the total use per 24 h is restricted to 3 tablets, using either 1 every 8 h or 2 initially followed by one 12 h later. While naproxen sodium is more rapidly absorbed than pure naproxen, effects are perhaps a little slower in onset than those of other OTC analgesics, a slight disadvantage in treating migraine [9]. However, once absorbed, the longer duration of action, and reliable potency, renders it particularly well suited to use in inflammatory conditions, e.g., arthritis or skeletal injuries, where OTC use can be continued for up to 10 days. This allowable duration of use raises slightly greater concerns about safety. Against this, in a manner somewhat similar to aspirin, there has been an extensive meta-analysis of the safety profile of naproxen sodium OTC [29]. From among 90 published studies, 46 randomized, placebo-controlled, double-blind clinical trials were selected for analysis that had used either 200 or 440 mg tablets of naproxen sodium in single, multiple, or PRN doses. In trials involving almost 10,000 subjects, using doses of 200–220, 400–440, 600–660, and 800–880 mg, in single- or multiple-dose studies, and examining comparisons of total AEs, moderate-to-severe AEs, and AEs involving the digestive tract, there was no situation in which the incidence of AEs in those on drug significantly exceeded the incidence seen in those on placebo statistically, although the reverse was not always true. Side effects that occurred in >1 % of naproxen-treated subjects were headache (4.9 % drug, 6.9 % placebo), nausea (4.4 %

drug, 4.8 % placebo), somnolence (2.4 % drug, 1.5 % placebo), dizziness (2.0 % drug, 2.1 % placebo), vomiting (1.8 % drug, 2.4 % placebo), and dyspepsia (1.9 % drug, 1.8 % placebo). Serious complications were rare and none were clearly attributable to drug. When used as recommended, the incidence of side effects of using the drug is low and comparable to that of placebo. These data support the conclusion that, like all other NSAIDs, naproxen OTC is safe.

Problems and Perspectives

Following over 40 years of publications about the dangers associated with using NSAIDs, there is considerable anxiety among pharmacists, members of the medical profession, and the general public about the safety of OTC analgesics. Much of the literature causing this anxiety is based on reports of adverse events occurring in patients that were using high doses, for long durations of exposure to drug and, particularly in observational studies, in heterogeneous populations where many other risk factors were present to varying extents. Such risk factors include increasing age, male gender, the presence of diagnosed or subclinical comorbid conditions (especially in the GI tract, liver or kidney), and co-therapy with other medications. These last include but are not limited to antithrombotic and anticoagulant drugs, selective serotonin reuptake inhibitors (SSRIs), corticosteroids, chemotherapeutic agents, amphetamines, and erosive formulations of common compounds such as potassium chloride.

Many observational studies also fail to distinguish between the adverse effects of drugs obtained OTC and those obtained by prescription, all reported AEs being pooled for analysis. Some of the subjects, falsely identified for analysis as using a prescription NSAID, in addition were taking another NSAID or aspirin obtained OTC, thus falsely increasing the apparent risk associated with a lower dose. Reports often fail to estimate the magnitude of the effects of dose, duration of use, or other risk factors. Often the precise site or nature of the complication is not identified, and a number of "complications" may be grouped together for analysis [e.g., perforations, ulcers, or bleeding (PUBs)], rendering it impossible to estimate the precise risk of the individual complication. For all these reasons, the incidences of complications and AEs seen in observational studies are nearly always higher than those reported in clinical trials, in which there is careful exclusion of any subject who might be at increased risk. To apply the results from longer-term, higher-dose studies to estimating the risks attending the short-term use of OTC doses of the compounds is scientifically invalid.

It seems clear from the data summarized in this chapter that, used as directed, OTC compounds are very safe: the occurrence of serious AEs is very rare, with incidences comparable to placebo (Table 8.1). Nonetheless, it must be recognized that there are circumstances where analgesics are obtained OTC but used beyond the limits recommended, leading to the occurrence of serious side effects, including hospitalization and death. Very few studies have tried to separate the hazards associated with such use or

Table 8.1 Key issues on OTC NSAID/analgesic drug use

1. In general these drugs are used for pain relief and are similarly effective
2. Rapidity of absorption is an important determinant of the speed of onset of the effects of any OTC drug
3. A common reason for lack of efficacy of any OTC analgesic is failure to take the prescribed dose
4. In general, the OTC analgesics when used as directed are all very safe, and no serious adverse events have attended their use
a. Some studies revealed that only a minority of pharmacists, who act as gatekeepers, followed the correct guidelines in dispensing OTC drugs
b. Paracetamol (acetaminophen) should be used with caution in heavy drinkers
c. Ibuprofen may interact with cardiovascular low-dose aspirin
d. Diclofenac has been retired as an OTC drug in some countries due to its CV effect
e. Aspirin has been associated with a small increase in dyspepsia rates when compared to placebo and in some studies compared to ibuprofen or paracetamol

misuse of OTC compounds, from those arising as the consequences of using prescription drugs.

A seminal case-control study by Lewis et al. [17] looked at the risk of serious upper gastrointestinal toxicity with over-the-counter nonaspirin nonsteroidal anti-inflammatory drugs (NANSAIDs), in 359 rigorously selected cases identified from 28 hospitals, and compared them with 1889 controls from the same area (Fig. 8.1). They found that OTC NANSAIDs (dose not stated) used on ≥4 days, during the week prior to hospitalization for GI toxicity, had an adjusted odds ratio (OR) of 1.83 [95 % CI: 1.14, 2.59], compared to an OR of 0.67 [95 % CI: 0.43,1.06] when used <4 times. Use of high-dose OTC NANSAIDs during the index week had an adjusted OR of 5.21 [95%CI: 2.32, 11.69], when the use of low doses carried an OR of 0.74 [95 % CI: 0.49, 1.12]. Affected cases were also more likely to be frequent users of both prescription and OTC NANSAIDs in ~1 % of the cases. These data suggest that the major danger mainly lay in using doses of NANSAIDs higher than those approved for OTC use, although additional unrecognized risk factors could have been present. Two other reviews, from which clear conclusions about NSAID dosages and durations of therapy that were associated with minor increases in toxicity are hard to ascertain, yielded broadly similar conclusions, but are product oriented and hard to interpret [30, 31]. It appeared that use of OTC drugs outside of guidelines contributed to small increases in the risk of GI complications. Of note, in none of the published studies has duration of exposure to an OTC drug been adequately studied. This could yet prove of some importance, particularly in assessing the risk of thrombotic events in those using specific OTC NANSAIDs while taking low-dose aspirin daily for cardiovascular prophylaxis.

A final issue concerning safety relates to the contribution of self-medication with OTC NSAIDs to adverse drug reactions (ADRs) requiring hospital admission. Between 2000 and 2008, in a hospital with a catchment area with over 500,000 population, 6887 patients were hospitalized with ADRs, and 266 (3.9 %) of these occurred in self-medicating patients: 143 (53.8 %) of the latter were due to OTC drugs, mainly NSAIDs causing GI complaints [32]. The most frequent occurrences of ADRs were due to OTC aspirin and prescription diclofenac, and most occurred in women aged 70–79 and men aged 60–69 years. This, while uncommon, has received little attention and stresses the need for greater awareness among physicians of the need to inquire about a patient's use of OTC compounds before prescribing potent drugs, particularly NSAIDs for elderly subjects.

Conclusions

Among a large number of analgesic and anti-inflammatory compounds, five enjoy major worldwide use, namely, ibuprofen, aspirin, paracetamol, diclofenac, and naproxen, their popularity varying from country to country. In OTC-approved doses, all are effective, with some minor variations in various conditions and between individuals. In these same doses, used for the limited durations recommended and following other stipulations included in their package inserts, all are safe and well tolerated, with only low (if any) incidences of minor side effects associated with their use. Risk/benefit considerations do not apply to OTC use. However, when OTC limits are ignored or exceeded, there are dangers associated with their use that vary with the compound and that increase with dose, duration of therapy, age, gender, co-therapy with other drugs, and the presence of comorbid conditions in the patient. When used to excess in low-risk patients, ibuprofen is probably associated with the least danger, but not necessarily with the comparable efficacy. Responsibility for ensuring proper use of each compound, in some countries, lies with the pharmacist who is providing the drug, but in other countries, where the drugs can be sold in any retail outlet without restriction, the efficacy and safety that attach to using any OTC compound depend heavily on patient intelligence and compliance with the accompanying directions. Serious AEs are very rare.

References

1. Moore RA, Derry C. Efficacy of OTC analgesics. Int J Clin Pract. 2013;67 Suppl 178:21–5.
2. Hinz B, Cheremina O, Brune K. Acetaminophen (paracetamol) is a selective cyclooxygenase-2-inhibitor in man. FASEB J. 2007;22:383–90.
3. Machado GC, Maher CG, Ferreira PH, et al. Efficacy and safety of paracetamol for spinal pain and osteoarthritis: systematic review and meta-analysis of randomized placebo controlled trials. BMJ. 2015;3015:h1225. doi:10.1136/bmj.h1225.
4. Moore N. Diclofenac Potassium 12.5 mg tablets for mild to moderate pain and fever: a review of its pharmacology, clinical efficacy and safety. Clin Drug Investig. 2007;27:163–95.
5. Rainsford KD. Ibuprofen: pharmacology, efficacy and safety. Inflammopharmacology. 2009;17:275–342.
6. McCarthy DM. Efficacy and gastrointestinal risk of aspirin used for the treatment of pain and cold. Best Pract Res Clin Gastroenterol. 2012;26:101–12.
7. Moore RA, Derry S, Wiffen PJ, et al. Overview review: Comparative efficacy of oral ibuprofen and paracetamol (acetaminophen) across acute and chronic pain conditions. Eur J Pain. 2015;19:1213–23.
8. Peters BH, Fraim CJ, Masel BE. Comparison of 650mg of aspirin and 1000 mg of acetaminophen with each other and placebo in moderately severe headache. Am J Med. 1983;74:36–42.
9. Law S, Derry S, Moore RA. Naproxen with or without an antiemetic for acute migraine headaches in adults. Cochrane Database Syst Rev. 2013;10, CD009455. doi:10.1002/14651858.CD009455.pub2.
10. Rainsford KD, Bjarnasson I. NSAIDs: take with food or after fasting? J Pharm Pharmacol. 2012;64:465–9.
11. Van Hoof JJ, Cents MHG, Megens NMJ, van der Tang SJ. Druggists and Pharmacists as gatekeepers: sales routines and compliance with sales protocols for over-the-counter naproxen 275mg medicines in the Netherlands. Health Policy. 2014;117:353–60.
12. Schiodt FV, Rochling GA, Casey DL, Lee WM. Acetaminophen toxicity in an urban county hospital. N Engl J Med. 1997;337:1112–7.
13. Whitcomb DC. Acetaminophen toxicity: the rest of the story. Gastroenterology. 1998;114:1105–6.
14. de Vries F, Setakis E, van Staa TP, et al. Concomitant use of ibuprofen and paracetamol and the risk of major clinical safety outcomes. Br J Clin Pharmacol. 2010;70:429–38.
15. Allmers H, Skudlick C, John SM. Acetaminophen Use: a risk for asthma? Curr Allergy Asthma Rep. 2009;9:164–7.
16. Roberts E, Nunes VD, Buckner S, et al. Paracetamol: not as safe as we thought? A systematic literature review of observational studies. Ann Rheum Dis. 2015;75 (3):552–9.
17. Lewis JD, Kimmel SE, Localio AR, et al. Risk of serious upper gastrointestinal toxicity with over-the-counter nonaspirin nonsteroidal anti-inflammatory drugs. Gastroenterology. 2005;129:1865–74.
18. Moore RA. Analgesic safety—myths, mysteries and misconceptions. Int J Clin Pract. 2015;69 Suppl 182:24–7.
19. Le Parc J-M, Van Ganze E, Moore N, et al. Comparative tolerability of paracetamol, aspirin and ibuprofen for short term analgesia in patients with musculoskeletal conditions: results in 4291 patients. Clin Rheumatol. 2002;21:28–31.
20. Rampal P, Moore N, Van Ganse V, et al. Gastrointestinal tolerability of ibuprofen compared with paracetamol and aspirin at over-the-counter doses. J Int Med Res. 2002;30:301–8.
21. Lanas A, McCarthy DM, Voelker M, et al. Short term acetylsalicylic acid (aspirin) use for pain, fever or colds—gastrointestinal adverse effects. Drugs R D. 2011;1193:1–12.
22. Derry C, Derry S, Moore RA, et al. Single dose ibuprofen for acute post-operative pain in adults. Cochrane Database Syst Rev. 2009;3, CD001548.
23. Rabbie R, Derry S, Moore RA. Ibuprofen with or without an anti-emetic for acute migraine headaches in adults. Cochrane Database Syst Rev. 2013;4, CD008039.
24. Goodson NJ, Brookhart AM, Symmons DP, et al. Nonsteroidal Anti-inflammatory drug use does not appear to be associated with increased cardiovascular mortality in patients with inflammatory polyarthritis: results from a primary care based inception cohort of patients. Ann Rheum Dis. 2009;68:367–72.
25. Mangoni AA, Woodman RJ, Gaganis P, et al. Use of nonsteroidal anti-inflammatory drugs and risk of incident myocardial infarction and heart failure, and all-cause mortality in the Australian veteran community. Br J Clin Pharmacol. 2010;69:689–700.
26. Sukkar E. Risk of heart problems prompts removal of OTC diclofenac from UK pharmacy shelves. Pharm J. 2015;294:7846.
27. Baron JA, Senn S, Voelker M, et al. Gastrointestinal adverse effects of short-term aspirin use: a met-analysis of published randomized controlled trials. Drugs R D. 2013;13:9–16.
28. Sarganas G, Buttery AK, Zhuang W, et al. Prevalence, trends, patterns and associations of analgesic use in Germany. BMC Pharmacol Toxicol. 2015;16:28.
29. Bansal V, Dex T, Proskin H, et al. A look at the safety profile of over-the-counter naproxen sodium: a meta-analysis. J Clin Pharmacol. 2001;41:127–38.
30. Biskupiak JE, Brixner DI, Howard K, Oderda GM. Gastrointestinal complications of over-the-counter nonsteroidal anti-inflammatory drugs. J Pain Palliat Care Pharmacother. 2006;20:7–14.
31. Michels S, Collins J, Reynolds MW, et al. Over the counter Ibuprofen and risk of gastrointestinal bleeding complications: a systematic literature review. Curr Med Res Opin. 2012;28:89–99.
32. Schmiedel S, Rottenlolber M, Hasford J, et al. Self-medication with over-the-counter and prescribed drugs causing adverse-drug-reaction-related hospital admissions; Results of a prospective multicenter study. Drug Saf. 2014;37:225–35.

Low-Dose Aspirin in the Cardiovascular System

9

Ruben Casado-Arroyo, Angel Lanas, and Pedro Brugada

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in the world [1]. The disruption of an atherosclerotic plaque may lead to platelet deposition and the formation of a thrombus that can induce an acute clinical cardiovascular event [2]. In this setting, a drug with a potent antithrombotic effect would have a significant impact on morbidity and mortality. The history of aspirin is one of the best examples of the success of translational research in medicine, from the description of its mechanism of action to the studies of clinical pharmacology and ending with a large number of adequately sized, placebo-controlled clinical trials (Table 9.1).

The efficacy of low-dose aspirin (LDA) as an antithrombotic agent has been evaluated in several populations, from healthy persons at low-high risk of vascular complications (primary prevention) to high-risk patients surviving a myocardial infarction or an ischemic stroke (secondary prevention). Studies have ranged from a few weeks to 10 years in duration (Table 9.1).

In other settings, the co-prescription of other antiplatelet agents and the use of oral anticoagulation with aspirin compared to aspirin alone in population with coronary artery disease have shown to reduce the risk of death, associated with myocardial infarction (MI) or stroke. This beneficial effect had to be balanced against the increased risk of upper GI bleeding (RR of 2.08 (CI 95 %, 1.34–3.21) in aspirin + another antiplatelet drug and 2.00 (CI 95 %, 1.15–3.45), respectively, in aspirin + oral anticoagulants compared with monotherapy with LDA [12]). In clinical practice, LDA is increasingly prescribed in both primary and secondary prevention.

In summary, the saturability of the antiplatelet effect of aspirin at low doses, the lack of dose-response relationship in clinical studies evaluating its antithrombotic effects, and the dose dependence of its side effects all support the use of LDA in the prevention of cardiovascular disorders. The use of the lowest effective dose of aspirin (75–100 mg/day for long-term treatment) is currently the most appropriate strategy to maximize its efficacy and minimize its toxicity.

R. Casado-Arroyo, M.D., Ph.D. (✉)
Department of Cardiology, Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium
e-mail: rbcasado@gmail.com

A. Lanas, M.D., Ph.D., A.G.A.F., A.C.G.F.
Universidad de Zaragoza, IIS Aragón, CIBER Enfermedades Hepáticas y Digestivas (CIBERehd), Zaragoza, Spain

Service of Digestive Diseases, University Hospital Lozano Blesa, Zaragoza, Spain
e-mail: alanas@unizar.es

P. Brugada, M.D., Ph.D.
Cardiovascular Division, Heart Rhythm Management Center, Cardiovascular Center, Free University of Brussels (UZ Brussels) VUB, Brussels, Belgium

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_9

Primary Prevention in Patients at Enhanced Risk of Vascular Complications

In the last 30 years, nine major trials have examined the benefit of aspirin for primary CVD prevention (Table 9.1). Trial results were mixed to some degree, but the evidence pointed that aspirin could decrease CVD risk, including MI and stroke. When cardiovascular and all-cause mortality was assessed, no statistically significant effect was observed.

The Antithrombotic Trialists' Collaboration (ATC) published a meta-analysis analyzing six primary prevention trials including over 95,000 low-risk individuals; aspirin yielded a significant reduction in serious vascular events (myocardial infarction, stroke, or vascular death) from an annual rate of 0.57–0.51 % [13]. This cardioprotective effect was mainly due to a reduction in nonfatal myocardial infarction, from 0.23 to 0.18 % per year. There was no reduction in vascular mortality and stroke, with or without aspirin. Aspirin treatment increased gastrointestinal (or extracranial) bleeds by about half, from 0.07 to 0.1 % per year.

The ATC showed that in primary prevention without previous disease, aspirin is of uncertain net value as the reduction in occlusive events (myocardial infarction, stroke, or vascular death) and needs to be weighed against any increase in major bleeds. Interestingly and unfortunately, the main risk factors for CVD were similar to those for major bleeding [13].

As a result, even for patients at moderately increased risk of coronary events, the absolute benefits and hazards of LDA added to a statin-based regimen of primary prevention are likely to be approximately evenly balanced (Table 9.2) [14].

If we analyze the effect of gender, the ATC meta-analysis suggests no differences in response to aspirin between men and women [13]. In the Women Heart Study [8], aspirin showed a significant benefit on the risk of a first stroke but not a first MI. Although there is not a clear evidence in the literature, some institutions make separate recommendations for women and men, based on their interpretation of the previous trials.

Regarding the use of aspirin in diabetic patients, a recent meta-analysis analyzed seven trials involving patients with diabetes and found no significant reduction in either serious cardiovascular events or all-cause mortality [15].

Probably the more neutral role of aspirin in this population may be the result of side effects, such as a higher bleeding risk and a different pharmacological profile of the drug in this population. In light of these findings, the American Diabetes Association now recommends that low-dose aspirin be prescribed primarily for men over age 50 and women over age 60 who have diabetes and are at high risk of cardiovascular events [16].

In resume, in the primary prevention setting, the current totality of evidence provides only modest support for a benefit of aspirin in patients without clinical CVD, which is offset by its risk. Weighing the overall benefit and risk requires careful consideration by the physician and patient before initiating aspirin for preventive therapy.

Further primary prevention trials of aspirin are currently ongoing with the aim of recruiting patients who are at relatively high cardiovascular risk because of diabetes mellitus and being on treatment with simvastatin (ACCEPT-D) and patients with diabetes mellitus and treated with aspirin/omega-3 fatty acid versus placebo control (ASCEND) and advanced age (ASPREE) or several risk factors that do not include diabetes (ARRIVE) [17–20].

In this evolving scenario, physicians should be aware of these ongoing trials and be ready to adapt practices, especially in patients on or who may benefit from combination therapy with aspirin and statins.

Secondary Prevention in High-Risk Patients of Vascular Complications

LDA remains the mainstay of antiplatelet treatment for patients with acute coronary syndrome (ACS) and acute myocardial infarction. It was

Table 9.1 Summary of trials evaluating aspirin for the primary prevention of serious cardiovascular events

	BDT [3]	PHS [4]	TPT [5]	HOT [6]	PPP [7]	WHS [8]	POPADAD [9]	JPAD [10]	AAA [11]
Publication	1988	1989	1998	1998	2001	2005	2008	2008	2010
Patients	5139	22,071	5085	18,790	4495	39,876	1276	2539	3350
Women (%)	0	0	0	47	58	100	56	45	72
Aspirin dosage	500 mg/day	325 every other day	75 mg/day (controlled release)	75 mg/day	100 mg/day	100 mg/every other day	100 mg/day	81 or 100 mg/day	100 mg/day
Duration of therapy	5.8 years	5 years	6.8 years	3.8 years	3.6 years	10.1 years	6.7 years	4.37 years	8.2 years
Blinding	Open label	Double blind	Double blind	Double blind	Open label	Double blind	Double blind	Open label	Double blind
Primary efficacy endpoint	CV death, nonfatal MI, and stroke or TIA	CV mortality	Ischemic heart disease	CV death, nonfatal MI, stroke	CV death, MI, stroke	CV death, nonfatal MI, stroke	CV death, nonfatal MI, stroke, or amputation for critical limb ischemia	CV death, nonfatal MI, stroke, UA, PVD, new angina	CV death, MI, stroke, revascularization
HR (95 % CI)	NS	RR 0.96 (0.6–1.54)	20 % (1–35 %) reduction	RR 0.9 (0.79–1.04)	0.71 (0.48–1.04)	0.91 (0.8–1.03)	0.98 (0.76–1.26)	0.80 (0.58–1.10)	1.03 (0.84–1.27)

Table 9.2 Benefit/risk ratio of aspirin treatment in different clinical situations [14]

Clinical situation	Benefit Major vascular event avoided per 1000/year	Risk Major GI bleeding event is caused per 1000/year	Benefit/risk
Low cardiovascular risk	1–2	The risk of GI bleeding for all groups (is) was considered to be constant, 1–2	0–1
Chronic stable angina	10		5–10
Prior myocardial infarction	15		7.5–15
Unstable angina	50		25–50

Table 9.3 Comparison of absolute effects of aspirin in primary and secondary prevention of cardiovascular disease [22]

	Absolute differences (per 1000/year)	
	Primary prevention	Secondary prevention
Major coronary event	−0.6	−10
Nonfatal MI	−0.5	−6.6
CHD mortality	−0.1	−3.4
Vascular death	−0.1	−2.9
Any serious vascular event	−0.6	−14.9

first demonstrated in the second International Study of Infarct Survival (ISIS-2) trial [21]. Aspirin use resulted in a significant reduction in nonfatal reinfarction, stroke, 5-week vascular mortality, and all-cause mortality. For the first time, it was shown that 1 month of low-dose aspirin started immediately after MI in 1000 patients would prevent 25 deaths and 10–15 nonfatal infarcts and strokes.

Afterward, the ATC analyzed 16 secondary prevention trials (17,000 individuals, 3306 vascular events) [13]. Aspirin use resulted in significant reductions in serious vascular events including stroke and coronary events in both men and women, and low-dose regimens were found to be as effective as higher doses. The proportional reduction in the risk of any serious vascular event did not differ significantly between primary and secondary prevention trials, but the absolute risk reduction was much smaller in primary than in secondary prevention (absolute benefits 0.06 % per year primary and 1.00 % per year secondary) (Table 9.3).

In the secondary prevention of CV events, the benefits of LDA are clear. Aspirin has been associated with a reduction of vascular mortality, yielding a 10 % in total mortality and a yearly absolute decrease of 1 % of major coronary events [13]. LDA represents the first option for the secondary prevention of recurrent vascular events in patients surviving a myocardial infarction or ischemic stroke, with clopidogrel providing a valid alternative [23].

In the secondary prevention trials (in which a smaller proportion of the strokes of known cause were hemorrhagic), aspirin significantly reduced the aggregate of all strokes. By contrast with primary prevention, in the secondary prevention trials, aspirin seemed to reduce vascular mortality yielding a 10 % reduction in the total mortality (RR = 0.90; CI 95 %, 0.82–0.99) [13].

The Veterans Administration (VA) Cooperative Study was a multicenter [24], double-blind, randomized trial that compared aspirin to placebo in men with a non-ST elevation ACS. Aspirin lowered the incidence of death or acute MI by 51 %. Although therapy was discontinued after 12 weeks, the mortality rate remained 43 % lower after 1 year of follow-up in the aspirin group. The Canadian multicenter trial [25] was a double-blind randomized trial that compared four regimens in patients with a non-ST elevation ACS: aspirin, sulfinpyrazone, combination therapy, and placebo. Treatment was initiated within 8 days after hospitalization and continued for 18 months. Aspirin administration resulted in a 71 % reduction in mortality and a 51 % reduction in the combined endpoint of death or nonfatal MI when compared to placebo. Prolonged follow-up showed that the benefit of LDA was maintained after 1 year of therapy.

Table 9.4 Recommendations for primary and secondary prevention in cardiovascular disease

Organization	Clinical recommendation	Recommendation LOE
Primary prevention		
AHA/ADA [27–29]	– Low-dose aspirin in persons at higher CHD risk (especially those with 10-year risk of CHD >10 %	IIa, LOE B.
	– Low-dose (75–162 mg/day) aspirin use for prevention is reasonable for adults with diabetes and no previous history of vascular disease who are at increased CVD risk (10-year risk of CVD events over 10 %) and who are not at increased risk for bleeding	IIa, LOE B.
	– Patients with asymptomatic carotid stenosis should be prescribed daily aspirin and a statin	I, LOE C.
USPSTF [30]	– Adults aged 50–59 years: recommend low-dose aspirin use for the primary prevention of cardiovascular disease (CVD) and colorectal cancer in adults who have a 10 % or greater 10-year CVD risk, are not at increased risk for bleeding	B.
	– Adults aged 60–69 years: recommend low-dose aspirin use in an individual basis. Persons who are not at increased risk for bleeding, have a life expectancy of at least 10 years, and are willing to take low-dose aspirin daily for at least 10 years are more likely to benefit	C
ESC [31]	– Antiplatelet therapy with aspirin is not recommended for people with diabetes who do not have clinical evidence of atherosclerotic disease	III, LOE A.
	– Aspirin or clopidogrel cannot be recommended in individuals without cardiovascular or cerebrovascular disease due to the increased risk of major bleeding	III, LOE B.
	– Antiplatelet therapy may be considered in hypertensive patients without a history of cardiovascular disease, but with reduced renal function or at high cardiovascular risk	IIb, LOE A.
Secondary prevention		
AHA [32]	– Patients with acute and chronic ischemic heart disease	I, LOE A.
	– Patients after a ST elevation myocardial infarction	I, LOE A.
	– Patients with unstable angina and non-ST-elevated myocardial infarction	I, LOE A.
	– For patients with stroke or TIA due to 50–99 % stenosis of a major intracranial artery, aspirin is recommended in preference to warfarin	I, LOE B.
ESC [33]	– Acute coronary syndromes in patients presenting without persistent ST segment elevation	I, LOE A.
	– Management of acute myocardial infarction in patients presenting with persistent ST segment elevation	I, LOE B.
	– Stable angina [35]	I, LOE A.
	– Aspirin is recommended immediately before and after carotid revascularization [36]	I, LOE A.
	– In the chronic phase (>12 months) after myocardial infarction, aspirin is recommended for secondary prevention	I, LOE A.
CCS [34]	– Aspirin is recommended indefinitely in all patients with ACS	Strong, high-quality evidence
	– Aspirin is recommended indefinitely in all patients after CABG	

Regarding the duration of therapy, aspirin therapy is associated with long-term benefit in patients treated after an acute myocardial infarction [26]. With all this data, the evidence strongly supports the use of LDA as maintenance therapy in patients who have undergone a percutaneous coronary intervention after unstable angina/non-ST elevation myocardial infarction.

The recommendations in primary and secondary prevention of cardiovascular disease are summarized in Table 9.4.

LDA Versus Thienopyridines

Several randomized trials of secondary prevention compared aspirin with clopidogrel or ticlopidine. The CAPRIE trial [23] found that clopidogrel had a modest and marginally significant advantage over aspirin for the prevention of stroke, MI, and vascular disease in patients with a recent stroke, MI, or peripheral artery disease (annual event rate 5.3 versus 5.8 %). Clopidogrel is an effective alternative in the approximately 5 % of patients who cannot tolerate aspirin.

Economic Implication of Aspirin Usage

Given the limited resources and the large number of patients to whom antiplatelet agents are applied, the efficient use of health-care resources with favorable indices of cost-effectiveness is of particular importance. The cost-effectiveness of aspirin treatment depends on the balance between the clinical effectiveness (ischemic events and bleeding complications) and cost.

Recent publications confirm that aspirin use remains suboptimal even in secondary prevention (where aspirin is an economically dominant strategy compared with no aspirin based on the absolute reduction of mortality). In a meta-analysis regarding the use of LDA therapy for secondary prevention, adherence was found to be approximately 65 % [35]. In another study performed in Wisconsin, aspirin was simultaneously underused by those at high CVD risk and overused by those at low CVD risk (31 % versus 18 %) [36].

In the primary prevention setting, a cost-effectiveness analysis of LDA performed in Europe suggested that LDA was cost saving compared with no treatment for patients at moderate risk of myocardial infarction or stroke. The result was economically favorable for aspirin in all countries when a patient's annual risk was >1 % [37]. Regarding the addiction of a proton pump inhibitor to patients of high risk of bleeding, a recent cost-effectiveness analysis showed that PPI co-therapy has the highest probability to be cost-effective in patients taking LDA for primary and secondary prevention of acute coronary syndrome in patients with elevated risk for upper GI bleeding. The addiction of PPI to LDA reduced the overall number of ACS. This assumption is due to the fact that patients treated with PPI co-therapy or the fixed combination had a significantly lower ACS risk compared to patients treated with ASA monotherapy or no medication. The reason is that concomitant prescription of PPI reduces GI side effects and thereby increases patients' compliance to ASA, which in turn reduces the probability of developing an ACS [38].

Atrial Fibrillation and Oral Antithrombotic Agents

Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia in the world. It affects 1.5–2 % of the general population, that is, more than 100 million people worldwide [38]. Untreated patients with nonvalvular AF (NVAF) are exposed to an annual risk of thromboembolic stroke of 5 %. Nowadays, anticoagulation is the unchallenged current treatment of choice for patients with AF at moderate to high risk of thromboembolic complications.

Antiplatelet therapy has been compared with placebo or no treatment in eight trials [39]. For primary prevention, aspirin was associated with a nonsignificative 19 % reduction (mainly due to positive results from the SPAF-1 trial) (95 % CI, -1–35 %) in stroke incidence with an absolute risk reduction of 0.8 % per year; number needed to treat (NNT) = 125. For secondary prevention among those with TIA or strokes, aspirin was associated with an absolute risk reduction of 2.5 % per year and a corresponding NNT of 40. Vitamin K antagonist (warfarin) has been extensively studied in AF. In six RCTs, adjusted-dose warfarin resulted in a 64 % RR reduction (95 % CI, 49–74 %) for ischemic and hemorrhagic stroke compared with placebo. The absolute risk reduction was 2.7 % per year, which yielded a NNT of 37 for 1 year to prevent 1 stroke and 12 for patients with prior stroke or TIA [39].

Regarding the novel oral anticoagulants, aspirin has been compared with new antithrombotic agents in the AVERROES study, a double-blind study of 5599 patients deemed unsuitable for warfarin therapy [40]. Subjects were randomized to apixaban 5 mg twice daily or to aspirin 81 or 325 mg once daily. The primary outcome of the study was the occurrence of a stroke or systemic embolism. After a mean follow-up of 1.1 years, the study was prematurely terminated due to the superiority of apixaban over aspirin. Major bleeding risk between the two treatments was similar. In this evolving scenario of AF, new elements should be considered like the direct oral anticoagulants with an improved benefit/risk profile compared with warfarin and the increasing awareness of potential nonvascular health benefits (e.g., prevention of cancer) of long-term aspirin therapy.

For all these reasons, current guidelines recommend the use of oral anticoagulation, aspirin, or no treatment for patients who present a CHA_2DS_2-VASc score of 1. In the case of a score equal or more than 2, oral anticoagulation is indicated (CHA_2DS_2-VASc score assigns one point for heart failure or ejection fraction $\leq$35 %, hypertension, diabetes, vascular disease, and female sex and two points for age >75 years and stroke, TIA, or systemic emboli) [41].

In summary, properly dosed anticoagulation is extremely effective in preventing AF-related strokes, reducing risk by two-thirds compared with no therapy and by one-half compared with aspirin.

In the near future, these novel therapeutic options and areas of knowledge will be integrated with an assessment of the individual AF patient's ischemic and bleeding risks, as well as values and preferences in order to perform a personalized antithrombotic therapy in AF.

Deep Vein Thrombosis and Pulmonary Embolization

Venous thromboembolism (VTE) includes deep vein thrombosis (DVT) and pulmonary embolism (PE). It is a frequent and potentially life-threatening event.

Acute pulmonary embolism (PE) is the most serious clinical presentation of venous thromboembolism [42]. It is the third most frequent cardiovascular disease with an overall annual incidence of 100–200 per 100,000 inhabitants. To date different agents are available for the effective treatment of acute VTE and the prevention of recurrence. Direct oral anticoagulants seem to have a more favorable risk-benefit profile compared to vitamin K antagonist.

The role of aspirin is mainly focused on the prevention of new events. In two recent trials with a total of 1224 patients, extended therapy with aspirin (after the termination of standard oral anticoagulation) was associated with a 30–35 % reduction in the risk of recurrence after unprovoked DVT and/or PE. This corresponds to less than half of the risk reduction achieved by oral anticoagulants, but it should be noted that the bleeding rates associated with aspirin were low [43, 44].

Strategies to Optimize the Use of Aspirin

Aspirin-induced impairment of primary hemostasis cannot be separated from its antithrombotic effect and its effect favoring bleeding. Evaluation of the individual CV and GI risk with different available tools [45] and the reduction of risk with modifiable measures are mandatory in the primary and secondary prevention of CV events. Table 9.5 presents ten strategies to optimize the use of LDA in clinical practice. This table reflects the best available evidence in the field of pharmacology and also in the prevention and treatment of cardiovascular diseases and cancer. It describes also the attitude in the case of acute bleeding.

Future

Due to the fact that most aspirin trials for primary CV prevention have enrolled individuals at low cardiovascular risk (<1 % event rates per person-years), several ongoing trials are investigating the safety and efficacy of LDA

Table 9.5 Ten strategies to optimize the use of aspirin

1. *Estimation of the risk* of coronary artery disease (Framingham, USPSTF, ASCVD, SCORE) and GI bleeding or both (CV/GI risk calculator) [45]
2. *Reduction of modifiable risk factors*: healthy lifestyle, cessation of smoking, reduction of obesity, statins and antihypertensive drug if indicated, prevention and treatment of diabetes and heart failure, eradication of *H. pylori*
3. *Use the lowest* dose of aspirin and avoid co-therapy with nonselective or COX-2 selective NSAIDs
4. *Co-therapy* with a gastroprotective drug if high risk of upper GI bleeding (PPI)
5. *Change* of aspirin for other antiplatelet agents *only if contraindication of aspirin use (e.g., allergy)* (clopidogrel)
6. *Management of bleeding and emergency care*: Minor bleeding may predict major bleeding and may lead to stoppage of effective antithrombotic therapy. Major bleeding is associated with higher mortality
7. *The interruption* of aspirin in the case of acute bleeding should be made *in an individual basis*
8. *Consider family history of cancer* (mainly GI)
9. *In the case of atrial fibrillation*: the CHA2DS2-VASc scoring system helps clinicians to determine stroke risk and to choose the optimal antithrombotic agent. The use of aspirin is advised if the score is 0
10. *Establish a partnership* between the doctor and patient according to patient's preferences

daily versus placebo at high level of CV (cardiovascular) risk [17–19, 46, 47]. Also, the clarification of the value of aspirin in the prevention cancer would extend the indications of aspirin treatment.

More research is needed in order to establish strategies for the identification of the risk factors for upper and lower GI bleeding.

Conclusions

Aspirin has proved to prevent fatal and nonfatal cardiovascular events in the secondary prevention of cardiovascular disease irrespective of age and gender. In contrast, in primary prevention, the cardiovascular benefits of adding LDA to statins and antihypertensive drugs are likely to be of similar magnitude and a safer profile.

Ongoing primary prevention trials may help assess the benefit/risk profile of LDA in preventing multiple outcomes (cardiovascular, dementia, and cancer).

A clinical risk-benefit analysis of the appropriate therapy should precede any indication of LDA, PPI, or both. Algorithms to integrate the stratification of risk on the GI bleeding risk with the ischemic/thrombotic side are needed, and some are already available for the clinician.

Acknowledgment This review was funded by a grant for international research, Foundation Horlait-Dapsens (RC).

References

1. World Health Organization. [homepage on the Internet]. http://www.who.int/en/, last accessed in January 2016.
2. Patrono C, García Rodríguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med. 2005;353:49–59.
3. Peto R, Gray R, Collins R, et al. Randomised trial of prophylactic daily aspirin in British male doctors. Br Med J (Clin Res Ed). 1988;296:313–6.
4. Final report on the aspirin component of the ongoing Physicians' Health Study. Steering Committee of the Physicians' Health Study Research Group. N Engl J Med. 1989;321(3):129–35.
5. Thrombosis prevention trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in the primary prevention of ischaemic heart disease in men at increased risk. The Medical Research Council's General Practice Research Framework. Lancet 1998; 351:233–41.
6. Hansson L, Zanchetti A, Carruthers SG, et al. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. Lancet. 1998;351:1755–62.
7. de Gaetano G, Collaborative Group of the Primary Prevention Project. Low-dose aspirin and vitamin E in people at cardiovascular risk: a randomised trial in general practice. Collaborative Group of the Primary Prevention Project. Lancet 2001;357(9250):89-95.
8. Ridker PM, Cook NR, Lee IM, et al. A randomized trial of low- dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med. 2005;352:1293–304.
9. Belch J, MacCuish A, Campbell I, et al. The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with

diabetes and asymptomatic peripheral arterial disease. BMJ. 2008;337:a1840.
10. Ogawa H, Nakayama M, Morimoto T, et al. Low-dose aspirin for primary prevention of atherosclerotic events in patients with type 2 diabetes: a randomized controlled trial. JAMA. 2008;300(18):2134–41.
11. Fowkes FR, Price JF, Stewart MW, et al. Aspirin for prevention of cardiovascular events in a general population screened for a low ankle brachial index: a randomized controlled trial. JAMA. 2010;303 (9):841–8.
12. García Rodríguez LA, Lin KJ, Hernández-Díaz S, Johansson S. Risk of upper gastrointestinal bleeding with low-dose acetylsalicylic acid alone and in combination with clopidogrel and other medications. Circulation. 2011;123(10):1108–15.
13. Antithrombotic Trialists' (ATT) Collaboration, Baigent C, Blackwell L, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373:1849–60.
14. Patrono C, Baigent C, Hirsh J, Roth G, American College of Chest Physicians. Antiplatelet drugs: American College of Chest Physicians evidence-based clinical practice guidelines (8th ed.). Chest. 2008;133(6 Suppl):199S–233S.
15. Zhang C, Sun A, Zhang P, Wu C, Zhang S, Fu M, Wang K, Zou Y, Ge J. Aspirin for primary prevention of cardiovascular events in patients with diabetes: A meta-analysis. Diabetes Res Clin Pract. 2010;87:211–8.
16. Pignone M, Alberts MJ, Colwell JA, Cushman M, Inzucchi SE, Mukherjee D, Rosenson RS, Williams CD, Wilson PW, Kirkman MS. Aspirin for primary prevention of cardiovascular events in people with diabetes: a position statement of the American Diabetes Association, a scientific statement of the American Heart Association, and an expert consensus document of the American College of Cardiology Foundation. Circulation. 2010;121:2694–701.
17. Nelson M, Reid C, Beilin L, Donnan G, Johnston C, Krum H. Aspirin in Reducing Events in the Elderly (ASPREE) Study Group. Rationale for a trial of low-dose aspirin for the primary prevention of major adverse cardiovascular events and vascular dementia in the elderly: Aspirin in Reducing Events in the Elderly (ASPREE). Drugs Aging. 2003;20 (12):897–903.
18. De Berardis G, Sacco M, Evangelista V, ACCEPT-D Study Group, et al. Aspirin and Simvastatin Combination for Cardiovascular Events Prevention Trial in Diabetes (ACCEPT-D): design of a randomized study of the efficacy of low-dose aspirin in the prevention of cardiovascular events in subjects with diabetes mellitus treated with statins. Trials. 2007;8:21.
19. ASPREE Investigator Group. Study design of ASPirin in Reducing Events in the Elderly (ASPREE): a randomized, controlled trial. Contemp Clin Trials. 2013;36(2):555–64.
20. ARRIVE Study Website. Aspirin to Reduce Risk of Initial Vascular Events (ARRIVE). Bayer HealthCare. Available at: http://www.arrive-study.com/EN/study.cfm. Accessed January 10, 2016.
21. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. Lancet. 1988;2:349–60.
22. Patrono C1, Andreotti F, Arnesen H, Badimon L, Baigent C, Collet JP, De Caterina R, Gulba D, Huber K, Husted S, Kristensen SD, Morais J, Neumann FJ, Rasmussen LH, Siegbahn A, Steg PG, Storey RF, Van de Werf F, Verheugt F. Antiplatelet agents for the treatment and prevention of atherothrombosis. Eur Heart J. 2011;32(23):2922–32.
23. Steering Committee CAPRIE. A randomized, blinded, trial of clopidogrel versus aspirin in patients at risk of ischemic events (CAPRIE). Lancet. 1996;348:1329–39.
24. Lewis Jr HD, Davis JW, Archibald DG, Steinke WE, Smitherman TC, Doherty JE, et al. Protective effects of aspirin against acute myocardial infarction and death in men with unstable angina. Results of a Veterans Administration Cooperative Study. N Engl J Med. 1983;309(7):396–403.
25. Cairns JA, Gent M, Singer J, Finnie KJ, Froggatt GM, Holder DA, et al. Aspirin, sulfinpyrazone, or both in unstable angina. Results of a Canadian multicenter trial. N Engl J Med. 1985;313(22):1369–75.
26. Van de Werf F, Bax J, Betriu A, Blomstrom Lundqvist C, Crea F, Falk V. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. Eur Heart J. 2008;29(23):2909–45.
27. Goff Jr DC, Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. J Am Coll Cardiol. 2014;63 (25 Pt B):2935–59.
28. Meschia JF, Bushnell C, Boden-Albala B, et al. Guidelines for the primary prevention of stroke: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2014;45(12):3754–832.
29. Preventive Services Task Force US. Aspirin for the prevention of cardiovascular disease: US Preventive Services Task Force recommendation statement. Ann Intern Med. 2009;150:396–404.
30. Bibbins-Domingo K, U.S. Preventive Services Task Force. Aspirin use for the primary prevention of cardiovascular disease and colorectal cancer: U.S. Preventive Services Task Force Recommendation Statement. Ann Intern Med. 2016;164(12):836–45.
31. European Guidelines on cardiovascular disease prevention in clinical practice (version2012): the

Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts).Eur J Prev Cardiol 2012; 19(4): 585-667.
32. Amsterdam EA, Wenger NK, Brindis RG, et al. 2014 AHA/ACC guideline for the management of patients with non-ST-elevation acute coronary syndromes: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;130 (25):2354–9.
33. Roffi M, Patrono C, Collet JP, Mueller C, Valgimigli M, Andreotti F, Bax JJ, Borger MA, Brotons C, Chew DP, Gencer B, Hasenfuss G, Kjeldsen K, Lancellotti P, Landmesser U, Mehilli J, Mukherjee D, Storey RF, Windecker S. 2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent st-segment elevation. Rev Esp Cardiol. 2015;68 (12):1125.
34. Tanguay JF, Bell AD, Ackman ML, et al. Focused 2012 update of the Canadian Cardiovascular Society guidelines for the use of anti- platelet therapy. Can J Cardiol. 2013;29(11):1334–45.
35. Naderi SH, Bestwick JP, Wald DS. Adherence to drugs that prevent cardiovascular disease: a meta-analysis on 376,162 patients. Am J Med 2012; 125: 882–7.e1.
36. Vanwormer JJ, Greenlee RT, McBride PE. Aspirin for primary prevention of CVD: are the right people using it? J Fam Pract. 2012;61:525–33.
37. de Groot NL, van Haalen HG, Spiegel BM, Laine L, Lanas A, Focks JJ, Siersema PD, van Oijen MG. Gastroprotection in low-dose aspirin users for primary and secondary prevention of ACS: results of a cost-effectiveness analysis including compliance. Cardiovasc Drugs Ther. 2013;27(4):341–57.
38. You JJ, Singer DE, Howard PA, et al. American College of Chest Physicians. Antithrombotic therapy for atrial fibrillation: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012;141(2 Suppl):e531S–75.
39. Hart RG, Pearce LA, Aguilar MI. Meta-analysis: antithrombotic therapy to prevent stroke in patients who have nonvalvular atrial fibrillation. Ann Intern Med. 2007;146:857–67.
40. Connolly SJ, Eikelboom J, Joyner C, et al. Apixaban in patients with atrial fibrillation. N Engl J Med. 2011;364:806–17.
41. January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC Jr, Conti JB, Ellinor PT, Ezekowitz MD, Field ME, Murray KT, Sacco RL, Stevenson WG, Tchou PJ, Tracy CM, Yancy CW; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. J Am Coll Cardiol. 2014:2;64(21):e1–76.
42. Heit JA. The epidemiology of venous thromboembolism in the community. Arterioscler Thromb Vasc Biol. 2008;28(3):370–2.
43. Becattini C, Agnelli G, Schenone A, Eichinger S, Bucherini E, Silingardi M, Bianchi M, Moia M, Ageno W, Vandelli MR, Grandone E, Prandoni P. Aspirin for preventing the recurrence of venous thromboembolism. N Engl J Med. 2012;366 (21):1959–67.
44. Brighton TA, Eikelboom JW, Mann K, Mister R, Gallus A, Ockelford P, Gibbs H, Hague W, Xavier D, Diaz R, Kirby A, Simes J. Low-dose aspirin for preventing re- current venous thromboembolism. N Engl J Med. 2012;367(21):1979–87.
45. Lanas A, Polo-Tomás M, Casado-Arroyo R. The aspirin cardiovascular/gastrointestinal risk calculator--a tool to aid clinicians in practice. Aliment Pharmacol Ther. 2013;37(7):738–48.
46. Teramoto T, Shimada K, Uchiyama S, et al. Rationale, design, and baseline data of the Japanese Primary Prevention Project (JPPP)-a randomised, open-label, controlled trial of aspirin versus no aspirin in patients with multiple risk factors for vascular events. Am Heart J. 2010;159:361–9. e4.
47. Reid CM, Storey E, Wong TY, et al. Aspirin for the prevention of cognitive decline in the elderly: rationale and design of a neuro-vascular imaging study (ENVIS-ion). BMC Neurol. 2012;12:3.

Adverse Effects of Low-Dose Aspirin in the Gastrointestinal Tract

10

Katsunori Iijima

Introduction

Aspirin is widely administered at relatively low doses (75–325 mg/day) as an antithrombotic drug to prevent cerebrovascular and cardiovascular disease. However, aspirin, even at low doses, exerts adverse effects on the gastrointestinal (GI) tract through its pharmacological inhibitory action on cyclooxygenase (COX), which leads to GI tract ulcers and GI tract bleeding. Low-dose aspirin (LDA)-induced upper GI tract injury has received much attention [1, 2]. However, currently, although upper GI bleeding is decreasing, lower GI bleeding is increasing [3, 4], and renewed attention is focused on mucosal injury in the more distal sites (small and large intestine). Because the prevalence of serious GI adverse events, such as GI bleeding, is considerably low [5], identifying potential risk factors is important for establishing an efficient preventive strategy for long-term LDA users. In this chapter, LDA-induced adverse effects on the upper and lower GI tract are briefly reviewed regarding their pathogenesis, epidemiology, risk factors, and prevention.

K. Iijima, M.D. (✉)
Department of Gastroenterology, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita, Akita 010-8543, Japan
e-mail: kiijima@med.akita-u.ac.jp

Upper GI Tract Injury

Pathogenesis

Aspirin induces gastroduodenal mucosal injury through topical irritation or systemic effects through the inhibition of COX-1, which regulates prostaglandin biosynthesis from arachidonic acid. However, enteric-coated aspirin, which is designed to reduce local damage, has failed to show any clear benefit over uncoated LDA for reducing upper GI bleeding [6, 7], suggesting that the systemic effects, rather than the topical actions, are mainly involved in the pathogenesis of LDA-induced gastroduodenal lesions. The depletion of mucosal prostaglandin induced via the pharmacological action of aspirin results in impaired epithelial defenses, such as impaired gastric mucus secretion [8, 9], because prostaglandin in the gastroduodenal mucosa plays a central role in controlling the epithelial defense mechanism [10, 11]. Gastric acid is likely to exacerbate aspirin-induced injury through HCl back diffusion, resulting in the formation of a deeper injury [12, 13]. An essential role of gastric acid in LDA-induced gastroduodenal injury is supported by the preventive effect of a potent inhibitor of gastric acid secretion, a proton pump inhibitor (PPI), on aspirin-induced gastroduodenal mucosal injury [14]. In addition, aspirin induces the adhesion of neutrophils to endothelial cells within the mucosal capillaries

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_10

by increasing intracellular adhesion molecule-1 (ICAM-1) expression, resulting in reduced blood flow with ischemic changes [15, 16].

Epidemiology

LDA induces a variety of upper GI adverse events, ranging from dyspeptic symptoms without macroscopic lesions to complicated peptic ulcers (bleeding and perforation) and even death.

Upper GI symptoms are common in LDA users, with a prevalence of up to 15–20 % of all such patients [17–19]. The extent and severity of endoscopic mucosal damage are not directly associated with an increased risk for dyspeptic symptoms [1, 19, 20]. Dyspepsia is not life threatening, but it can be a serious problem because it may decrease adherence to LDA due to troublesome symptoms [17]. Constant administration of LDA is a critical issue because the cessation of LDA is a significant risk factor for adverse cardiovascular events [18].

Endoscopic controlled studies have revealed that a variety of severe gastroduodenal mucosal injury, including petechiases, erosions, and ulcers, can be induced by LDA administration. Erosive lesions are frequently seen in up to 60 % of LDA users [19, 20]. In a recent, large-scale prospective study comprising 1454 LDA users in Japan, the prevalence of gastroduodenal erosions was reported to be 29.2 % [21]. In addition, the prevalence of gastroduodenal ulcers ranged from 5 to 30 % [19, 20]. The wide range of prevalence of LDA-induced gastroduodenal erosions and/or ulcers is partly due to the geographic differences resulting from the diverse effects of *H. pylori* infection on gastroduodenal lesions, such that the prevalence is higher in Western countries than that in Asia [22], or to the timing of the endoscopic examination. Notably, only 20 % of gastroduodenal ulcers were associated with the manifestation of dyspeptic symptoms[1]; thus, the clinical significance for the majority of asymptomatic erosions or ulcers is obscure because such tiny lesions likely heal spontaneously over a period of time.

Relatively few gastroduodenal ulcers lead to ulcer complications (bleeding or perforation) that could be life threatening. The annual incidence rate of major GI bleeding in a double-blind randomized, placebo-controlled trial of LDA users ranged from 0.07 to 1.57 %, and the relative risk of major GI bleeding in LDA users in relation to controls ranges from 1.5 to 2.6 [5, 19]. Perforation is also a rare event in LDA users, with an incidence of 32.7 per 100,000 patient-years in patients older than 65 years [19, 20].

Risk Factors

Because of the large number of patients taking LDA to prevent cerebrovascular and cardiovascular diseases and the relatively rare incident rate of complicated upper GI adverse events in aspirin users, identifying high-risk groups for upper GI adverse events from LDA users and targeting them as a prevention therapy should be an effective strategy [23].

A history of peptic ulcer, particularly associated complicated (bleeding or perforated) ulcers, is the most important risk factor for gastroduodenal adverse events in LDA users. Previous observational studies have indicated that a history of peptic ulcers increases the risk of the upper GI complication by approximately two- to threefold, and for a history of complicated peptic ulcers, the risk further increased five- to sixfold compared with the absence of such histories [24–26].

Older age (60 year or more) is generally considered a risk factor for gastroduodenal complications in patients taking LDA, although there are few studies to support this indication [23]. Otherwise, general comorbid diseases accompanied by aging rather than aging itself may be a therapeutic target for prevention in LDA users because after ulcers start bleeding, the comorbidity is associated with a fatal condition in peptic ulcer bleeding [27, 28].

The concomitant usage of some other drugs enhances LDA-induced upper GI adverse events.

To date, it has been established that co-prescribed nonaspirin nonsteroidal anti-inflammatory drugs (NSAIDs), including COX-2-selective inhibitors, antiplatelet agents, anticoagulants, and oral corticosteroids, increase the risk of adverse upper GI events in LDA users compared with monotherapy [23–26]. The interactions between these drugs and LDA were confirmed not only by many observational studies but also by more recent large-scale analyses using a network of healthcare databases [29, 30]. Because most of these drugs have injurious effects on the upper GI mucosa, it is not surprising that the concomitant use of these drugs exacerbates LDA-induced upper GI mucosal injury.

For the interaction between *H. pylori* infection and LDA use, thus far, observational studies have shown conflicting results, with some showing an additive effect, others showing antagonizing effects, and still others showing no overall interaction [23]. A recent systematic review of *H. pylori* and the risk of upper GI bleeding in aspirin users concluded that the current data are not sufficient to allow a meta-analysis of the issue [31]. A series of recent studies in which individual gastric acid secretion levels were measured in chronic LDA users indicated that *H. pylori* infection could have biphasic effects on drug-induced gastroduodenal injury, depending on the gastric acid secretion level. The infection exacerbates mucosal injury in the presence of sufficient gastric acid, whereas the infection protects the mucosa from injury through a hypochlorhydric state accompanied by the infection [32–35]. In patients with *H. pylori* infection, evaluation of serum pepsinogen levels or endoscopic findings prior to the commencement of aspirin administration may help identify a high-risk group for upper GI adverse events from LDA users by extracting hyperchlorhydric subjects [32, 33].

Prevention

According to several guidelines [36, 37], patients with the above risk factors for upper GI bleeding must be coadministered preventive gastroprotective drugs when LDA is started.

The effectiveness of potent antisecretory drugs, PPIs, on upper GI adverse events in LDA users has been consistently reported in several randomized controlled trials [38–40]; PPIs completely prevent the recurrence of upper GI events in LDA users, even in high-risk groups with histories of complicated peptic ulcers [14]. Therefore, co-therapy with PPIs is recommended for LDA users with risk factors based on an expert consensus [36, 37], and prevention therapy is broadly implemented in clinical practice. In addition, PPIs may be effective in alleviating dyspeptic symptoms, even in the absence of endoscopic abnormality [41, 42], which could boost patient compliance with LDA treatment. Therefore, the broader application of PPIs to a wide range of LDA users, regardless of the presence or absence of accompanying risk factors, would be an alternative strategy for managing aspirin users in the expectation of boosting patient adherence to long-term LDA treatment [14]. However, other than the cost-effectiveness of the broader application of PPIs, recent studies in animal models and humans have indicated that PPI administration may enhance LDA-induced mucosal injury at more distal sites (small intestine) instead of preventing gastroduodenal mucosal injury, as discussed below. Hence, preventive therapy with PPIs should remain targeted to LDA users with risk factors.

By contrast, although a previous study indicated the significant effectiveness of a histamine H_2 receptor antagonist (H2RA) on upper GI adverse events in LDA users compared to placebo [43], other studies have demonstrated that the preventive effect by H2RA is significantly inferior to that of PPIs [44, 45]. Therefore, co-therapy with H2RA is unsatisfactory for the prevention of adverse upper GI events in LDA users.

Misoprostol, a prostaglandin E1 analogue, shows preventive effects on NSAID-induced gastroduodenal mucosal injury, although the use of the drug was restricted due to its severe abdominal side effects, such as diarrhea and

nausea. However, a previous study demonstrated that a lower dose of misoprostol (100 mg/day) significantly reduces gastroduodenal mucosal injury in LDA users without any abdominal side effects [46]. Rebamipide, a drug that stimulates gastric mucosal prostaglandin E1 and enhances the accompanying gastric mucus secretion [47, 48], also has potential effectiveness in the prevention of LDA-induced gastroduodenal mucosal injury without any serious side effects [49, 50]. However, these are small and explorative studies, and large-scale, clinical studies are required to introduce misoprostol or rebamipide in the clinical setting for the prevention of LDA-induced gastroduodenal mucosal injury.

H. pylori eradication therapy should be considered in *H. pylori*-positive LDA users with a history of peptic ulcers because a recent study indicated the long-term efficacy of the treatment for the recurrence of ulcer complications [51], as recommended by a recent European guideline on the management of *H. pylori* infection [52]. By contrast, the efficacy of eradication therapy for adverse gastroduodenal events in the remaining unselected *H. pylori*-positive LDA users without a history of peptic ulcers remains to be clarified [23]. This issue has significant implications in countries where the *H. pylori* infection rate remains high in the elderly, who are occasionally subjected to daily LDA intake, although the *H. pylori* infection rate is decreasing worldwide.

Lower GI Tract Injury

Pathogenesis

NSAIDs/aspirin also injures the small and large intestines with both topical effects on the epithelium and systemic effects via the suppression of epithelial prostaglandin biosynthesis. The latter mechanism is primarily involved in aspirin-induced enteropathy. The pathogenesis of NSAIDs/aspirin is multifactorial, and the following factors may be involved in the manifestation of drug-induced enteropathy: increased intestinal permeability, decreased intestinal mucus secretion, neutrophil infiltration and accompanying free radial formation, and bacterial infection [53–55]. Increased intestinal permeability may allow bile acid, pancreatic juice, and bacteria to invade the intestinal epithelium, leading to the induction of an inflammatory reaction in the tissue [53–55]. Consequently, inflammation in the small intestine is frequently manifested as mucosal lesions, such as erosions and ulcers. A previous study demonstrated that NSAIDs do not induce small intestine injury in germ-free rats [56]; therefore, bacteria in the small intestine may play an essential role in the formation of the ultimate mucosal lesions.

Nonetheless, caution should be used when extrapolating these mechanisms to the pathogenesis of aspirin-induced enteropathy occurring in humans because the majority of findings are from NSAID-induced enteropathy in animal models. In fact, experimental animal studies showed that orally administered aspirin, even at large doses, does not induce mucosal injury in the small intestine [53–55]. The difference in susceptibility to small intestine injury between NSAIDs and aspirin may be due to the difference in exposure time to each drug in the small intestine because the enterohepatic circulation of NSAIDs results in multiple time exposures of small intestine to the drug, whereas there is little enterohepatic circulation for aspirin [53–55]. However, previous studies showed increased intestinal permeability in aspirin users, even at a low dose, in humans [57, 58]. Thus, LDA may induce small intestine mucosal injury via the same mechanism as ordinary NSAIDs.

Epidemiology

Until recently, it was believed that damage to the human GI tract by LDA is mainly confined to the upper portion of GI tract up to the duodenum because the drug is primarily absorbed in the stomach and duodenum and rarely reaches the small intestine. Consequently, epidemiological studies on LDA-associated lower GI tract adverse events were limited, although the upper GI tract has been extensively investigated. However, with the advent of capsule endoscopy and

double balloon enteroscopy, the presence of small bowel injury can now be observed. Since the first case report of severe enteropathy in a LDA user by Leung et al. in 2007 [59], LDA-induced mucosal injury of the lower GI tract has received a great deal of attention.

The causal relationship of LDA to small intestine mucosal injury was confirmed using capsule endoscopy in healthy volunteers, in whom the short-term (1–2 weeks) administration of LDA significantly increased small intestine lesions compared with controls [57, 60]. Several studies using capsule endoscopy revealed the high prevalence (80–100 %) of small intestine injury of any degree in chronic LDA users with a wide spectrum of lesions, including multiple petechiae, loss of villi, erosions, and ulcers, although each study included a small number of patients (10–30 patients) [54]. A portion of these lesions may contribute to the pathogenesis of unexplained iron deficiency anemia or hypoalbuminemia, both of which are significantly more frequently observed in chronic LDA users [61, 62]. However, the clinical significance of the remaining vast majority of small intestine lesions observed in LDA users remains uncertain; therefore, human studies on small intestinal mucosal injury in LDA users should be interpreted with caution.

Colonic diverticular bleeding is the most frequent cause of lower GI bleeding, constituting 40 % of episodes of severe hematochezia [63]. Several studies have indicated that LDA use is a significant increased risk factor for diverticular bleeding [64–66], and a recent meta-analysis confirmed this association [67]. LDA use may facilitate bleeding of the colonic diverticulum partly due to its antithrombotic property.

No previous study has investigated the prevalence of lower GI bleeding in LDA users. The incidence of lower GI bleeding is 20–80/100,000, based on some population-based studies from the USA, Spain, and Iceland [3, 63, 68]. Because LDA use is associated with an increased risk of lower GI bleeding, with an odds ratio of 2–3 [69], the incidence of lower GI bleeding in LDA users should be severalfold higher than in the general population; however, it remains considerably low, in contrast to the high prevalence of lower GI tract mucosal lesions in LDA users.

Risk Factors

Considering the relatively low prevalence of lower GI bleeding in chronic aspirin users, the identification of risk factors is important for targeting patients with a potential preventive therapy. However, the risk factors for lower GI tract injury in LDA users are only now being identified and thus remain largely unknown.

Using capsule endoscopy in 205 chronic LDA users, Endo et al. identified the use of enteric-coated aspirin and PPI use as independent risk factors for the presence of small intestinal mucosal breaks [70]. Enteric-coated aspirin is designed to dissolve in the proximal small intestine to prevent gastric damage; however, this formula may allow aspirin to contact the intestinal mucosa at a high concentration, resulting in exacerbation of small intestinal injury. By contrast, the finding that PPI use exacerbates small intestinal injury in LDA users is supported by animal model studies by Wallace et al., who found that PPI use provokes dysbiosis in the small intestine through potent suppression of gastric acid, leading to exacerbation of NSAID-induced mucosal injury at that site in rats [71]. More recently, two case-control studies with lower GI bleeding as a primary outcome reported conflicting results for the association between PPI use and lower GI bleeding in LDA users. Lanas et al. reported that PPI use is weakly but significantly associated with an increased risk of lower GI bleeding in NSAIDs and/or LDA users [69], whereas Nagata et al. demonstrated that PPI use was not associated with the risk of lower GI bleeding in the entire cohort and even among LDA users [72]. Whether PPI use paradoxically exacerbates small intestinal injury in LDA users but PPI effectively prevents upper GI mucosal injury is a critical issue to be solved when managing aspirin users. An additional high-quality study to determine the association between PPI use and small intestinal mucosal

injury in LDA users is required to establish a comprehensive strategy for the prevention of mucosal injury to the overall (upper and lower) GI tract.

Prevention

Recently, there have been some attempts to prevent LDA-induced small intestinal injury, although these studies are all small and explorative. Using capsule endoscopy, Watanabe et al. demonstrated that misoprostol is effective in reducing the number of mucosal breaks of the small intestine in chronic LDA users, although a substantial portion (3 of 11) of patients experienced severe side effects of misoprostol (diarrhea) and dropped out of the study [73]. Endo et al., using capsule endoscopy in randomized controlled trial, indicated that probiotic (*Lactobacillus casei*) administration significantly decreased the number of mucosal breaks of the small intestine in chronic LDA users, with no side effects of the probiotics during the study period of 3 months [74]. This result is supported by an animal model study that showed the effectiveness of probiotics (*Lactobacillus casei* strain Shirota) on indomethacin-induced small intestinal injury [75].

Some studies have demonstrated the effectiveness of rebamipide in prevention of small intestinal injury in LDA users. As discussed above, rebamipide may exert its beneficial effects on not only the gastroduodenal mucosa but also the small intestinal mucosa by increasing mucosal prostaglandin biosynthesis [47]. Mizukami et al., using capsule endoscopy in healthy volunteers, indicated the significant effectiveness of rebamipide in reducing the number of subjects with small intestinal mucosal breaks [76]. More recently, Watanabe et al. used capsule endoscopy in a randomized, double-blind placebo-controlled trial and demonstrated that a high dose of rebamipide, but not placebo, significantly decreased the number of mucosal breaks in the small intestine in chronic LDA users without any side effects [77]. These studies indicate that probiotics and rebamipide are promising agents to prevent LDA-induced small intestinal injury. However, the number and presence of mucosal breaks were defined as primary outcomes for these studies, and the clinical significance of these lesions remains obscure because of the high prevalence of these lesions in the small intestines of LDA users. Further studies are required to investigate the effectiveness of candidate drugs on LDA-related small intestinal adverse lesions using the more rigorous clinical outcomes, such as decreased GI bleeding.

By contrast, there is no established prophylaxis for lower GI bleeding, including diverticular bleeding, in LDA users at present. In particular, diverticular bleeding usually occurs in the absence of mucosal injury [78], and a different preventive strategy other than the administration of muco-protective drugs is required to reduce the risk of LDA-related diverticular bleeding.

Conclusion

A preventive strategy for LDA-induced upper GI adverse events is being established that targets high-risk patients with preventive co-therapy using PPI administration. However, the epidemiology, risk factors, and treatment for LDA-induced lower GI adverse events are largely unknown at present. Because the incidence of lower GI bleeding is increasing in LDA users but that of the upper GI bleeding is decreasing, a preventive strategy for drug-induced lower GI adverse events must be established immediately.

References

1. Yeomans ND, Lanas AI, Talley NJ, Thomson AB, Daneshjoo R, Eriksson B, et al. Prevalence and incidence of gastroduodenal ulcers during treatment with vascular protective doses of aspirin. Aliment Pharmacol Ther. 2005;22:795–801.
2. Serrano P, Lanas A, Arroyo MT, Ferreira IJ. Risk of upper gastrointestinal bleeding in patients taking

low-dose aspirin for the prevention of cardiovascular diseases. Aliment Pharmacol Ther. 2002;16:1945–53.
3. Lanas A, García-Rodríguez LA, Polo-Tomás M, Ponce M, Alonso-Abreu I, Perez-Aisa MA, Perez-Gisbert J, Bujanda L, Castro M, Muñoz M, Rodrigo L, Calvet X, Del-Pino D, Garcia S. Time trends and impact of upper and lower gastrointestinal bleeding and perforation in clinical practice. Am J Gastroenterol. 2009;104:1633–41.
4. Casado Arroyo R, Polo-Tomas M, Roncalés MP, Scheiman J, Lanas A. Lower GI bleeding is more common than upper among patients on dual antiplatelet therapy: long-term follow-up of a cohort of patients commonly using PPI co-therapy. Heart. 2012;98:718–23.
5. Sostres C, Gargallo CJ. Gastrointestinal lesions and complications of low-dose aspirin in the gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2012; 26:141–51.
6. García Rodríguez LA, Hernández-Díaz S, de Abajo FJ. Association between aspirin and upper gastrointestinal complications: systematic review of epidemiologic studies. Br J Clin Pharmacol. 2001;52:563–71.
7. Derry S, Loke YK. Risk of gastrointestinal haemorrhage with long term use of aspirin: meta-analysis. BMJ. 2000;321(7270):1183–7.
8. Iijima K, Ara N, Abe Y, Koike T, Iwai W, Iwabuchi T, Ichikawa T, Kamata Y, Ishihara K, Shimosegawa T. Association of gastric acid and mucus secretion level with low-dose aspirin-induced gastropathy. J Gastroenterol. 2012;47:150–8.
9. Iijima K, Iwabuchi T, Ara N, Koike T, Shinkai H, Kamata Y, Ichikawa T, Ishihara K, Shimosegawa T. Reactive increase in gastric mucus secretion is an adaptive defense mechanism against low-dose aspirin-induced gastropathy. Dig Dis Sci. 2013; 58:2266–74.
10. Wallace JL. Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? Physiol Rev. 2008;88:1547–65.
11. Laine L, Takeuchi K, Tarnawski A. Gastric mucosal defense and cytoprotection: bench to bedside. Gastroenterology. 2008;135:41–60.
12. Kitahora T, Guth PH. Effect of aspirin plus hydrochloric acid on the gastric mucosal microcirculation. Gastroenterology. 1987;93:810–7.
13. Rowe PH, Starlinger MJ, Kasdon E, Hollands MJ, Silen W. Parenteral aspirin and sodium salicylate are equally injurious to the rat gastric mucosa. Gastroenterology. 1987;93:863–71.
14. Sugano K. Prevention of Upper Gastrointestinal Ulcer and Complications in Low-Dose Aspirin Users. Curr Pharm Des. 2015;21:5082–8.
15. Wallace JL, Keenan CM, Granger DN. Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. Am J Physiol. 1990;259(3 Pt 1):G462–7.
16. Malfertheiner P, Chan FK, McColl KE. Peptic ulcer disease. Lancet. 2009;374(9699):1449–61. Review.
17. Cayla G, Collet JP, Silvain J, Thiefin G, Woimant F, Montalescot G. Prevalence and clinical impact of Upper Gastrointestinal Symptoms in subjects treated with low dose aspirin: the UGLA survey. Int J Cardiol. 2012;156(1):69–75.
18. Biondi-Zoccai GG, Lotrionte M, Agostoni P, Abbate A, Fusaro M, Burzotta F, Testa L, Sheiban I, Sangiorgi G. A systematic review and meta-analysis on the hazards of discontinuing or not adhering to aspirin among 50,279 patients at risk for coronary artery disease. Eur Heart J. 2006;27:2667–74.
19. Hsu PI, Tsai TJ. Epidemiology of Upper Gastrointestinal Damage Associated with Low-Dose Aspirin. Curr Pharm Des. 2015;21(35):5049–55.
20. Lanas A, Gargallo CJ. Management of low-dose aspirin and clopidogrel in clinical practice: a gastrointestinal perspective. J Gastroenterol. 2015;50: 626–37.
21. Uemura N, Sugano K, Hiraishi H, Shimada K, Goto S, Uchiyama S, Okada Y, Origasa H, Ikeda Y, MAGIC Study Group. Risk factor profiles, drug usage, and prevalence of aspirin-associated gastroduodenal injuries among high-risk cardiovascular Japanese patients: the results from the MAGIC study. J Gastroenterol. 2014;49:814–24.
22. Iijima K, Shimosegawa T. Geographic differences in low-dose aspirin-associated gastroduodenal mucosal injury. World J Gastroenterol. 2015;21:7709–17.
23. Iijima K, Shimosegawa T. Risk Factors for Upper GI Damage in Low-Dose Aspirin Users and the Interaction Between H. pylori Infection and Low-Dose Aspirin Use. Curr Pharm Des. 2015;21:5056–65.
24. Laine L. Review article: gastrointestinal bleeding with low-dose aspirin—what's the risk? Aliment Pharmacol Ther. 2006;24:897–908. Review.
25. Valkhoff VE, Sturkenboom MC, Kuipers EJ. Risk factors for gastrointestinal bleeding associated with low-dose aspirin. Best Pract Res Clin Gastroenterol. 2012;26:125–40.
26. Lanas A, Scheiman J. Low-dose aspirin and upper gastrointestinal damage: epidemiology, prevention and treatment. Curr Med Res Opin. 2007;23:163–73. Review.
27. Leontiadis GI, Molloy-Bland M, Moayyedi P, Howden CW. Effect of comorbidity on mortality in patients with peptic ulcer bleeding: systematic review and meta-analysis. Am J Gastroenterol. 2013;108: 331–45.
28. Marmo R, Koch M, Cipolletta L, Bianco MA, Grossi E, Rotondano G, PNED 2 Investigators. Predicting mortality in patients with in-hospital nonvariceal upper GI bleeding: a prospective, multicenter database study. Gastrointest Endosc. 2014; 79:741–9.
29. García Rodríguez LA, Lin KJ, Hernández-Díaz S, Johansson S. Risk of upper gastrointestinal bleeding with low-dose acetylsalicylic acid alone and in combination with clopidogrel and other medications. Circulation. 2011;123:1108–15.

30. Masclee GM, Valkhoff VE, Coloma PM, de Ridder M, Romio S, Schuemie MJ, Herings R, Gini R, Mazzaglia G, Picelli G, Scotti L, Pedersen L, Kuipers EJ, van der Lei J, Sturkenboom MC. Risk of upper gastrointestinal bleeding from different drug combinations. Gastroenterology. 2014; 147:784–92.
31. Fletcher EH, Johnston DE, Fisher CR, Koerner RJ, Newton JL, Gray CS. Systematic review: Helicobacter pylori and the risk of upper gastrointestinal bleeding risk in patients taking aspirin. Aliment Pharmacol Ther. 2010;32:831–9.
32. Iijima K, Ara N, Abe Y, Koike T, Iwabuchi T, Shinkai H, Uno K, Endo H, Asano N, Shimosegawa T. Biphasic effects of *H. pylori* infection on low-dose aspirin-induced gastropathy depending on the gastric acid secretion level. J Gastroenterol. 2012;47:1290–7.
33. Iijima K, Ara N, Abe Y, Koike T, Iwai W, Uno K, Asano N, Imatani A, Ohara S, Shimosegawa T. Gastric acid secretion level modulates the association between Helicobacter pylori infection and low-dose aspirin-induced gastropathy. J Gastroenterol. 2011;46:612–9.
34. Iijima K, Koike T, Ara N, Nakagawa K, Kondo Y, Uno K, Hatta W, Asano N, Imatani A, Shimosegawa T. Identification of a high-risk group for low-dose aspirin-induced gastropathy by measuring serum pepsinogen in H. pylori-infected subjects. J Gastroenterol. 2015;50:305–12.
35. Hatta W, Iijima K, Koike T, Kondo Y, Ara N, Asanuma K, Uno K, Asano N, Imatani A, Shimosegawa T. Endoscopic findings for predicting gastric acid secretion status. Dig Endosc. 2015; 27:582–9.
36. Abraham NS, Hlatky MA, Antman EM, Bhatt DL, Bjorkman DJ, Clark CB, Furberg CD, Johnson DA, Kahi CJ, Laine L, Mahaffey KW, Quigley EM, Scheiman J, Sperling LS, Tomaselli GF, ACCF/ACG/AHA. ACCF/ACG/AHA 2010 expert consensus document on the concomitant use of proton pump inhibitors and thienopyridines: a focused update of the ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use. Am J Gastroenterol. 2010;105:2533–49.
37. Chan FK, Goto S, Wu MS, Abola MT, Yeoh KG, Sutrisna B, Chua SS, Mahachai V, Turajane T, Wu B, Zeng QY, Sugano K. Burden of nonsteroidal anti-inflammatory and antiplatelet drug use in Asia: a multidisciplinary working party report. Clin Gastroenterol Hepatol. 2012;10:753–60.
38. Sugano K, Choi MG, Lin JT, Goto S, Okada Y, Kinoshita Y, Miwa H, Chiang CE, Chiba T, Hori M, Fukushima Y, Kim HS. Chang CY; on behalf of the LAVENDER Study Group. Multinational, double-blind, randomised, placebo-controlled, prospective study of esomeprazole in the prevention of recurrent peptic ulcer in low-dose acetylsalicylic acid users: the LAVENDER study. Gut. 2014;63:1061–8.
39. Scheiman JM, Devereaux PJ, Herlitz J, et al. Prevention of peptic ulcers with esomeprazole in patients at risk of ulcer development treated with low-dose acetylsalicylic acid: a randomised, controlled trial (OBERON). Heart. 2011;97:797–802.
40. Yeomans N, Lanas A, Labenz J, et al. Efficacy of esomeprazole (20 mg once daily) for reducing the risk of gastroduodenal ulcers associated with continuous use of low-dose aspirin. Am J Gastroenterol. 2008;103:2465–73.
41. Ekström P, Carling L, Wetterhus S, Wingren PE, Anker-Hansen O, Lundegårdh G, Thorhallsson E, Unge P. Prevention of peptic ulcer and dyspeptic symptoms with omeprazole in patients receiving continuous non-steroidal anti-inflammatory drug therapy. A Nordic multicentre study. Scand J Gastroenterol. 1996;31:753–8.
42. Yap PR, Goh KL. Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) Induced Dyspepsia. Curr Pharm Des. 2015;21:5073–81.
43. Taha AS, McCloskey C, Prasad R, Bezlyak V. Famotidine for the prevention of peptic ulcers and oesophagitis in patients taking low-dose aspirin (FAMOUS): a phase III, randomised, double-blind, placebo-controlled trial. Lancet. 2009;374 (9684):119–25.
44. Lin KJ, Hernández-Díaz S, García Rodríguez LA. Acid suppressants reduce risk of gastrointestinal bleeding in patients on antithrombotic or anti-inflammatory therapy. Gastroenterology. 2011;141: 71–9.
45. Ng FH, Wong SY, Lam KF, Chu WM, Chan P, Ling YH, Kng C, Yuen WC, Lau YK, Kwan A, Wong BC. Famotidine is inferior to pantoprazole in preventing recurrence of aspirin-related peptic ulcers or erosions. Gastroenterology. 2010;138:82–8.
46. Donnelly MT, Goddard AF, Filipowicz B, Morant SV, Shield MJ, Hawkey CJ. Low-dose misoprostol for the prevention of low-dose aspirin-induced gastroduodenal injury. Aliment Pharmacol Ther. 2000;14: 529–34.
47. Arakawa T, Kobayashi K, Yoshikawa T, Tarnawski A. Rebamipide: overview of its mechanisms of action and efficacy in mucosal protection and ulcer healing. Dig Dis Sci. 1998;43(9 Suppl):5S–13.
48. Iijima K, Ichikawa T, Okada S, Ogawa M, Koike T, Ohara S, Shimosegawa T. Rebamipide, a cytoprotective drug, increases gastric mucus secretion in human: evaluations with endoscopic gastrin test. Dig Dis Sci. 2009;54:1500–7.
49. Tozawa K, Oshima T, Okugawa T, Ogawa T, Ohda Y, Tomita T, Hida N, Fukui H, Hori K, Watari J, Nakamura S, Miwa H. A randomized, double-blind, placebo-controlled study of rebamipide for gastric mucosal injury taking aspirin with or without clopidogrel. Dig Dis Sci. 2014;59:1885–90.
50. Yamamoto T, Isono A, Mishina Y, Ebato T, Shirai T, Nakayama S, Nagasawa K, Abe K, Hattori K, Ishii T, Kuyama Y. Gastroduodenal mucosal injury in patients

taking low-dose aspirin and the role of gastric mucoprotective drugs: possible effect of rebamipide. J Clin Biochem Nutr. 2010;47:27–31.
51. Chan FK, Ching JY, Suen BY, Tse YK, Wu JC, Sung JJ. Effects of Helicobacter pylori infection on long-term risk of peptic ulcer bleeding in low-dose aspirin users. Gastroenterology. 2013;144:528–35.
52. Malfertheiner P, Megraud F, O'Morain CA. et al; European Helicobacter Study Group. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. Gut. 2012;61:646–64.
53. Pavlidis P, Bjarnason I. Aspirin Induced Adverse Effects on the Small and Large Intestine. Curr Pharm Des. 2015;21(35):5089–93.
54. Sostres C, Lanas A. Epidemiology of Low Dose Aspirin Damage in the Lower Gastrointestinal Tract. Curr Pharm Des. 2015;21:5094–100.
55. Higuchi K, Umegaki E, Watanabe T, Yoda Y, Morita E, Murano M, Tokioka S, Arakawa T. Present status and strategy of NSAIDs-induced small bowel injury. J Gastroenterol. 2009;44: 879–88. Review.
56. Robert A, Asano T. Resistance of germ-free rats to indomethacin induced intestinal inflammation. Prostaglandins. 1977;14:333–41.
57. Smecuol E, Pinto Sanchez MI, Suarez A, Argonz JE, Sugai E, Vazquez H, Litwin N, Piazuelo E, Meddings JB, Bai JC, Lanas A. Low-dose aspirin affects the small bowel mucosa: results of a pilot study with a multidimensional assessment. Clin Gastroenterol Hepatol. 2009;7:524–9.
58. Hilsden RJ, Meddings JB, Sutherland LR. Intestinal permeability changes in response to acetylsalicylic acid in relatives of patients with Crohn's disease. Gastroenterology. 1996;110:1395–403.
59. Leung WK, Bjarnason I, Wong VW, Sung JJ, Chan FK. Small bowel enteropathy associated with chronic low-dose aspirin therapy. Lancet. 2007;369 (9561):614.
60. Endo H, Hosono K, Inamori M, Kato S, Nozaki Y, Yoneda K, Akiyama T, Fujita K, Takahashi H, Yoneda M, Abe Y, Kirikoshi H, Kobayashi N, Kubota K, Saito S, Matsuhashi N, Nakajima A. Incidence of small bowel injury induced by low-dose aspirin: a crossover study using capsule endoscopy in healthy volunteers. Digestion. 2009; 79:44–51.
61. Moore RA, Derry S, McQuay HJ. Faecal blood loss with aspirin, nonsteroidal anti-inflammatory drugs and cyclo-oxygenase-2 selective inhibitors: systematic review of randomized trials using autologous chromium-labelled erythrocytes. Arthritis Res Ther. 2008;10:R7. Review.
62. Fortun PJ, Hawkey CJ. Nonsteroidal antiinflammatory drugs and the small intestine. Curr Opin Gastroenterol. 2005;21:169–75. Review.
63. Longstreth GF. Epidemiology and outcome of patients hospitalized with acute lower gastrointestinal hemorrhage: a population-based study. Am J Gastroenterol. 1997;92:419–24.
64. Strate LL, Liu YL, Huang ES, Giovannucci EL, Chan AT. Use of aspirin or nonsteroidal anti-inflammatory drugs increases risk for diverticulitis and diverticular bleeding. Gastroenterology. 2011;140:1427–33.
65. Yamada A, Sugimoto T, Kondo S, Ohta M, Watabe H, Maeda S, Togo G, Yamaji Y, Ogura K, Okamoto M, Yoshida H, Kawabe T, Kawase T, Omata M. Assessment of the risk factors for colonic diverticular hemorrhage. Dis Colon Rectum. 2008;51:116–20.
66. Nagata N, Niikura R, Aoki T, Shimbo T, Kishida Y, Sekine K, Tanaka S, Watanabe K, Sakurai T, Yokoi C, Akiyama J, Yanase M, Mizokami M, Uemura N. Colonic diverticular hemorrhage associated with the use of nonsteroidal anti-inflammatory drugs, low-dose aspirin, antiplatelet drugs, and dual therapy. J Gastroenterol Hepatol. 2014;29:1786–93.
67. Yuhara H, Corley DA, Nakahara F, Nakajima T, Koike J, Igarashi M, Suauki T, Mine T. Aspirin and non-aspirin NSAIDs increase risk of colonic diverticular bleeding: a systematic review and meta-analysis. J Gastroenterol. 2014;49:992–1000.
68. Hreinsson JP, Gumundsson S, Kalaitzakis E, Björnsson ES. Lower gastrointestinal bleeding: incidence, etiology, and outcomes in a population-based setting. Eur J Gastroenterol Hepatol. 2013;25:37–43.
69. Lanas A, Carrera-Lasfuentes P, Arguedas Y, García S, Bujanda L, Calvet X, Ponce J, Perez-Aísa Á, Castro M, Muñoz M, Sostres C, García-Rodríguez LA. Risk of upper and lower gastrointestinal bleeding in patients taking nonsteroidal anti-inflammatory drugs, antiplatelet agents, or anticoagulants. Clin Gastroenterol Hepatol. 2015;13:906–12.
70. Endo H, Sakai E, Taniguchi L, Kessoku T, Komiya Y, Ezuka A, Kawamura H, Taguri M, Higurashi T, Ohkubo H, Yamada E, Takahashi H, Inamori M, Maeda S, Sakaguchi T, Hata Y, Nagase H, Nakajima A. Risk factors for small-bowel mucosal breaks in chronic low-dose aspirin users: data from a prospective multicenter capsule endoscopy registry. Gastrointest Endosc. 2014;80:826–34.
71. Wallace JL, Syer S, Denou E, de Palma G, Vong L, McKnight W, Jury J, Bolla M, Bercik P, Collins SM, Verdu E, Ongini E. Proton pump inhibitors exacerbate NSAID-induced small intestinal injury by inducing dysbiosis. Gastroenterology. 2011;141:1314–22.
72. Nagata N, Niikura R, Aoki T, Sakurai T, Moriyasu S, Shimbo T, Sekine K, Okubo H, Watanabe K, Yokoi C, Yanase M, Akiyama J, Uemura N. Effect of proton-pump inhibitors on the risk of lower gastrointestinal bleeding associated with NSAIDs, aspirin, clopidogrel, and warfarin. J Gastroenterol. 2015;50:1079–86.
73. Watanabe T, Sugimori S, Kameda N, Machida H, Okazaki H, Tanigawa T, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Higuchi K, Arakawa T. Small bowel injury by low-dose enteric-coated aspirin and treatment with misoprostol: a pilot study. Clin Gastroenterol Hepatol. 2008;6:1279–82.

74. Endo H, Higurashi T, Hosono K, Sakai E, Sekino Y, Iida H, Sakamoto Y, Koide T, Takahashi H, Yoneda M, Tokoro C, Inamori M, Abe Y, Nakajima A. Efficacy of Lactobacillus casei treatment on small bowel injury in chronic low-dose aspirin users: a pilot randomized controlled study. J Gastroenterol. 2011;46:894–905.
75. Watanabe T, Nishio H, Tanigawa T, Yamagami H, Okazaki H, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Asahara T, Nomoto K, Higuchi K, Takeuchi K, Arakawa T. Probiotic Lactobacillus casei strain Shirota prevents indomethacin-induced small intestinal injury: involvement of lactic acid. Am J Physiol Gastrointest Liver Physiol. 2009;297: G506–13.
76. Mizukami K, Murakami K, Abe T, Inoue K, Uchida M, Okimoto T, Kodama M, Fujioka T. - Aspirin-induced small bowel injuries and the preventive effect of rebamipide. World J Gastroenterol. 2011;17:5117–22.
77. Watanabe T, Takeuchi T, Handa O, Sakata Y, Tanigawa T, Shiba M, Naito Y, Higuchi K, Fujimoto K, Yoshikawa T, Arakawa T. A multicenter, randomized, double-blind, placebo-controlled trial of high-dose rebamipide treatment for low-dose aspirin-induced moderate-to-severe small intestinal damage. PLoS One. 2015;10, e0122330.
78. von Rahden BH, Germer CT. Pathogenesis of colonic diverticular disease. Langenbecks Arch Surg. 2012;397:1025–33.

Aspirin in the Treatment and Prevention of Cardiovascular Disease: Need for Individual Clinical Judgments

11

Sarah K. Wood, Angel Lanas, and Charles H. Hennekens

Introduction

Cardiovascular disease (CVD), which includes coronary heart disease (CHD) and stroke, is by far the leading cause of death in most developed countries and is rapidly becoming the leading cause of death in the world. This alarming and potentially avoidable situation results mainly from major increases in cigarette smoking, obesity, and physical inactivity in both developed and developing countries [1]. In this context, the availability of an adjunctive drug therapy that is readily available at low cost represents a desirable possible option which requires a reliable totality of evidence that includes large-scale randomized trials designed to test the hypothesis.

The totality of evidence on aspirin in a very wide range of high-risk patients supports its routine prescription by health-care providers to decrease their risks of subsequent occlusive CVD events, but, in low-risk primary prevention subjects, the absolute benefits on occlusion may not outweigh the absolute risks on bleeding [2].

In this chapter, we review the benefits and risks of aspirin in high-risk secondary and primary prevention patients as well as moderate- and low-risk primary prevention subjects. We quantitate the relative and absolute benefits on various manifestations of occlusive vascular diseases as well as the relative and absolute risks on gastrointestinal and bleeding consequences. Although aspirin is available in many countries over the counter, its utilization to treat and prevent chronic conditions such as cardiovascular disease should be based on an individual judgment of the responsible clinician that weighs the absolute benefits against the absolute risks.

Aspirin and Its Beneficial Effects on Occlusive CVD Events

Who Does Need Aspirin?

How Much Is the Magnitude of the Benefit?

In secondary prevention of CVD, the Antithrombotic Trialist's Collaboration performed the most comprehensive, worldwide meta-

S.K. Wood, M.D.
Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL, USA

A. Lanas, M.D., Ph.D., A.G.A.F., A.C.G.F.
Universidad de Zaragoza, IIS Aragón, CIBER Enfermedades Hepáticas y Digestivas (CIBERehd), Aparato Digestivo, Zaragoza, Spain

Service of Digestive Diseases, University Hospital Lozano Blesa, Zaragoza, Spain
e-mail: angel.lanas@gmail.com

C.H. Hennekens, M.D. (✉)
First Sir Richard Doll Professor & Senior Academic Advisor to the Dean, Charles E. Schmidt College of Medicine, Florida Atlantic University, Boca Raton, FL, USA
e-mail: PROFCHHMD@prodigy.net

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_11

analysis of 195 randomized trials of antiplatelet therapy, principally with aspirin, among more than 135,000 high-risk patients with prior evidence of cardiovascular disease, including prior or acute myocardial infarction, prior or acute stroke, or transient ischemia attacks, and other high-risk groups such as unstable angina, chronic stable coronary disease, and peripheral artery disease, as well as patients with coronary artery bypass grafts or percutaneous coronary interventions [3]. Aspirin produced a statistically significant and clinically important 22 % reduction in risk of subsequent vascular event. In this wide range of patients with prior cardiovascular disease, there were absolute reductions of approximately 36 vascular events per 1000 patients with a prior myocardial infarction treated for a mean of 27 months, 36 events per 1000 patients with a previous stroke or transient ischemic attack treated for 29 months, and 22 events per 1000 patients with other high-risk conditions treated for 22 months. With respect to dose of aspirin, in indirect comparisons as well as direct comparisons in three trials testing this hypothesis, there were no significant differences in efficacy or safety between doses of 75–150 mg/day and 160–325 mg/day. In addition, the most plausible mechanisms of aspirin are on thrombosis and statins on atherosclerosis, suggesting that the benefits of both drugs used simultaneously would be additive [4]. Importantly, relevant information on this hypothesis was generated from a meta-analysis of randomized trials of statins in secondary prevention which aspirin was used in varying frequencies. In this meta-analysis, the combination of aspirin and statins conferred, at the very least, additive clinical benefits than either agent alone on myocardial infarction, occlusive stroke, and death from cardiovascular disease. In fact, the probability that the benefits were greater than just additive was 92 %. Finally, these benefits were apparent in the two largest individual trials, namely, LIPD and CARE, that comprised this meta-analysis [5].

With respect to aspirin given during acute myocardial infarction, the Second International Study of Infarct Survival (ISIS-2) randomized 17,187 patients within 24 h on onset of their symptoms of acute myocardial infarction in a 2×2 factorial design to aspirin (162.5 mg), streptokinase (SK) (1.5 million units), both active treatments, or both placebos [6]. At 35 days, the primary prespecified endpoints of total mortality were reduced to 23 % by aspirin, 25 % by SK, and 42 % by aspirin and SK together. For aspirin, the mortality benefits were similar regardless of whether administration was within 1 h or up to 24 h after onset of symptoms of acute MI. In contrast, those treated within 6 h with SK had a 30 % reduction in mortality and with SK and aspirin a 52 % reduction. Among those assigned at random to aspirin, there were statistically significant and clinically important reductions in vascular deaths of 23 %, nonfatal reinfarction of 49 %, and nonfatal stroke of 46 %. Major bleeds requiring transfusions were similar in the aspirin and placebo groups (0.4 %). After 35 days of treatment with aspirin, there were no excess risks of cerebral hemorrhages and only a slight increase in major bleeds. In terms of absolute risk reductions of vascular events, there was an avoidance of 38 events per 1000 patients with an acute myocardial infarction treated for one month [6].

In acute occlusive stroke, there are two landmark trials of aspirin. In each trial, occlusive stroke was initially diagnosed by the responsible clinician and subsequently confirmed by CT scanning. The International Stroke Trial (IST) randomized 19,435 patients to 300 mg aspirin daily or open control [7]. The Chinese Acute Stroke Trial (CAST) randomized about 20,000 patients to 160 mg aspirin daily or placebo [8]. Each showed benefits, and a meta-analysis showed a statistically significant and clinically important 11 % reduction in vascular events as well as nonfatal stroke and vascular deaths. In terms of absolute risk reductions, for every 1000 patients with acute occlusive stroke, treatment with aspirin avoided nine vascular events. Thus, for all patients with acute occlusive stroke, aspirin should be administered promptly and continued long term [7, 8].

As regards primary prevention, the Physician's Health Study was the first to

demonstrate a statistically significant benefit of aspirin on first myocardial infarction in 22,071 apparently healthy men [9, 10]. Since that time, there have been five additional major trials in men and women which comprise over 90,000 subjects. A comprehensive meta-analysis of these six major primary prevention trials using individual participant data provided more reliable comparison of the benefits and risks of aspirin in apparently healthy people. While all four of the proportional reductions in major coronary events and in ischemic stroke in the primary and in the secondary prevention trials were similar to each other, vascular mortality was not significantly reduced in the primary prevention trials. Since the numbers of fatal events were far smaller in the primary prevention trials, a proportional reduction comparable with that in the secondary prevention trials could not be excluded. Regardless of the similarities in proportional reductions, the absolute benefits of aspirin are far smaller in the primary than in the secondary prevention trials due to the far lower absolute risks of the apparently healthy subjects [11].

In the primary prevention trials, there were no significant modifications of the benefits of aspirin by age, smoking history, blood pressure, total cholesterol, body mass index, history of diabetes, or sex. In addition, an earlier suggestion that the beneficial effects of aspirin in primary prevention might differ between men and women has not been supported by the more robust data from the secondary prevention trials [12].

In primary prevention, aspirin reduces risk of a first myocardial infarction, but the data on stroke and vascular deaths remain inconclusive. In addition, the average absolute risk of subjects randomized in the primary prevention trials was so low that it is not possible to get reliable estimates of the benefit-to-risk ratio in primary prevention in subjects at moderate risk. Nonetheless, to maximize the benefit-to-risk ratio in primary prevention, most current guidelines recommend that aspirin be given to those above a certain level of absolute risk at baseline. These guidelines implicitly assume, perhaps erroneously, that risks of bleeding remain constant and that the GI risk in all individuals is similar. While the currently available trial results could well help inform appropriate individual clinical judgments on use of long-term aspirin, they do not seem to justify general guidelines advocating the routine use of aspirin in all apparently healthy individuals above a moderate level of risk of coronary heart disease. The ongoing trials in moderate- to high-risk primary prevention may facilitate a reliable benefit-to-risk ratio of aspirin in primary prevention among subjects at moderate risk [11]. Nobody would disagree that a nonfatal myocardial infarction or stroke is more likely to be disabling than a nonfatal bleed, but any judgment about the use of aspirin in primary prevention should be made on an individual clinical basis. Thus, in primary prevention, at present, the appropriate and judicious use of aspirin by clinicians based on individual clinical judgments that weigh the absolute benefits on first myocardial infarction against the absolute risks of the drug will avoid premature morbidity and possibly mortality from cardiovascular disease.

Aspirin and Risks of Bleeding in the Gastrointestinal Tract

Extent of the Damage to Upper and Lower GI Tract: How much Is the Risk? Who Is at Risk?

Due to its antiplatelet effect, aspirin increases the risk of bleeding in different organs and systems. A recent meta-analysis with data from 31 trials reporting on any bleeds showed a significantly increased risk of bleeding using low-dose aspirin compared with controls (OR, 1.54; 95 % CI, 1.36–1.74; $P < .001$). Seventy one individuals (95 % CI, 63–90) had to be treated to harm (NNH value) a patient with any bleed. The incidence rate difference was 8.1 (95 % CI, 4.0–12.2) per 1000 person-years. The authors also found an association between dose of aspirin and increased risks of bleeding (B coefficient, 0.60; 95 % CI, 0.18–1.02; $P = .004$) and also an

increased risk for hemorrhagic strokes (92 vs. 56; Peto OR, 1.67; 95 % CI, 1.12–2.48; $P = .008$; I2 = 21 %) [13].

The most frequent site of significant bleeding events associated with aspirin treatment occurs in the GI tract. Aspirin induces a wide spectrum of adverse events in GI tract, including symptoms without lesions to bleeding from the upper and lower GI tract, although the most common cause is peptic ulcer bleeding. Upper GI symptoms include those related to gastroesophageal reflux and dyspepsia which can be present in up to 15–20 % of patients taking aspirin [14, 15]. These symptoms are associated with decreased adherence or even aspirin therapy discontinuation as high as 50 %, which is associated with a threefold increased risk of CV events. Clinical symptoms are not predictive of the presence of mucosal damage. Endoscopic-controlled studies have shown that most patients taking aspirin have gastroduodenal erosions. However, the incidence of ulcers is lower [16, 17]. A study of 187 patients taking aspirin without gastro-protectant drugs showed an ulcer point prevalence of 11 % (95 % CI 6.3–15.1 %) and projected a yearly ulcer incidence of 28 %. Only 20 % of patients with ulcer had dyspeptic symptoms, not significantly different from patients without ulcer [14].

It is estimated that aspirin use is associated with a two–fourfold increase in symptomatic or complicated ulcers [13, 18]. The estimated average excess risk is five cases per 1000 aspirin users per year [19]. A meta-analysis of 33 RCTs involving 87,581 individuals with 338,735 person-years of follow-up evaluation [13] found an increased risk of any GI bleeds with low-dose aspirin use (OR, 1.31; 95 % CI, 1.21–1.42; $P < .001$), translating into an NNH of 166 (95 % CI, 125–250; IRD, 2.1; 95 % CI, 0–4.7 per 1000 person-years). Neither fatal GI bleeds (OR, 0.94; 95 % CI, 0.47–1.87; $P = .87$) nor fatal hemorrhagic strokes (33 vs. 23; Peto OR, 1.42; 95 % CI, 0.84–2.41) were associated significantly with aspirin use. Observational studies have reported in general higher-risk estimates of upper GI bleeding [20, 21].

Another meta-analysis of individual participant data in six primary prevention trials (95,000 individuals at low average risk) and 16 secondary prevention trials (17,000 individuals at high average risk) [11, 22] found in primary prevention studies a small increase in hemorrhagic stroke with aspirin treatment vs. placebo (0.04 %vs. 0.03 %, $p = 0.05$), with no significant differences in vascular mortality (0.19 % vs. 0.19 % per year, $p = 0.7$). Aspirin allocation increased major gastrointestinal and extracranial bleeds (0.10 %vs. 0.07 % per year, $p < 0.0001$). In this study, the main risk factors for coronary disease were also risk factors for bleeding. In the secondary prevention trials, aspirin allocation was not associated with that increase in hemorrhagic stroke. Aspirin increased major gastrointestinal and other extracranial bleeds by about half in the primary prevention trials (0·10 % vs. 0.07 % per year; RR 1·54 [1.30–1·82], $p < 0.0001$). The excess risk was mainly due to nonfatal bleeds, perhaps by chance. In secondary prevention trials, there was an excess of major bleeds among aspirin-treated patients (RR 2·69 [1.25–5.76], $p = 0.01$) (Table 11.1).

The association of aspirin use with adverse effects on the lower GI tract is less documented. A systematic review found a small increase of fecal blood loss (0.5–1.5 mL per day) in aspirin users [23]. One study conducted in healthy volunteers showed that enteric-coated aspirin treatment was associated with small intestine mucosal damage in 50 % of volunteers; a few volunteers developed asymptomatic deep ulcers. These lesions could explain why some aspirin users develop bleeding of "unknown" source, iron deficiency anemia, or hypoproteinemia. A study in health professionals concluded that aspirin increases significantly the risk of diverticulitis and diverticular bleeding, reporting an HR = 1.25 (95 % CI 1.05–1.47) for diverticulitis and an HR = 1.70 (95 % CI 1.21–2.39) for diverticular bleeding [24]. Another Japanese study also found association of aspirin and other antiplatelet agents with diverticular bleeding [25]. A more recent study by Lanas et al. [26] quantified the relative risk of upper and lower GI

Table 11.1 Absolute and relative effects of aspirin of three major outcomes in primary and secondary prevention trials in a meta-analysis of the Antithrombotic Trialist's (ATT) Collaboration [11]

Variable	Primary prevention (660,000 person-years)	Secondary prevention (43,000 person-years)	Rate ratio primary prevention (95 % CI)	Rate ratio secondary prevention (95 % CI)	*P* value for heterogeneity
Major coronary events	934 vs. 1115	995 vs. 1214	0.82 (0.75–0.90)	0.80 (0.73–0.88)	0.7
Vascular death	619 vs. 637	825 vs. 896	0.97 (0.87–1.09)	0.91 (0.82–1.00)	0.4
Major extracranial bleed	335 vs. 219	23 vs. 6	1.54 (1.30–1.82)	2.69 (1.25–5.76)	0.2

bleeding associated with use of NSAIDs, antiplatelet drugs, and anticoagulants. NSAIDs, anticoagulants, aspirin, and nonaspirin antiplatelet agents were associated with both upper and lower GI bleeding. The adjusted relative risks of upper and lower GI bleeding for aspirin were 1.7 (95 % CI 1.2–2.6) and 2.7 (95 % IC 1.8–4.1), respectively, whereas for nonaspirin antiplatelets were 2.8 (95 % CI 1.4–25.8) and 2.8 (95 % IC 1–3.2), respectively.

Risk Factors

The risk of upper GI complications differs among aspirin users. Several risk factors for GI bleeding in patients treated with antiplatelet aspirin therapy have been reported and include history of peptic ulcer disease, older age, concomitant use of NSAIDs or other antiplatelet agents or anticoagulants, severe comorbidity, aspirin dose, and *H. pylori* infection. Other potential risk factors have been also mentioned (corticosteroids, alcohol use, and high body mass). Importantly, one of the most recent meta-analyses [11, 22] concluded that GI and CV risk factors were similar including age, male sex, diabetes, smoking, and high blood pressure. The relative risk of GI bleeding increases with the number of risk factors present in the patient.

History of complicated and uncomplicated peptic ulcer is the most important risk factor in aspirin users [27, 29]. The meta-analysis of serious vascular events and major bleeds in six primary prevention trials and 16 secondary prevention trials showed that age (per decade) was associated with an increased risk of major extracranial bleed (RR 2.15, CI 95 %, 1.93–2.39) [11].

Aspirin use is frequent among NSAID users (20–25 % in clinical trials), mainly in the elderly. The combination increases further two–threefold the risk compared to monotherapy with aspirin [27, 28, 30]. The GI benefits of selective COX-2 inhibitors over nonselective NSAIDs are reduced with the coadministration of aspirin, although a meta-analysis of all available trials that included patients treated with aspirin and nonselective or selective NSAIDs showed a lower risk of GI complications in patients taking a selective COX-2 inhibitors plus aspirin, compared with those taking nonselective NSAIDs plus aspirin [RR 0.72 (95 % CI 0.62–0.95)] [31]. It must be pointed out that these studies were nonrandomized trials and the data were obtained from indirect comparisons.

Dual antiplatelet therapy (aspirin plus other antiplatelet agents) is common in several clinical scenarios. Dual therapy increases the risk of GI bleeding to a higher degree (two- to threefold) than aspirin alone. The absolute risk increase was in the range of 0.6–2.0 % [32]. In the CHARISMA (clopidogrel for high atherothrombotic risk and ischemic stabilization, management, and avoidance) trial, patients treated with the combination therapy had a higher risk of moderate to severe bleeding during the first year when compared to aspirin alone [33]. However, not all studies have confirmed these results [27, 34].

Anticoagulants do not affect directly the GI mucosa but have a high anti-hemostatic effect. Most data suggest that concomitant use of anticoagulant and aspirin increases the risk of GI bleeding to a higher degree than aspirin alone [13].

A recent systematic review evaluated the influence of *H. pylori* on upper GI bleeding risk in aspirin users. Authors concluded that current data do not allow performing meta-analyses and that no firm conclusion could be drawn on this issue [35]. We have recently performed the largest case-control study evaluating the interaction between *H. pylori* infection and aspirin or NSAID use. *H. pylori* infection, NSAID use, and aspirin treatment were independent risk factors for upper GI bleeding but found no interaction between aspirin and *H. pylori* infection [36]. In contrast, a cohort study showed that patients with peptic ulcer bleeding history treated with aspirin in whom *H. pylori* was eradicated had a similar recurrent bleeding rate not far different to those aspirin-treated patients with no risk factor [37]. Sub-analysis of this cohort showed however that in the presence of other concomitant gastrotoxic medication, the risk of bleeding was higher than in the average-risk cohort.

Iijima and colleagues evaluated the possible biphasic effects of *H. pylori* infection on aspirin-induced gastropathy depending on the gastric acid secretion level. They concluded that in the presence of sufficient amounts of gastric acid, *H. pylori* and aspirin could synergistically damage the gastric mucosa, while in the absence of sufficient gastric acid, the infection could even suppress the aspirin-induced gastropathy. These biphasic effects could explain the controversy data in the literature about the role of *H. pylori* infection in the GI risk in aspirin users [38].

Finally, aspirin dose is another important aspect to consider related to its GI risk of bleeding. Although studies are somehow conflicting, most data show that the GI risk with aspirin is dose dependent and that 75–100 mg daily is enough to obtain the CV benefits. Therefore, the current recommendation is to use the lowest possible aspirin dose ($\leq$100 mg/day) for the prevention of CV event. The risk of GI complications with aspirin seems to be higher in the first month of treatment, whereas with longer durations, the risk decreases and then remains constant over time [21, 39, 40]. This effect has been explained as the consequence of gastric adaptation to aspirin [41]. The use of enteric-coated or enteric-buffered preparations does not reduce the risk of GI complications [42]. The reason for this is explained on the understanding that the main effect of aspirin on the gastric mucosa depends on the systemic effects rather than in the local "topical" effects of this compound.

Aspirin Beyond the CV System and the GI Tract

With respect to other effects of aspirin, randomized data suggest benefits of low dose in the prevention of migraine [43] and high dose in the treatment [44]. Observational data suggest that elderly individuals who self-select for aspirin have lower rates of loss of cognitive function, but this hypothesis requires direct testing in randomized trials of sufficient size and duration [45].

In addition, a recently reported meta-analysis of randomized trials, most of which were not designed a priori to test the hypothesis, suggests beneficial effects of aspirin on overall cancer mortality [46].

Thus, the randomized data on prevention and treatment of colon polyps as well as primary prevention of colon cancer are more reliable than the data from other shorter-term trials which, in turn, are more reliable than the observational data on other cancers

How Do I Identify the Actual Risk of My Patient?

On the basis of the available evidence, the recommendation of aspirin in primary prevention depends on the accurate assessment of

Table 11.2 Cardiovascular risk evaluation

Very high
(a) CV disease documented by either invasive or noninvasive techniques, myocardial infarction acute coronary syndrome, coronary revascularization, cerebrovascular event, peripheral arterial disease
(b) Diabetes mellitus type 2 or type 1 associated with target organs (e.g., microalbuminuria 30–300 mg/24 h)
(c) Moderate to severe renal disease (estimated glomerular flow <60 mL/min/1.73 m^2)
(d) SCORE ≥ 10 %
High
(a) Any individual risk factor seriously increased such as familiar hypercholesterolemia or severe arterial hypertension
(b) SCORE ≥5 % y <10 %
Moderate
SCORE de ≥1 y <5 %
Low
SCORE <1 % y ausencia de otros factores de riesgo

Table 11.3 Gastrointestinal risk estimations

GI risk	Complications per 100 patients-year	NNT
Low		
No risk factors	<1.5	>120
Moderate		
Presence of 1–2 risk factors, e.g., age >65 or any combination (1–2)	2–10	10–100
High		
History of GI bleeding, therapy with ACOs, or 3 or more risk factors	>20	<10

cardiovascular risk as part of the decision-making process. CV risk can be easily estimated today by using the Framingham's 10-year risk estimations or the Systematic Coronary Risk Evaluation (SCORE), a European cardiovascular disease risk assessment. Framingham's tables for CV risk were based on the method reported on the paper published by Wilson and colleagues [47] for the prediction of cardiovascular heart disease (angina pectoris, recognized and unrecognized myocardial infarction, coronary insufficiency, and coronary heart disease death), and it is accessible online and in multiple apps. The risk is based on several key variables including age, gender, blood pressure, cholesterol levels, and smoking (http://www.framinghamheartstudy.org/ risk/coronary.html#tab3). The CV risk estimation with Framingham's tables seems to apply better for American countries and the UK.

The SCORE system provides an estimation of the 10-year risk of **fatal** cardiovascular disease stratification in the primary prevention of CV disease [48]. The CV risk can be grouped into low, moderate, and high based on a six-step method that combines a calculation of the 10-year risk for coronary heart disease and for noncoronary cardiovascular disease, together with the presence of CV risk factors, such as smoking, cholesterol, and systolic blood pressure. CV risk is estimated to be low when that risk is <2 %, moderate when the risk is placed between 2 and 5 %, and high when >5 %. Diabetes and previous CV pathologies (acute myocardial infarction, angina, and ictus) are not included in these models since they are categorized directly in the high-CV-risk group (Table 11.2). There is sufficient evidence to be considered; these CV risk estimators are accurate and are widely accepted and used worldwide.

GI Risk Estimations

GI risk estimations are not as standardized as those described for the CV risk. Based on previous reports [49, 50], patients may be classified into three GI categories when taking NSAIDs (Table 11.3). That risk can be used for any

patient who may receive potential gastrotoxic drug such as aspirin. Patients at low GI risk are considered patients without any of the risk factors mentioned in the previous section. Patients at moderate GI risk include those with at least one of the following GI risk factors: (1) age 60 or older, (2) concomitant use of NSAIDs, (3) concomitant use of corticosteroids, (4) concomitant use of other antiplatelet agents, (5) history of symptomatic peptic ulcer, and (6) history of dyspepsia. Patients at high GI risk were those with either a GI bleeding history or concomitant use of anticoagulants or the presence of three risk factors of those described for moderate GI risk. These levels of risk were based on the estimated incidence of events obtained from combining different risk factors that would put patients at a similar risk level to those with a history of bleeding peptic ulcers. In this way, in low-GI-risk patients, the expected rate of upper GI complications should not exceed 1.5 events per 100 patients/year, whereas for those at moderate GI risk, the rate should be between 1.5 and 10, and for high-GI-risk patients, the rate should be greater than ten events per 100 patients/year. Patients being treated with anticoagulants (warfarin or coumadin or NOACs) were considered high risk, because bleeding events in anticoagulated patients can be more severe, when taking other gastrotoxic drugs.

A recent study has provided an aspirin GI risk calculator tool [51] that may help physicians in assessing the actual risk of their patients and use appropriate therapy. The authors of this tool used data reported by Hernandez-Diaz and Garcia-Rodriguez [19] as a baseline for the construction of tables and algorithms. This study characterized aspirin users together with major gastrointestinal risk factors and provided incidence rates as well as excess risk of upper GI complications linked to low-dose aspirin. These estimations were based on data from the UK General Practice Research Database and systematic reviews of the literature. Based on those reports, the ASA Risk Calculator (available online at www.asariskcalculator.es) assumes an overall baseline upper GI bleeding incidence rate of 1 per 1000 person-years and then constructs absolute incidence rates within each risk subgroup based on pooled estimates and 95 % confidence interval reported from different meta-analyses. The calculator assumes that the pooled relative risks of upper GI bleeding was 2.0 for aspirin at doses <300 mg/day. The major risk factors for the development of upper GI bleeding were age, male gender, history of complicated peptic ulcer, history of uncomplicated peptic ulcer, and concomitant use of NSAIDs, anticoagulants, or clopidogrel. Based on those estimations, and as reported in their original article, the calculator shows that in patients with low CV risk, the use of aspirin induces more GI harm than CV benefits in almost all clinical scenarios. In patients with high CV risk, aspirin is recommended, but the GI harms may overcome sometimes the CV benefits in some patients. Eventually, the calculator provided recommendations that the use of PPI with/without *H. pylori* eradication can reduce the harm and increases the CV benefits of aspirin in most clinical scenarios. However, in patients with complicated peptic ulcer history and other risk factors, the CV benefits may still be offset by GI harm.

Can We Reduce the GI Risk?

Reducing the GI risk linked to aspirin treatment will increase the net beneficial CV effect and increase treatment compliance. There are several strategies to minimize the upper GI damage induced by cardiovascular aspirin: (1) reducing modifiable risk factors (including eradication of *H. pylori* infection), (2) using the most appropriate aspirin dose, and (3) using gastroprotective agents.

Reducing Modifiable Risk Factors

Avoidance of Concomitant Gastrotoxic Medication

Concomitant treatment of aspirin with NSAIDs (nonselective and COX-2 selective), other

antiplatelet agents, anticoagulants, and to a lesser extent corticosteroids increases the risk of developing upper GI bleeding complications and probably lower GI bleeding as well. Guidelines strongly suggest avoiding the combination of NSAIDs and aspirin if possible. In addition, the concomitant use of aspirin with ibuprofen, and perhaps naproxen, should be avoided because these NSAIDs interfere with the antiplatelet effect of aspirin. This is due to competition between both drugs for a common docking site within the COX-1 channel. If these combinations were used, aspirin should be taken first and well before dosing with ibuprofen or naproxen, but still interaction is possible, especially if a patient takes enteric-coated aspirin where aspirin is being released slowly for several hours and the T_{max} takes 4–5 h to occur [14–16].

Eradication of *H. pylori* Infection

H. pylori eradication is controversial in patients without history of peptic ulcer taking aspirin. This aspect has been commented above in the risk factors section. Several studies have evaluated the effect of *H. pylori* eradication in the prevention of peptic ulcer bleeding recurrence in aspirin users. The most important study was conducted by Chan and colleagues and compared long-term PPI treatment vs. *H. pylori* eradication in *H. pylori*-positive patient with a recent peptic ulcer GI bleeding event. The re-bleeding rate was similar in both groups at 6 months of follow-up [1.9 % vs. 0.9 %, respectively, (absolute difference 1 %; 95 % CI 1.9–3.9 %)] [55]. However, the small sample size and short time of follow-up could have prevented a different outcome and conclusion. The largest long-term prospective cohort study has been published recently [37]. Over nine hundred patients were divided into three cohorts and were followed up for 10 years or until death. The cohorts were (1) *H. pylori*-positive patients with bleeding ulcers in which *H. pylori* infection was eradicated, (2) *H. pylori*-negative patients with bleeding ulcers, and (3) new users of aspirin without prior peptic ulcer. None of them received regular PPI treatment. The incidence of upper GI bleeding was not significantly different between the *H. pylori*-eradicated cohort (1.09; 95 % CI 0.61–1.98) and the average-risk cohort of patients without history of peptic ulcer (0.67; 95 %CI 0.42–1.06). Sub-analysis of the *H. pylori*-related peptic ulcer bleeding cohort showed that in the presence of other risk factors, these patients had higher risk of ulcer bleeding compared to the non-peptic ulcer history cohort. Moreover, *H. pylori*-negative patients with bleeding ulcers had a high risk of recurrent bleeding. The impact of these findings is reduced because of the lack of direct comparisons and the clinical differences between the cohorts. The ongoing HEAT (Helicobacter Eradication Aspirin Trial) study aimed at the evaluation of the effect of *H. pylori* eradication on the incidence of upper GI bleeding in aspirin-treated patients can provide quality evidence on the role of eradication in primary CV prevention in aspirin users (ClinicalTrials.gov, NCT01506986), but this will take a few years to be known. In the meantime, guidelines recommend that *H. pylori* infection should be tested and treated in all patients with previous ulcer history. Still those at high risk should receive prevention therapy with antisecretory agents.

Aspirin Dose

As commented above, the GI bleeding risk is dose dependent, whereas the maximal CV beneficial effect can be obtained with 75–100 mg of aspirin daily. Based on these widely assumptions and evidence, today these low doses of aspirin are prescribed worldwide for this indication, and all risk-benefit balances are based on these doses that provide the best outcomes.

Gastroprotective Agents

Very few studies have evaluated the effect of misoprostol on aspirin-related GI injury. An endoscopic study showed that misoprostol significantly lowered the incidence of erosions in healthy volunteers taking LDA [56]. Misoprostol

has shown also to reduce the incidence of small bowel erosions in aspirin users [57].

Data with H_2 blockers are a bit more consistent. These drugs can suppress gastric acid production by up to 70 % over 24 h. Two prospective case-control studies developed by Lanas and colleagues showed conflictive data on the efficacy of H_2 receptor antagonist. In the first study published in 2001, the risk of upper GI bleeding in patient taking LDA was not significantly reduced by H_2 receptor antagonist use (OR 0. 5, 95 % CI 0.2–1.2) [28]. However, in the second study published in 2007, H_2 receptor antagonists reduced significantly the risk of upper GI bleeding in LDA users (RR 0.40, 95 % CI 0.19–0.73) [58]. As in many other observational studies, confounding factors may have affected the outcome and explain the differences between these two studies.

The most important clinical trial with H_2 blockers was conducted in Scotland and compared high-dose famotidine (20 mg/12 h) for 12 weeks vs. placebo in aspirin users without ulcers at baseline [59]. Patients treated with famotidine had a significantly lower incidence of ulcers than placebo group (3, 8 % vs. 23, 5 %, respectively). However, there were several relevant concerns to consider: (1) rate of *H. pylori* infection was higher in placebo group and (2) some patients of famotidine group did not have final endoscopy evaluation. In any case, due to the potential interaction of PPIs with clopidogrel, the use of famotidine in patients taking dual antiplatelet therapy has been recommended. However, most guidelines still recommend the use of PPI in high-risk patients taking aspirin [32].

PPIs are potent inhibitors of gastric acid secretion. Several studies have explored the impact of PPI on reducing endoscopic damage and the risk of GI complications in users of aspirin. Today, considerable evidence support that PPIs are more effective than H_2 blockers as gastroprotective agents in antiplatelet users [60] by comparing directly PPI (pantoprazole 20 mg) with high dose of famotidine (40 mg twice) in the prevention of recurrence of uncomplicated or complicated peptic ulcer in aspirin users. Recurrent GI bleeding and uncomplicated ulcer were significantly more common in the famotidine group than in the pantoprazole group (7.7 % vs. 0 %, $p < 0.05$ and 12.3 % vs. 0 %, $p < 0.05$, respectively). A recent nested case-control study investigated the impact of different prevention strategies against GI complications in aspirin or NSAID users; 2049 cases and 20,000 controls were included [61]. The risk of upper GI bleeding associated with PPI use was 0.5 events per 100 patient-years among aspirin users, 0.18 among clopidogrel users, and 0.17 among dual antiplatelet therapy users. The corresponding estimates for H_2 receptor antagonist tended to be smaller.

Many endoscopic studies have shown the high efficacy of different PPI compounds at standard doses in the prevention of upper gastrointestinal mucosal damage [58]. Sugano et al. [62] published this year the LAVENDER study. This was a double bind, randomized, placebo-controlled, and prospective trial that evaluated the efficacy of esomeprazole (20 mg once daily) for 72 weeks in the prevention of recurrent peptic ulcer in aspirin users. Authors concluded that esomeprazole 20 mg over 48 weeks prevented the recurrence of peptic ulcers. Interestingly 45 % of patients were *H. pylori* positive, which suggests that esomeprazole protected against ulcer recurrence irrespective of *H. pylori* status. The recent published PLANETARIUM study evaluated the efficacy, dose-response relationship (10 mg, 5 mg, and active control), and safety of rabeprazole for peptic ulcer recurrence over 24 weeks in Japanese patients on aspirin treatment. The cumulative recurrence rates of peptic ulcers were 1.4 and 2.8 % in rabeprazole groups (5 mg and 10 mg, respectively), significantly lower than in the active control group (21.7 %). In rabeprazole groups, there were not bleeding ulcers. Therefore, rabeprazole prevented recurrence of peptic ulcers without evidence of a major dose-response effect in patients on aspirin therapy [63].

The efficacy of PPI in the prevention of recurrence of ulcer complications has also been confirmed in several studies. Lai and colleagues [64] performed a RCT that compared lansoprazole (30 mg/day) with placebo in aspirin

users with history of peptic ulcer and who had already received *H. pylori* eradication therapy. Patients were treated with lansoprazole or placebo for one year. Patients on lansoprazole had significantly less recurrence of ulcer complications than those treated with placebo (1.6 % vs. 14.8 %). This study suggested that *H. pylori* eradication was not sufficient to prevent ulcer bleeding recurrence in high-risk aspirin users. Combined treatment (*H. pylori* eradication plus PPI) seems the most adequate therapy for these patients.

As we commented above, dual antiplatelet therapy with aspirin and clopidogrel increases the risk of GI bleeding. The use of this therapy is increasing, especially in patients with coronary stents. The COGENT study [65] evaluated both the occurrence of CV and GI events in this clinical scenario. Patients receiving dual antiplatelet therapy with clopidogrel and aspirin were randomized to omeprazole or placebo. A total of 3873 patients were included and 51 patients had a GI event (bleeding, symptomatic ulcers, or erosions, obstruction, or perforation). In the omeprazole group, the event rate was 1.1 % compared with 2.9 % in placebo group (HR 0.34, 95 % CI, 0.18–0.63, $p < 0.001$). The rate of upper GI bleeding was also significantly lower in PPI group (HR 0.13, 95 % CI, 0.03–0.56). No differences in CV events were present at the end of the study between the two arms, which rejected the hypothesis that omeprazole and clopidogrel interaction could have a clinical impact on the occurrence of CV events in patients taking dual therapy plus a PPI [65].

Prevention of Lower GI Bleeding with Aspirin

Very few studies have focused on the prevention of lower damage associated with aspirin. Only a preliminary work has suggested that co-therapy with probiotics can reduce the risk of developing anemia in patients who take aspirin and PPI [66]. This approach is based on the growing perception that the microbiota has a role in the mucosal damage induced by NSAIDs and aspirin in the small bowel affecting its permeability. PPI co-therapy would change the microbiota profile making the environment more susceptible to damage by these compounds.

A Rational Therapeutic Approach to Common Clinical Scenarios

Secondary Prevention

During Occlusive CVD

The Second International Study of Infarct Survival (ISIS-2) randomized 17,187 patients within 24 h on onset of their symptoms of acute myocardial infarction in a 2 × 2 factorial design to aspirin (162.5 mg), streptokinase (SK) (1.5 million units), both active treatments, or both placebos [6]. At 35 days, the primary prespecified endpoints of total mortality were reduced to 23 % by aspirin, 25 % by SK, and 42 % by aspirin and SK together. For aspirin, the mortality benefits were similar regardless of whether administration was within 1 h or up to 24 h after onset of symptoms of acute MI. In contrast, those treated within 6 h with SK had a 30 % reduction in mortality and with SK and aspirin a 52 % reduction.

Among those assigned at random to aspirin, there were statistically significant and clinically important reductions in vascular deaths of 23 %, nonfatal reinfarction of 49 %, and nonfatal stroke of 46 %. Major bleeds requiring transfusions were similar in the aspirin and placebo groups (0.4 %). After 35 days of treatment with aspirin, there were no excess risks of cerebral hemorrhages and only a slight increase in major bleeds. In terms of absolute risk reductions of vascular events, there was an avoidance of 38 events per 1000 patients with an acute myocardial infarction treated for one month.

With respect to the benefit-to-risk ratio, aspirin given within 24 h of onset of symptoms of acute myocardial infarction avoided 23 deaths with no increase in cerebral hemorrhage. In contrast, SK given within 12 h avoided 30 deaths but caused three cerebral hemorrhages. As regards benefit to cost, the cost per life saved during

acute MI is about $88,000 for tPA, $12,000 for SK, and $13 for aspirin [67].

There are two landmark trials of aspirin in acute occlusive stroke. In each trial, occlusive stroke was initially diagnosed by the responsible clinician and subsequently confirmed by CT scanning. The International Stroke Trial (IST) randomized 19,435 patients to 300 mg aspirin daily or open control (IST Lancet). The Chinese Acute Stroke Trial (CAST) randomized about 20,000 patients to 160 mg aspirin daily or placebo [8]. Each showed benefits and a meta-analysis showed a statistically significant and clinically important 11 % reduction in vascular events as well as nonfatal stroke and vascular deaths. In terms of absolute risk reductions, for every 1000 patients with acute occlusive stroke, treatment with aspirin avoided nine vascular events.

Thus, for all patients suffering acute myocardial infarction or acute occlusive stroke, aspirin should be administered promptly and continued long term [2].

Among Survivors of Occlusive CVD

The Antithrombotic Trialist's Collaboration performed the most comprehensive, worldwide meta-analysis of 195 randomized trials of antiplatelet therapy, principally with aspirin, among more than 135,000 high-risk patients with prior evidence of cardiovascular disease, including prior or acute myocardial infarction, prior or acute stroke, or transient ischemia attacks, and other high-risk groups such as unstable angina, chronic stable coronary disease, and peripheral artery disease, as well as patients with coronary artery bypass grafts or percutaneous coronary interventions [3]. Aspirin produced a statistically significant and clinically important 22 % reduction in risk of subsequent vascular event. In this wide range of patients with prior cardiovascular disease, there were absolute reductions of approximately 36 vascular events per 1000 patients with a prior myocardial infarction treated for a mean of 27 months, 36 events per 1000 patients with a previous stroke or transient ischemic attack treated for 29 months, and 22 events per 1000 patients with other high-risk conditions treated for 22 months. With respect to dose of aspirin, in indirect comparisons as well as direct comparisons in three trials testing this hypothesis, there were no significant differences in efficacy or safety between doses of 75–150 mg/day and 160–325 mg/day.

During Occlusive CVD in At-Risk GI Patients

Patients who develop an occlusive CVD and are at risk of GI complications based on the presence of risk factors should receive GI prevention therapy. This approach would prevent a GI bleeding event that may jeopardize the success of the CV therapy. The most recommended approach in that scenario is the prescription of a PPI at standard doses p.o. or endovenously if no oral feeding is permitted. In that case, a loading dose of 40 mg of pantoprazole or any other available PPI is required followed by 40 mg/day till oral route is reintroduced. In patients receiving dual antiplatelet therapy with aspirin and clopidogrel, the potential metabolic interaction of PPI with clopidogrel has been a hot topic for debate, and different regulatory bodies have suggested to avoid PPI therapy, especially omeprazole and esomeprazole [68]. Available clinical evidence [65], however, speaks against these strong recommendations, and different guidelines suggest PPI (any) therapy when the GI risk is high. Famotidine is an alternative, but prescribers should be aware that its efficacy in the prevention of GI events is lower than PPIs. At discharge, patients should be maintained on GI prevention therapy as risk factors are present (see next section).

Among Survivors of Occlusive CVD in At-Risk GI Patients

Patients who have survived to an occlusive CVD are at high risk of new CV events, and therefore, they will be taking aspirin or any other antiplatelet agent or combination of them. In many cases, they are also at increased GI risk, even when prescribed aspirin at doses as low as 75 mg/day. Special caution should be taken in those over the age of 70 or those who had a previous ulcer history. The best therapeutic approach is to prescribe co-therapy with a PPI at standard doses (20 mg of omeprazole, 20 mg

of pantoprazole, 20 mg of esomeprazole, 15 mg of lansoprazole, or 20 mg of rabeprazole). In patients who take clopidogrel concomitantly with aspirin, a PPI is also recommendable if the GI risk is high. As commented above, famotidine could be prescribed at high dose but in the understanding that its efficacy is lower than that observed, for example, with pantoprazole. Finally, in case of patients with ulcer history, *H. pylori* eradication should be carried out followed by PPI therapy. Use of other gastrotoxic drugs should be avoided [69].

High-Risk Primary Prevention

Patients with Metabolic Syndrome

Patients with metabolic syndrome, a constellation of obesity, dyslipidemia, hypertension, and insulin resistance leading to diabetes, have 10-year risks of a first CHD event of 16–18 %. In addition to therapeutic lifestyle changes, such patients are most likely to derive net benefits from the additive or more benefits from statins and aspirin.

Diabetics

In observational studies of diabetes of long duration, their risks of a first CHD event are comparable to those with a prior event so some guidelines recommend aspirin. These studies, however, are generally of diabetics of long duration so any decision to use aspirin, pending the outcome of ASCEND [70], should be an individual clinical judgment that weighs the absolute risk of occlusion against the absolute risk of bleeding.

Subjects 70 and older: It is well described that the elderly have higher risks of occlusion, but they also have higher risks of bleeding. Thus, any decision to use aspirin should be an individual clinical judgment [70].

Low- to Moderate-Risk Primary Prevention

Men under 50 and women under 60 H&W. In general, such apparently healthy men and women will have an absolute risk of a first CHD event that is lower than the absolute risk of bleeding. Thus, the clinician should make individual judgments that may be influenced by other risk factors such as obesity, physical inactivity, and family history of a premature CHD event which is generally considered to have been 55 or less in a male or 65 or less in a female first-degree relative.

Special Categories of Patients

On Aspirin Who Need NSAIDs

Patients on aspirin who need NSAIDs is a common clinical scenario, since patients who suffer from OA or AR among other rheumatic diseases often are older and have high CV risk or had suffered from a CV event [50]. Furthermore, ns-NSAIDs and coxibs are associated with increased CV risk. Only naproxen at 500 mg b.i.d. has been shown not to be associated with increased risk. In these last circumstances, NSAID (ns-NSAIDs or coxibs) use should be avoided. If there is high CV risk but patients had not suffered a previous CV event, NSAID treatment should be taken at the lowest possible dose and for the shorter period of time. Naproxen is the NSAID of choice since its CV risk is lower than any other NSAIDs or coxibs. Ibuprofen, and even probably naproxen, should be avoided if patients are taking aspirin since these drugs, especially ibuprofen, interact with the antiplatelet effect of aspirin. Taking these drugs before the aspirin dosing may not be sufficient, since interaction may still occur, especially if patients take enteric-coated aspirin. In these circumstances, the Tmax of aspirin lasts 4–5 h after dosing [54]. Recent guidelines have provided useful recommendation on what to do in these clinical scenarios (Fig. 11.1).

Need Aspirin but at High GI Risk

As commented above for patients who receive aspirin in secondary prevention, patients who need aspirin in a primary prevention setting should be questioned for careful evaluation of the GI risk factors. When the CV benefits

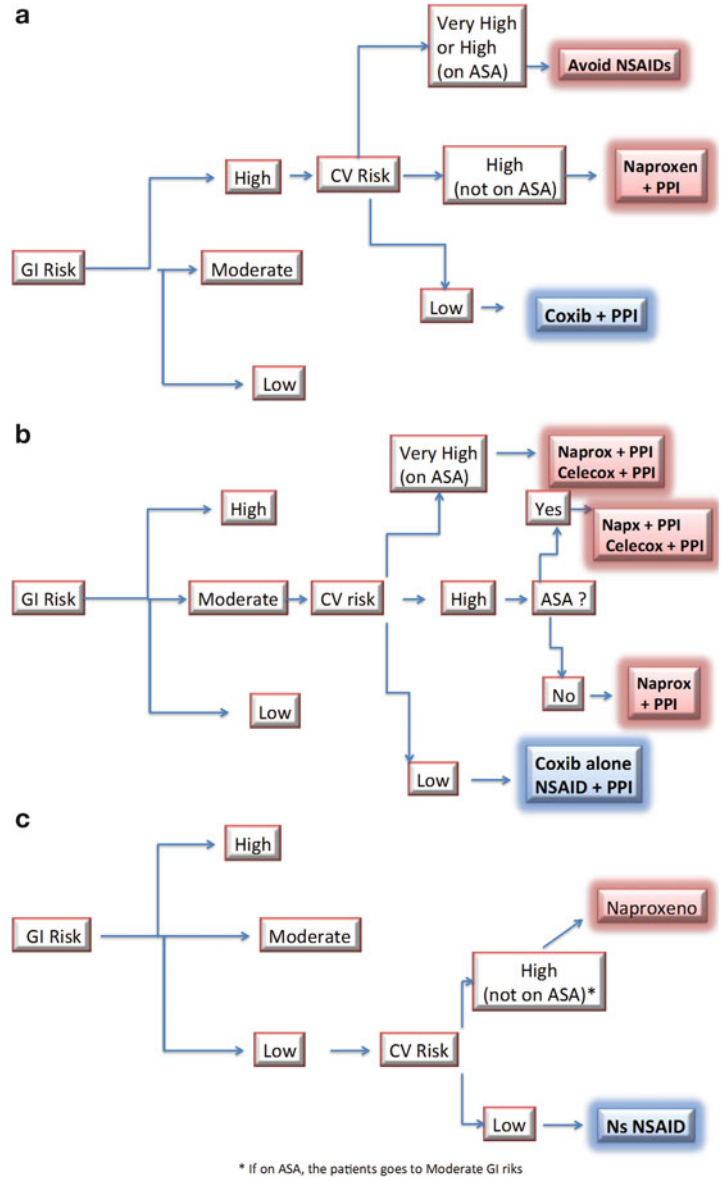


Fig. 11.1 (**a**–**c**) Prescription recommendations on NSAID use in patients taking ASA according to GI and CV risk (Adapted from Lanas et al. Gastroenterol Hepatol 2013 [71])

are outweighed by the GI risk, we recommend prescribing therapy with a PPI. Common risk factors are age 70 or older, ulcer history, concomitant treatment with NSAIDs, corticosteroids, or anticoagulants or other antiplatelet agents. There is no need for high dose of PPI; standard dose is enough. There are several guidelines instructing on this (refs), but also risk calculators are available online that will help the practitioner on the clinical decision process for individual cases (www.asariskcalculator.com) [51].

Need Aspirin but Develop a GI Complication

The occurrence of a gastrointestinal bleeding complication in patients treated with aspirin for cardiovascular prevention, especially secondary prevention, is a difficult clinical challenge, since discontinuation of platelet inhibition in patients

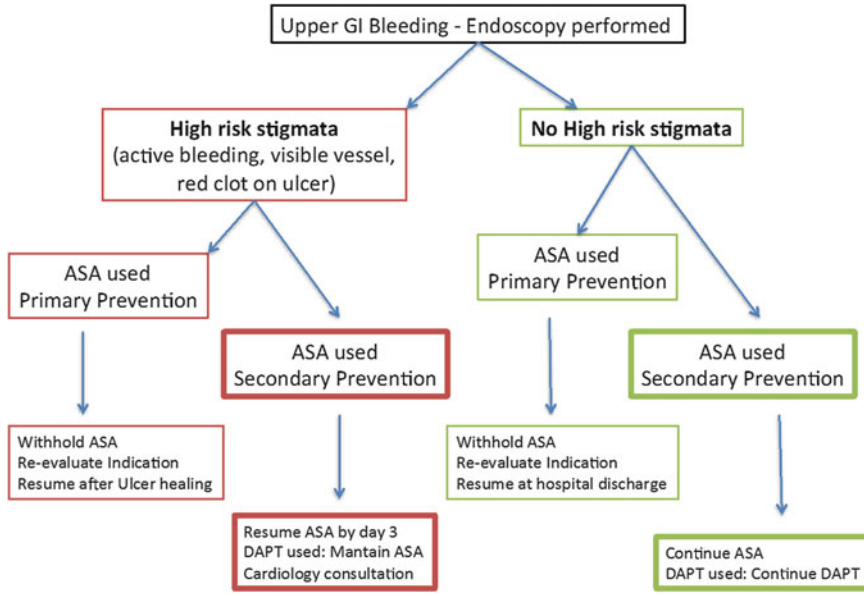


Fig. 11.2 Clinical management in patients who develop an acute upper GI bleeding event when taking ASA for cardiovascular prevention (Adapted from reference Gralnek et al. [74])

may have fatal consequences and this need to be balanced against the potential fatal outcome of a severe GI bleeding event.

There are two different clinical scenarios: (1) patients treated with aspirin for CV prevention who develop upper GI bleeding and (2) hospitalized patients who have just undergone a stent placement and develop an acute GI bleeding event. Based on the accumulated evidence, current guidelines state that early reintroduction (within 3 days) of aspirin is highly recommended in patients taking aspirin for secondary cardiovascular prevention (Fig. 11.2). The first RCT [72] that specifically evaluated the effect of no interruption of aspirin in patients who presented an acute upper GI bleeding event while taking cardiovascular aspirin concluded that these patients had lower all-cause 8-week mortality rates when compared to those where aspirin was interrupted and not reinitiated (1.3 % vs. 12.9 %). The difference was mainly due to lower mortality attributable to CV complications (1.3 % vs. 10.3 %). The recurrent ulcer bleeding at 30 days was higher in the early aspirin group (10.3 % vs. 5.4 %). Other more recent studies have confirmed these results [73]. If aspirin was indicated for the primary prevention, a reconsideration of the actual indication of aspirin is warranted, and the time for aspirin reintroduction can be prolonged at least till hospital discharge and preferably till the ulcer is healed.

In patients who have undergone a stent placement, the risk of thrombosis depends on the time interval between stent implantation and discontinuation of antiplatelet therapy. Dual antiplatelet therapy is recommended for at least 12 months after drug-eluting stents and at least 4 weeks after placement of a bare metal stent. Based on current evidence, and in agreement with expert consensus reports [32], patients with active ulcer bleeding should be treated endoscopically followed by high-dose PPI therapy. If endoscopy shows peptic ulcer with low-risk stigmata, aspirin should not be withdrawn. However, if endoscopy shows high-risk stigmata, aspirin should be stooped and reintroduced early, preferably within a 3–5-day window. If acute bleeding occurs soon after the placement of a coronary stent, the risk of thrombosis is very high. We believe that early endoscopy followed by a high dose of PPI is the

best option and usually dual antiplatelet should be maintained, at least clopidogrel. These clinical decisions may be difficult, and a close collaboration between the gastroenterologist and cardiologist is required.

Future Perspectives

Aspirin use in primary prevention of cardiovascular disease is controversial, and as shown in this review, current evidence is insufficient upon which to make general guidelines for aspirin. One explanation can be that studies have included patients at low cardiovascular risk with estimated coronary event rates <1 % per person-years. The scientific community is expectant to see the outcomes of five ongoing clinical primary prevention trials that have enrolled patients at high CV risk. The safety and efficacy of daily aspirin in this setting is very important. These results will need to be balanced against the detrimental effect of bleeding in the GI tract and the CNS. However, comparisons should be fair, and perhaps the right balance in terms of benefits and risk should be made based on the number of deaths induced and the number of deaths avoided. In this equation, the number of cancer deaths avoided should be placed in the equation if ongoing studies show which populations benefit from aspirin use in this regard.

The exact impact of adverse events of aspirin treatment in the lower GI tract should be better defined and these affects also be added in the risk-benefit balance. At the same time, current and future therapies of GI prevention in both the upper GI tract should be further investigated. In this way, the modification of the intestinal microbiota should be explored, and well-designed trials should be put in place sooner than later.

The outcomes of new antiplatelet agents, or combinations of them, now in the market require further evaluation in terms of benefits and adverse events since current evidence is deficient.

The assessment of CV, GI, and cancer risk and the benefits of aspirin at individual level may be difficult to determine in clinical practice. In order to overcome this problem and provide appropriate therapeutic strategies in clinical practice, new electronic tools based on the most recent and contrasted evidence are needed. The prospect of a personalized choice for any antiplatelet therapy in the individual patient appears realistic in the near future.

References

1. Hennekens CH. Increasing global burden of cardiovascular disease; current knowledge and future directions for research on risk factors. Special report. Circulation. 1998;97:1095–102.
2. Hennekens CH, Dalen JE. Aspirin in the treatment and prevention of cardiovascular disease: Past and current perspectives and future directions. Am J Med. 2013;126(5):373–8.
3. Antithrombotic Trialists Collaboration, Barnett H, Bousser M-G, Boysen G, Breddin K, Britton M, Buring J, Cairns J, Canner P, Collins R, Cortellaro M, Daniels A, Deykin D, Elwood P, Elwin E-E, Eschwege E, Farrell B, Fields WS, Gent M, Gray R, Guiraud-Chaumeil B, Hennekens CH, Klimt C, Lewis HD, Loria Y, Lowenthal A, MacMahon C, Miller J, Peto R, Qizilbash N, Reuther R, Richards S, Rosen A, Sandercock P, Sorensen PS, Sweetnam D, Taylor W, Thibult N, van Gijn J, Vogel G, Warlow C, Yusuf S. Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction and stroke in high-risk patients. Br Med J. 2002;24:71–86.
4. Hebert PR, Pfeffer MA, Hennekens CH. Use of statins and aspirin to reduce cardiovascular disease. J Cardiovasc Pharmacol Ther. 2002;7:77–80.
5. Hennekens CH, Sacks F, Tonkin A, Jukema W, Byington RP, Pitt B, Berry DA, Berry SM, Ford NF, Walker AJ, Nataragan K, Sheng-Lin C, Fiedorek FT, Belder R. Additive benefits of pravastatin and aspirin to decrease risks of cardiovascular disease: Randomized and observational comparisons of secondary prevention trials and their meta-analysis. Arch Intern Med. 2004;164(1):40–4.
6. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group (Australia: Hunt D; Austria: Dienstl F; Belgium: De Backer G; Canada Cairns J; Denmark Pedersen F; Finland: Kala R; France: Boissel J-P; Germany: Schroder R; Ireland: Horgan J; New Zealand Fitzpatrick M; Norway: Kjekshus Collins R, Peto R, Sleight P; US: Hennekens CH: Randomized trial of intravenous streptokinase, oral aspirin, both or neither among 17,187 cases of suspected acute myocardial infarction. Lancet 1988; 2:349-360.
7. International Stroke Trial Collaborative Group. The International Stroke Trial (IST): a randomized trial of

aspirin, subcutaneous heparin, both, or neither among 19,435 patients with acute ischemic stroke. Lancet. 1997;349:1569–81.
8. Chinese Acute Stroke Trial (CAST) Collaborative Group. CAST: randomized placebo-controlled trial of early aspirin use in 20,000 patients with acute ischemic stroke. Lancet. 1997;349:1641–9.
9. Steering Committee of the Physicians' Health Study Research Group. Preliminary report on the aspirin component of the ongoing Physicians' Health Study. N Engl J Med. 1988;318:262–4.
10. Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. N Engl J Med. 1989;321:129–35.
11. Antithrombotic Trialists Collaboration Primary Prevention Writing Group , Baigent C, Blackwell L, Buring J, Collins R, Emberson J, Godwin J, Hennekens C, Kearney P, Meade T, Patrono C, Peto R, Roncaglioni R, Zanchetti A. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373:1849–60.
12. Hennekens CH, Hollar D, Baigent C. Gender differences in response to aspirin in cardiovascular disease: An hypothesis formulated, but not tested. Nat Clin Pract Cardiovasc Med. 2006;3:4–5.
13. Lanas A, Wu P, Medin J, Mills EJ. Low doses of acetylsalicylic acid increase risk of gastrointestinal bleeding in a meta-analysis. Clin Gastroenterol Hepatol. 2011;9(9):762–8.
14. Yeomans ND, Lanas AI, Talley NJ, Thomson AB, Daneshjoo R, Eriksson B, Appelman-Eszczuk S, Långström G, Naesdal J, Serrano P, Singh M, Skelly MM, Hawkey CJ. Prevalence and incidence of gastroduodenal ulcers during treatment with vascular protective doses of aspirin. Aliment Pharmacol Ther. 2005;22(9):795–801.
15. Cayla G, Collet JP, Silvain J, Thiefin G, Woimant F, Montalescot G. Prevalence and clinical impact of Upper Gastrointestinal Symptoms in subjects treated with low dose aspirin: the UGLA survey. Int J Cardiol. 2012;156(1):69–75. doi:10.1016/j.ijcard.2010.10.027. Epub 2010 Nov 18.
16. Muller P, Fuchs W, Simon B. Studies on protective effect of lansoprazole on human gastric mucosa against low-dose acetylsalicylic. An endoscopic controlled double-blind study. Arzneimittelforschung. 1997;47:758–60.
17. Moore A, Bjarnason I, Cryer B, Garcia-Rodriguez L, Goldkind L, Lanas A, et al. Evidence for endoscopic ulcers as meaningful surrogate endpoint for clinically significant upper gastrointestinal harm. Clin Gastroenterol Hepatol. 2009;7(11):1156.
18. Weil J, Colin-Jones D, Langman M, Lawson D, Logan R, Murphy M, et al. Prophylactic aspirin and risk of peptic ulcer bleeding. BMJ. 1995;310:827–30.
19. Hernández-Díaz S, García Rodríguez LA. Cardioprotective aspirin users and their excess risk of upper gastrointestinal complications. BMC Med. 2006;4:22.
20. Chan FK, To KF, Wu JC, Yung MY, Leung WK, Kwok T, et al. Eradication of Helicobacter pylori and risk of peptic ulcers in patients starting long-term treatment with non-steroidal anti-inflammatory drugs: a randomised trial. Lancet. 2002;359(9300):9–13.
21. Lanas A, García-Rodríguez LA, Arroyo MT, Gomollón F, Feu F, González-Pérez A, Zapata E, Bástida G, Rodrigo L, Santolaria S, Güell M, de Argila CM, Quintero E, Borda F. Piqué JM; Asociación Española de Gastroenterología. Risk of upper gastrointestinal ulcer bleeding associated with selective cyclo-oxygenase-2 inhibitors, traditional non-aspirin non-steroidal anti-inflammatory drugs, aspirin and combinations. Gut. 2006;55(12):1731–8. Epub 2006 May 10.
22. Hennekens CH, Baigent C. Prevention. Aspirin in primary prevention–good news and bad news. Nat Rev Cardiol. 2012;9:262–3.
23. Moore RA, Derry S, McQuay HJ. Faecal blood loss with aspirin, nonsteroidal anti-inflammatory drugs and cyclo-oxygenase-2 selective inhibitors: systematic review of randomized trials using autologous chromium-labelled erythrocytes. Arthritis Res Ther. 2008;10(1):R7.
24. Strate LL, Liu YL, Huang ES, Giovannucci EL, Chan AT. Use of aspirin or nonsteroidal anti-inflammatory drugs increases risk for diverticulitis and diverticular bleeding. Gastroenterology. 2011;140(5):1427–33.
25. Nagata N, Niikura R, Aoki T, Shimbo T, Kishida Y, Sekine K, Tanaka S, Watanabe K, Sakurai T, Yokoi C, Akiyama J, Yanase M, Mizokami M, Uemura N. Colonic diverticular hemorrhage associated with the use of nonsteroidal anti-inflammatory drugs, low-dose aspirin, antiplatelet drugs, and dual therapy. J Gastroenterol Hepatol. 2014;29(10):1786–93.
26. Lanas A, Carrera P, Arguedas Y, Garcia S, Bujanda L, Calvet X, Ponce J, Perez-Asisa A, Castro M, Muñoz M, Sostres C. and Garcia Rodrigez. Risk of Upper and Lower Gastrointestinal Bleeding in Patients Taking Non-steroidal Anti-inflammatory Drugs, Antiplatelet Agents, or Anticoagulants. Clin Gastroenterol Hepatol. 2015;13(5):906–12.
27. Cea Soriano L, Rodriguez LA. Risk of upper gastrointestinal bleeding in a cohort of new users of low-dose ASA for secondary prevention of cardiovascular outcomes. Front Pharmacol. 2010;1:126.
28. Lanas A, Bajador E, Serrano P, Fuentes J, Carreno S, Guardia J, et al. Nitrovasodilators, low-dose aspirin, other nonsteroidal antiinflammatory drugs, and the risk of upper gastrointestinal bleeding. N Engl J Med. 2000;343:834–9.
29. Lanas A, Fuentes J, Benito R, Serrano P, Bajador E, Sainz R. Helicobacter pylori increases the risk of upper gastrointestinal bleeding in patients taking low-dose aspirin. Aliment Pharmacol Ther. 2002;16:779–86.
30. Sorensen HT, Mellemkjaer L, Blot WJ, Nielsen GL, Steffensen FH, McLaughlin JK, et al. Risk of upper gastrointestinal bleeding associated with use of low-dose aspirin. Am J Gastroenterol. 2000;95:2218–24.

31. Rostom A, Muir K, Dube C, et al. Gastrointestinal safety of cyclooxygenase-2 inhibitors: a Cochrane Collaboration systematic review. Clin Gastroenterol Hepatol. 2007;5:818–28.
32. Bhatt DL, Scheiman J, Abraham NS, Antman EM, Chan FK, Furberg CD, et al. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID use: a report of the American College of Cardiology Foundation Task Force on Clinical Expert Consensus Documents. J Am Coll Cardiol. 2008;52:1502–17.
33. Berger PB, Bhatt DL, Fuster V, Steg PG, Fox KA, Shao M, et al. Bleeding complications with dual antiplatelet therapy among patients with stable vascular disease or risk factors for vascular disease: results from the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management, and Avoidance (CHARISMA) trial. Circulation. 2010;121:2575–83.
34. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med. 2001;345:494–502.
35. Fletcher EH, Johnston DE, Fisher CR, Koerner RJ, Newton JL, Gray CS. Systematic review: Helicobacter pylori and the risk of upper gastrointestinal bleeding risk in patients taking aspirin. Aliment Pharmacol Ther. 2010;32(7):831–9.
36. Sostres C, Carrera-Lasfuentes P, Benito R, Roncales P, Arruebo M, Arroyo MT, Bujanda L, García-Rodríguez LA, Lanas A. Peptic Ulcer Bleeding Risk. The Role of Helicobacter Pylori Infection in NSAID/Low-Dose Aspirin Users. Am J Gastroenterol. 2015;110(5):684–9.
37. Chan FK, Ching JY, Suen BY, Tse YK, Wu JC, Sung JJ. Effects of Helicobacter pylori infection on long-term risk of peptic ulcer bleeding in low-dose aspirin users. Gastroenterology. 2013;144(3):528–35.
38. Iijima K, Ara N, Abe Y, Koike T, Iwabuchi T, Shinkai H, Uno K, Endo H, Asano N, Shimosegawa T. Biphasic effects of H. pylori infection on low-dose aspirin-induced gastropathy depending on the gastric acid secretion level. J Gastroenterol. 2012;47(12):1290–7. doi:10.1007/s00535-012-0598-8.
39. Casado-Arroyo R, Gargallo C, Lanas Arbeloa A. Balancing the risk and benefits of low-dose aspirin in clinical practice. Best Pract Res Clin Gastroenterol. 2012;26:173–84.
40. Sugano K. Prevention of upper gastrointestinal ulcer and complications in low-dose aspirin users. Curr Pharm Des. 2015;21:5082–8.
41. Graham DY, Smith JL, Spjut HJ, Torres E. Gastric adaptation. Studies in humans during continuous aspirin administration. Gastroenterology. 1988;95:327–33.
42. de Abajo FJ, Garcia Rodriguez LA. Risk of upper gastrointestinal bleeding and perforation associated with low-dose aspirin as plain and enteric-coated formulations. BMC Clin Pharmacol. 2001;1:1.
43. Buring JE, Peto R, Hennekens CH. Low-dose aspirin for migraine prophylaxis. JAMA. 1990;264:1711–3.
44. Diener HC, Lampl C, Reimnitz P, Voelker M. Aspiring in the treatment of acute migraine attacks. Expert Rev Neurother. 2006;6(4):563–73.
45. Stürmer T, Glynn RJ, Field TS, Taylor JO, Hennekens CH. Aspirin use and cognitive function in the elderly. Am J Epidemiol. 1996;143(7):683–91.
46. Rothwell P, Fowkes GR, Belch JFF, Ogawa H, Warlow C, Meade TW. Effect of daily aspirin on long term risk of death due to cancer: analysis of individual patient data from randomized trials. Lancet. 2011;377:31–41.
47. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998;97:1837–47.
48. Casado-Arroyo R, Bayrak F, Sarkozy A, Chierchia GB, de Asmundis C, Brugada P. Role of ASA in the primary and secondary prevention of cardiovascular events. Best Pract Res Clin Gastroenterol. 2012;26(2):113–23.
49. Lanas A, Tornero J, Zamorano JL. Assessment of gastrointestinal and cardiovascular risk in patients with osteoarthritis who require NSAIDs: the LOGICA study. Ann Rheum Dis. 2010;69(8):1453–8.
50. Lanas A, Garcia-Tell G, Armada B, Oteo-Alvaro A. Prescription patterns and appropriateness of NSAID therapy according to gastrointestinal risk and cardiovascular history in patients with diagnoses of osteoarthritis. BMC Med. 2011;9:38.
51. Lanas A, Polo-Tomás M, Casado-Arroyo R. The aspirin cardiovascular/gastrointestinal risk calculator–a tool to aid clinicians in practice. Aliment Pharmacol Ther. 2013;37(7):738–48.
52. Capone ML, Sciulli MG, Tacconelli S, Grana M, Ricciotti E, Renda G, Di Gregorio P, Merciaro G, Patrignani P. Pharmaco-dynamic interaction of naproxen with low-dose aspirin in healthy subjects. J Am Coll Cardiol. 2005;45:1295–301.
53. Anzellotti P, Capone ML, Jeyam A, Tacconelli S, Bruno A, Tontodonati P, Di Francesco L, Grossi L, Renda G, Merciaro G, Di Gregorio P, Price TS, Garcia Rodriguez LA, Patrignani P. Low-dose naproxen interferes with the antiplatelet effects of aspirin in healthy subjects: recommendations to minimize the functional consequences. Arthritis Rheum. 2011;63:850–9.
54. Patrignani P, Tacconelli S, Piazuelo E, Di Francesco L, Dovizio M, Sostres C, Marcantoni E, Guillem-Llobat P, Del Boccio P, Zucchelli M, Patrono C, Lanas A. Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and traditional indirect biomarkers of drug action. J Thromb Haemost. 2014;12(8):1320–30.
55. Chan FK, Chung SC, Suen BY, Lee YT, Leung WK, Leung VK, Wu JC, Lau JY, Hui Y, Lai MS, Chan HL, Sung JJ. Preventing recurrent upper gastrointestinal bleeding in patients with Helicobacter pylori infection who are taking low-dose aspirin or naproxen. N Engl J Med. 2001;344(13):967–73.

56. Donnelly MT, Goddard AF, Filipowicz B, Morant SV, Shield MJ, Hawkey CJ. Low-dose misoprostol for the prevention of low-dose aspirin-induced gastroduodenal injury. Aliment Pharmacol Ther. 2000;14(5):529–34.
57. Watanabe T, Sugimori S, Kameda N, Machida H, Okazaki H, Tanigawa T, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Higuchi K, Arakawa T. Small bowel injury by low-dose enteric-coated aspirin and treatment with misoprostol: a pilot study. Clin Gastroenterol Hepatol. 2008;6(11):1279–82.
58. Lanas A, García-Rodríguez LA, Arroyo MT, Bujanda L, Gomollón F, Forné M, Aleman S, Nicolas D, Feu F, González-Pérez A, Borda A, Castro M, Poveda MJ. Arenas J; Investigators of the Asociación Española de Gastroenterología (AEG). Effect of antisecretory drugs and nitrates on the risk of ulcer bleeding associated with nonsteroidal anti-inflammatory drugs, antiplatelet agents, and anticoagulants. Am J Gastroenterol. 2007;102(3):507–15.
59. Taha AS, McCloskey C, Prasad R, Bezlyak V. Famotidine for the prevention of peptic ulcers and oesophagitis in patients taking low-dose aspirin (FAMOUS): a phase III, randomized, double-blind, placebo-controlled trial. Lancet. 2009;374(9684):119–25.
60. Ng FH, Wong SY, Lam KF, Chu WM, Chan P, Ling YH, et al. Famotidine is inferior to pantoprazole in preventing recurrence of aspirin-related peptic ulcers or erosions. Gastroenterology. 2010;138(1):82–8.
61. Lin KJ, Hernandez-Diaz S, Garcia Rodriguez LA. Acid suppressants reduce risk of gastrointestinal bleeding in patients on antithrombotic or anti-inflammatory therapy. Gastroenterology. 2011;141(1):71–9.
62. Sugano K, Choi MG, Lin JT, Goto S, Okada Y, Kinoshita Y, Miwa H, Chiang CE, Chiba T, Hori M, Fukushima Y, Kim HS, Chang CY, Date M, LAVENDER Study Group. Multinational, double-blind, randomised, placebo-controlled, prospective study of esomeprazole in the prevention of recurrent peptic ulcer in low-dose acetylsalicylic acid users: the LAVENDER study. Gut. 2014;63(7):1061–8. doi:10.1136/gutjnl-2013-304722. Epub 2013 Dec 10.
63. Iwakiri R, Higuchi K, Kato M, Fujishiro M, Kinoshita Y, Watanabe T, Takeuchi T, Yamauchi M, Sanomura M, Nakagawa H, Sugisaki N, Okada Y, Ogawa H, Arakawa T, Fujimoto K. Randomised clinical trial: prevention of recurrence of peptic ulcers by rabeprazole in patients taking low-dose aspirin. Aliment Pharmacol Ther. 2014;40(7):780–95. doi:10.1111/apt.12907. Epub 2014 Aug 6.
64. Lai KC, Lam SK, Chu KM, Wong BC, Hui WM, Hu WH, et al. Lansoprazole for the prevention of recurrences of ulcer complications from long-term low-dose aspirin use. N Engl J Med. 2002;346(26):2033–8.
65. Bhatt DL, Cryer BL, Contant CF, Cohen M, Lanas A, Schnitzer TJ, et al. Clopidogrel with or without omeprazole in coronary artery disease. N Engl J Med. 2010;363(20):1909–17.
66. Endo H, Higurashi T, Hosono K, Sakai E, Sekino Y, Iida H, Sakamoto Y, Koide T, Takahashi H, Yoneda M, Tokoro C, Inamori M, Abe Y, Nakajima A. Efficacy of Lactobacillus casei treatment on small bowel injury in chronic low-dose aspirin users: a pilot randomized controlled study. J Gastroenterol. 2011;46(7):894–905.
67. Hennekens CH, Jonas MA, Buring JE. The benefits of aspirin in acute myocardial infarction. Still a well-kept secret in the United States. Arch Intern Med. 1994;154(1):37–9.
68. Laine L, Hennekens C. Proton pump inhibitor and clopidogrel interaction: fact or fiction? Am J Gastroenterol. 2010;105(1):34–41.
69. Lanas A, Gargallo CJ. Management of low-dose aspirin and clopidogrel in clinical practice: a gastrointestinal perspective. J Gastroenterol. 2015;50(6):626–37.
70. Hennekens CH, DeMets DL. Prevention: Aspirin in primary prevention needs individual judgements. Nat Rev Cardiol. 2014;11(8):438–40.
71. Lanas A, Benito P, Alonso J, Hernández-Cruz B, Barón-Esquivias G, Perez-Aísa A, Calvet X, García-Llorente JF, Gobbo M, Gonzalez-Juanatey JR. Safe prescription recommendations for non steroidal anti-inflammatory drugs: consensus document elaborated by nominated experts of three scientific associations (SER-SEC-AEG). Gastroenterol Hepatol. 2014;37:107–27.
72. Sung JJ, Lau JY, Ching JY, Wu JC, Lee YT, Chiu PW, Leung VK, Wong VW, Chan FK. Continuation of low-dose aspirin therapy in peptic ulcer bleeding: a randomized trial. Ann Intern Med. 2010;152(1):1–9.
73. Derogar M, Sandblom G, Lundell L, Orsini N, Bottai M, Lu Y, Sadr-Azodi O. Discontinuation of low-dose aspirin therapy after peptic ulcer bleeding increases risk of death and acute cardiovascular events. Clin Gastroenterol Hepatol. 2013;11(1):38–42.
74. Gralnek IM, Dumonceau JM, Kuipers EJ, Lanas A, Sanders DS, Kurien M, et al. Diagnosis and management of nonvariceal upper gastrointestinal hemorrhage: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. Endoscopy. 2015;47(10):a1–46. doi:10.1055/s-0034-1393172.

Part IV

NSAIDs and Aspirin in Cancer Prevention

Molecular and Experimental Basis for COX Inhibition in Cancer

12

Annalisa Bruno, Melania Dovizio, and Paola Patrignani

Introduction

Experimental, epidemiological, and randomized clinical studies have led to accumulate numerous pieces of evidence suggesting that the pharmacological inhibition of cyclooxygenase (COX)-isozymes by nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, has a protective effect against tumor development [1]. NSAIDs are a group of chemically heterogeneous molecules which act by inhibiting the synthesis of prostanoids through the prevention of arachidonic acid (AA) binding in the active site of COX-1 and COX-2. An important prostanoid involved in tumorigenesis is prostaglandin (PG)E_2. Enhanced biosynthesis of PGE_2 has been detected in various types of human malignancies including colorectal, lung, breast, and head and neck cancer and is often associated with a poor prognosis [2–5]. This prostanoid binds to and activates G-protein-coupled prostaglandin E1-4 receptors (EP1-4) and exerts a profound influence over the adhesive, migratory, and invasive behavior of cells during the development and progression of cancer [6]. Moreover, PGE_2 may contribute to the formation, maintenance, and expansion of cancer stem cells (CSCs), which have the capacity for self-renewal, differentiation, and resistance to cytotoxic agents [7]. Finally, enhanced PGE_2 production can generate an immunosuppressive microenvironment that allows advantages for tumor formation and progression [8].

The role of COX-2-dependent PGE_2 in intestinal tumorigenesis is strongly supported in humans by the results of clinical studies showing that selective COX-2 inhibitors (named coxibs) cause the reduction of polyp number and size in familial adenomatous polyposis (FAP) patients [9] and prevent polyp reoccurrence in patients with sporadic adenomas [10–12]. However, the role of prostanoids in colorectal carcinogenesis is more complex in the light of the findings that the antiplatelet agent low-dose aspirin may cause similar effects, in the same clinical conditions [13]. In fact, it is unlike that low-dose aspirin acts through an inhibitory effect on COX-2 activity expressed in colorectal adenomas; in contrast, several lines of evidence suggest that the drug acts by affecting COX-1-dependent prostanoid biosynthesis, mainly thromboxane (TX)A_2, in platelets.

The efficacy of the antiplatelet agent low-dose aspirin has opened new avenues in the understanding of the mechanisms involved in colorectal cancer (CRC) development and progression

A. Bruno, Ph.D. • M. Dovizio, Ph.D.
P. Patrignani, Ph.D. (✉)
Department of Neuroscience, Imaging and Clinical Science, Section of Cardiovascular and Pharmacological Sciences, CeSI-MeT, "G. d'Annunzio" University School of Medicine, Chieti, Italy
e-mail: ppatrignani@unich.it

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_12

and in the development of novel therapeutic anticancer strategies.

The aim of the present chapter is to enlighten the biology and functions of COX-1 and COX-2 and the derived prostanoids PGE_2 and TXA_2 in relation to tumorigenesis (mainly in the colorectum) and cancer metastasis. Moreover, we will characterize the different mechanisms of action of NSAIDs selective for COX-2 (coxibs) versus low-dose aspirin in regard to their anticancer effects.

Prostanoid Signaling in Cancer and Metastasis

The results of clinical studies showing the chemopreventive effect of NSAIDs in cancer [1] support the notion that COX-derived prostanoids play an important role in tumor development and progression. Here, a detailed description of the biosynthesis and activities of prostanoids (Fig. 12.1) and their implication in intestinal tumorigenesis is reported.

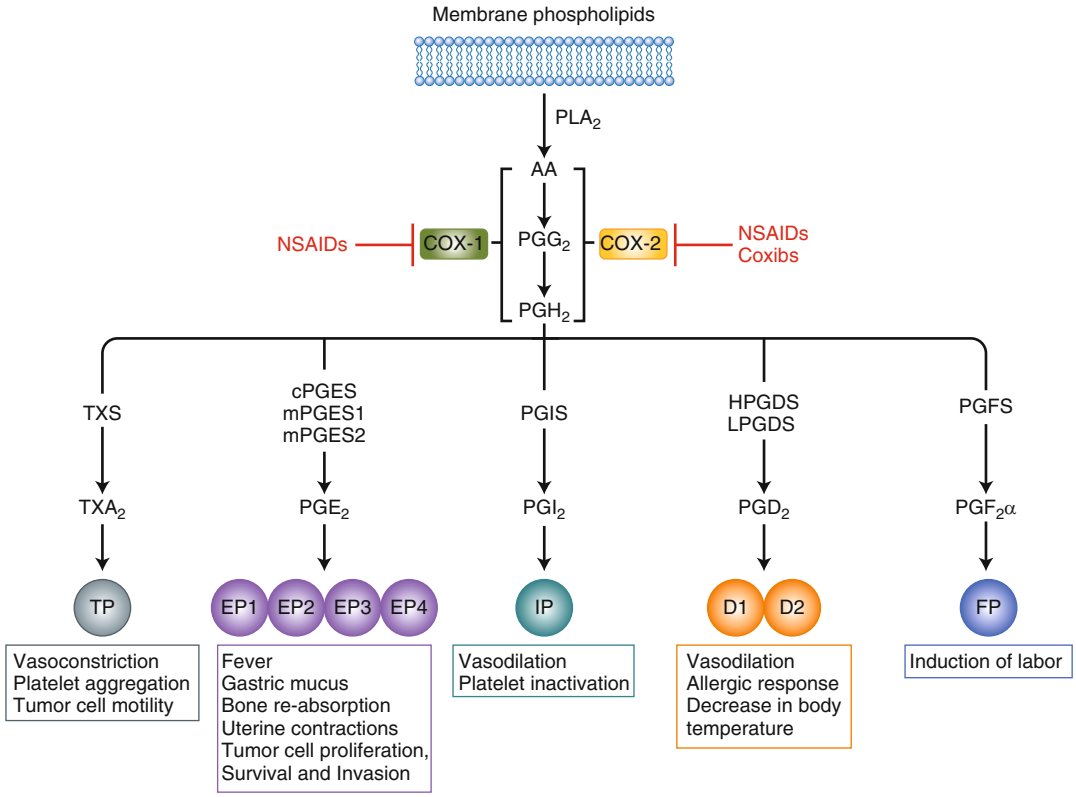


Fig. 12.1 Prostanoid biosynthesis and main functions in physiological and pathological settings. Arachidonic acid can be metabolized through the cyclooxygenase isozymes: COX-1 and COX-2. The derived prostaglandins (PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$) and TXA_2 exert a variety of biological functions through their respective cognate receptors. *AA* arachidonic acid, *COX* cyclooxygenase, *PG* prostaglandin, *PLA_2* phospholipase A2, *TXA_2* thromboxane A_2, *TXS* thromboxane synthase, *cPGES* cytosolic PGE_2 synthase, *mPGES* microsomal PGE_2 synthase, *PGIS* PGI_2 synthase, *H-PGDS* hematopoietic PGD_2 synthase, *L-PGDS* lipocalin PGD_2 synthase, *PGFS* $PGF_{2\alpha}$ synthase, *TP* TXA_2 receptor, *EP* PGE_2 receptor, *IP* PGI_2 receptor, *DP* PGD_2 receptor *FP*, PGF_{2a} receptor, *NSAIDs* nonsteroidal anti-inflammatory drugs, *Coxibs* COX-2 selective inhibitors

Prostanoid Generation: The Activity of COX Isozymes

COX-1 and COX-2 (also known as PGG/H synthase-1 and synthase-2, respectively) are homodimers of 576 and 581 amino acids, respectively [14]. Each subunit of the dimer contains the cyclooxygenase and peroxidase active sites which contribute to catalyze the rate-limiting step of prostanoid biosynthesis, i.e., the production of PGH_2 from AA, which is released from membrane phospholipids by phospholipases (PL), mainly cytosolic (c) PLA_2 upon cellular activation [15]. PGH_2 is then transformed to prostanoids by the activity of different terminal synthases. Prostanoids are a family of bioactive lipids which comprises PGE_2, $PGF_{2\alpha}$, PGD_2, prostacyclin (PGI_2), and TXA_2 (Fig. 12.1).

Despite COX-1 and COX-2 produce the same prostanoids, their cellular levels are influenced by the extent of expression of the corresponding genes. The genes of COX-1 (*PTGS1*) and COX-2 (*PTGS2*) have a different regulation: (1) *PTGS2* is an immediate early response gene that is normally absent from most cells but is highly induced at sites of inflammation and during tumor progression [16], while (2) *PTGS1* is a housekeeping enzyme responsible for maintaining basal prostanoid levels that are important for tissue homeostasis [16].

Prostanoids play important roles in many physiological and pathophysiologic processes, including inflammation and its resolution, erosion of cartilage and juxta-articular bone, gastrointestinal (GI) cytoprotection and ulceration, angiogenesis and cancer, hemostasis and thrombosis, renal hemodynamics and progression of kidney disease, and atheroprotection and progression of atherosclerosis [17–19]. COXs act as oxidase enzymes that first peroxidate AA to form the hydroperoxyendoperoxide PGG_2, which links two oxygen molecules across carbons 9 and 1. As second coordinate enzymatic function, COXs reduce a hydroperoxy group at carbon 15 of PGG_2 to form the intermediate product PGH_2 which serves as substrate for a variety of PG synthases involved in prostanoid biosynthesis [14] (Fig. 12.1). The generation of PGE_2 is catalyzed by three different synthases: a cytosolic PGE synthase (cPGES) and two membrane-bound PGESs, i.e., mPGES-1 and mPGES-2 [20]. Whereas cPGES and mPGES-2 are constitutive enzymes, mPGES-1 is encoded by an inducible gene. It is thought that the coordinated expression of COX-2 and mPGES-1 is responsible for enhanced biosynthesis of PGE_2 which occurs in inflammation and cancer [20]. The biosynthesis of PGD_2 is regulated by the activity of two PGD synthases, lipocalin (L-PGDS) and hematopoietic (H-PGDS) [21]. Finally, the biosynthesis of TXA_2 and PGI_2 involves the activity of TX-synthase (TXS) and PGI-synthase (PGIS), respectively [19] (Fig. 12.1).

Prostanoids are second messengers which can cross the cell membrane, diffuse through the extracellular space, and interact with high-affinity G-protein-coupled receptors (GPCRs) on the same cell or in neighboring cells. The prostanoid receptor family consists of eight rhodopsin-like (class A) GPCRs, each being the product of an individual gene: DP1 (for PGD_2); EP1, EP2, EP3, and EP4 (for PGE_2); FP (for $PGF_{2\alpha}$); IP (for PGI_2); and TP (for TXA_2) [14]. The prostanoid receptor named chemoattractant receptor-homologous molecule expressed on T-helper type 2 cells (CRTH2) or DP2, characterized by a higher sequence homology with other leukocyte chemoattractant receptors than prostanoid receptors [22], also acts as receptor for PGD_2.

The specific action of the different prostanoids in a particular type of tissue predominantly depends on the cell-type-specific expression of their receptors as well as prostanoid production. In addition to their biosynthesis, the extracellular levels of prostanoids also depend on a carrier-mediated transport process, as well as inactivation in the cytoplasm. These processes are regulated by prostaglandin transporter (PGT, an influx transporter), multidrug resistance-associated protein 4 (MRP4, an efflux transporter), and hydroxyprostaglandin dehydrogenase 15-(NAD) (HPGD, also known

15-PGDH). For example, PGE_2 and $PGF_{2\alpha}$ are rapidly metabolized in vivo by 15-PGDH to stable 13,14-dihydro-15-keto-PGE_2 (PGEM) and 13,14-dihydro-15-keto-PGF2α, respectively.

Involvement of PGE_2-EP Signaling in Tumorigenesis

Among prostanoids, pro-inflammatory PGE_2 has a predominant role in promoting tumor growth [23, 2–3]. PGE_2 is the most abundant prostaglandin that is found in various human malignancies, including colon, lung, breast, and head and neck cancer, and is often associated with a poor prognosis [4, 5]. By contrast, 15-PGDH is highly expressed in normal tissues but is ubiquitously lacking in human colon, gastric, lung, and breast cancer [24–27]. The lack of 15-PGDH expression in these tumors results in increased endogenous PGE_2 levels.

The role of PGE_2 in colon cancer progression arose from studies with the $APC^{Min/+}$mouse model of intestinal neoplasia. This genetic animal model for FAP maintains inactivating mutations in the adenomatous polyposis coli (*APC*) gene [28]. Treatment of these animals with PGE_2 promoted a dramatic increase in small and large intestinal tumor burden [23]. Moreover, studies in humans revealed that adenoma regression was more effective when PGE_2 tissue levels were profoundly inhibited by treatment with NSAIDs [29]. The activation of the canonical Wnt pathway in the colonic epithelium is a key event in polyp formation, and this event is associated with the upregulation of several genes involved in tumor development and progression [30]. Among them, overexpression of COX-2 plays a central role in intestinal tumorigenesis. In fact, elevated levels of COX-2-derived PGE_2 are associated with (1) resistance to apoptosis, through the upregulation of the antiapoptotic protein Bcl-2 and the induction of nuclear factor-kB (NF-kB) transcriptional activity [23], (2) stimulation of cell proliferation, (3) stimulation of cell migration, and (4) angiogenesis [23] (Fig. 12.2a).

In addition, PGE_2 may contribute to the formation, maintenance, and expansion of CSCs, by activating NF-kB, via EP4-PI3K and EP4-mitogen-activated protein kinase signaling, and promotes the formation of liver metastases in mice [7] (Fig. 12.2a). Enhanced PGE_2 production, which occurs in chronic inflammation, can generate an immunosuppressive microenvironment that allows advantages for tumor formation and progression [8]. The well-recognized role of PGE_2 during tumor promotion coupled with findings demonstrating that long-term use of NSAIDs may be associated with GI toxicity [31] and increased risk of adverse cardiovascular (CV) events [32, 33], provided the rationale for the identification of novel enzymatic targets within the AA pathway, including the PGE_2 terminal synthases [34].

Role of mPGES1 in Tumorigenesis

mPGES-1 is a member of the membrane-associated proteins involved in eicosanoid and glutathione metabolism (MAPEG) superfamily, showing significant homology with other MAPEG superfamily proteins. mPGES-1 is expressed at minimal levels in most normal tissues, although abundant and constitutive expression is detected in a limited number of organs, such as the lung, kidney, and reproductive organs. mPGES-1 is also induced by cytokines and various growth factors [35].

mPGES-1 is functionally coupled with COX-2, and its expression is often concomitantly induced with COX-2 overexpression, thus contributing to the efficient generation of PGE_2 during inflammation [36]. However, studies using diverse stimuli provided evidence that COX-2 and mPGES-1 can be independently regulated [37]. This observation suggested the possibility that the pharmacological targeting of mPGES-1 may result in the suppression of PGE_2 production by mechanisms that circumvent the CV toxicity associated with inhibition of COX-2 activity by NSAIDs, both traditional(t) and coxibs [38].

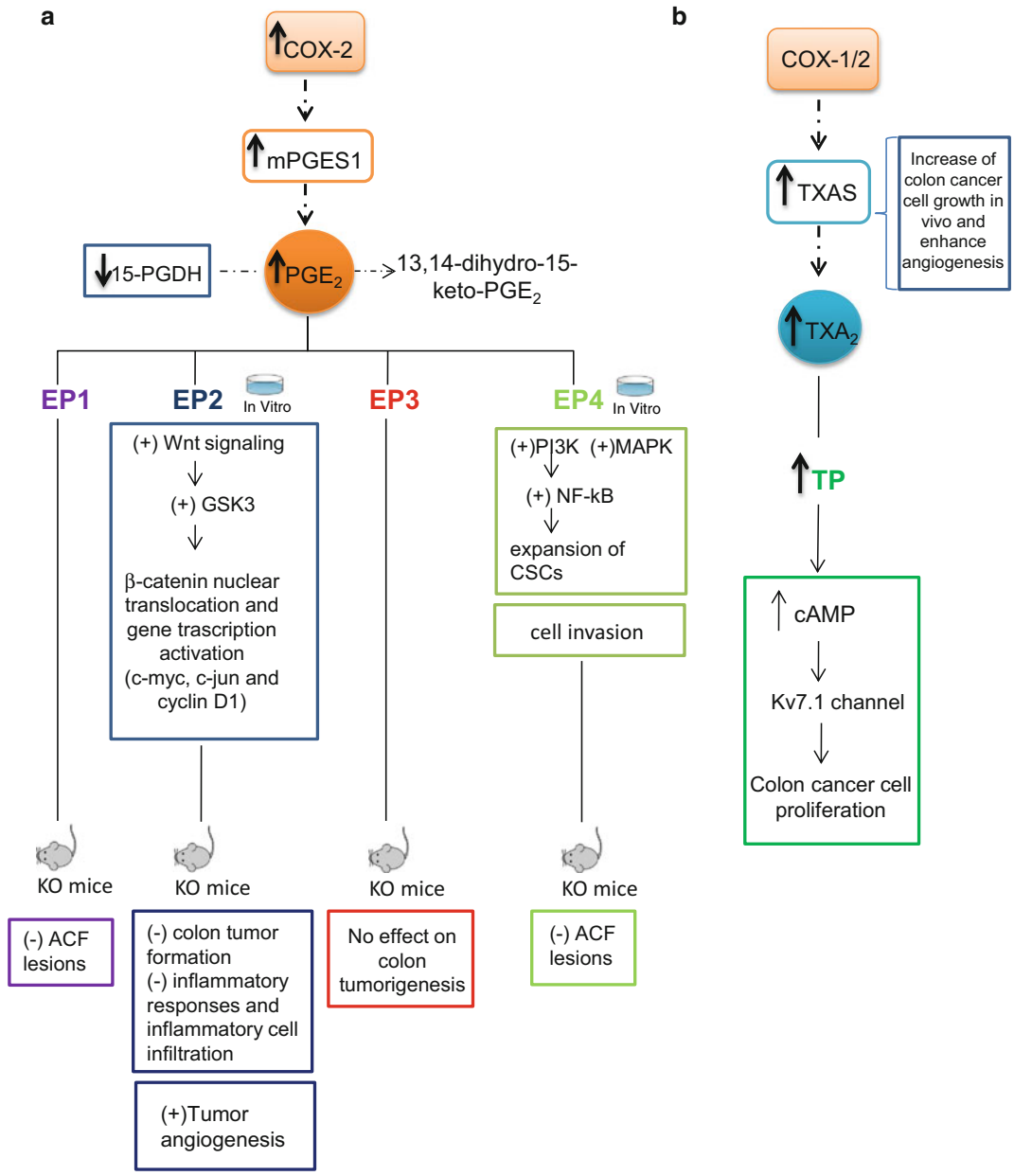


Fig. 12.2 PGE_2-EP (**a**) and TXA_2-TP (**b**) signaling in intestinal tumorigenesis. In intestinal tumorigenesis, the increased levels of PGE_2 derived from COX-2 and mPGES1 overexpression couples with the reduction of the PGE_2-degrading enzyme, 15-PGDH (**a**). In addition, in this setting, the increase of TXAS expression leads to the enhanced biosynthesis of TXA_2 (**b**). The interaction of PGE_2 and TXA_2 with their receptors exerts a protumorigenic effect through several mechanisms. In addition, the implication of EP receptors in tumorigenesis was demonstrated by several in vivo studies using specific knockout mice. *COX* cyclooxygenase, *PG* prostaglandin, *TXA_2* thromboxane A_2, *TXAS* thromboxane synthase, *mPGES* microsomal PGE_2 synthase, *TP* TXA_2 receptor, *EP* PGE_2 receptor, *KO* knockout, *15-PGDH* 15-hydroxyprostaglandin dehydrogenase, *GSK* glycogen synthase kinase 3, *ACF* aberrant crypt foci, *PI3K* phosphatidylinositol 3-kinase, *MAPK* mitogen-activated protein kinase, *NF-kB* nuclear factor kappa-light-chain-enhancer of activated B cells, *CSC* cancer stem cell, *cAMP* cyclic adenosine monophosphate, *Kv channel* voltage-gated K+ channel

On the basis of evidence from cell culture studies, several in vivo studies have been performed to address the impact of mPGES-1 targeting on colon tumorigenesis, but the results of these studies are conflicting [39]. In particular, mPGES-1 knockout (KO) mice in a mutant *APC* background showed a significant reduction in the number and size of intestinal tumors. In contrast, Elander et al. reported that genetic deletion of mPGES-1 resulted in accelerated intestinal tumorigenesis in $APC^{Min/+}$ mice [40]. Environmental factors (i.e., *Helicobacter pylori* infection) may represent a possible explanation of these different responses along with genetic differences in the mouse models used. mPGES-1 KO mice were also protected against azoxymethane (AOM)-induced colon cancer with reduced number of aberrant crypt foci (ACF), putative preneoplastic lesions of the colon, and up to 90 % decrease in tumor load in the distal colon [41]. Genetic deletion of mPGES-1 in a HER2 receptor-driven breast cancer mouse model also showed reduced number of larger tumors, in addition to suppression of angiogenesis in mammary glands [42]. In a study using xenografts from prostate cancer cells (RM9), Takahashi et al. showed that the number of lung metastases and tumor in the lung was reduced in mice treated with the coxib celecoxib. The same effect was obtained in mPGES-1 KO mice. The reduction in lung metastases was also coupled to a decrease in angiogenesis in the lung tissue [43]. It has been shown that the tumor growth in mouse xenografts using a lung cancer cell line (A549) and a prostate cancer cell line (DU145) with mPGES-1 knockdown (KD) was considerably slowed compared to cells with endogenous mPGES-1 expression [44]. Altogether these results have successfully demonstrated that the selective inhibition of mPGES-1 is a possible way to avoid general and detrimental downregulation of prostaglandins resulting from COX inhibition, but there is no selective mPGES-1 inhibitors on the market. Recent progress in the field has generated inhibitors active against both human and murine enzymes which will be an important tool to evaluate in animal studies the antitumor effect as well as to rule out toxic side effects of this pharmacological approach. Moreover, the possible shunting to other prostanoids needs to be investigated thoroughly, and the pharmacological inhibition of mPGES-1 also needs to be carefully compared to COX-1 and COX-2 selective inhibitors both in terms of antitumor efficacy and side effects.

Role of EP Receptors in Tumorigenesis

PGE_2 acts by the activation of four subtypes of receptor, known as EP1, EP2, EP3, and EP4 (Figs. 12.1 and 12.2a). These receptors belong to three clusters within the G-protein-coupled receptor superfamily of seven transmembrane-spanning proteins. EP2 and EP4 can form one cluster, transferring the signaling through increased cyclic AMP (cAMP) mediated by Gαs. EP3 is coupled to Gi and causes the decrease of cAMP formation. EP1 can increase the intracellular calcium through Gq [14]. There are several lines of evidence indicating the involvement of EP receptors in tumor progression. For example, PGE_2 regulates tumor growth by stimulating angiogenesis via EP2 [45], and EP4 is overexpressed in epithelial cancers and colorectal adenomas, mediating the functions of PGE_2 in cancer cell invasion and metastasis formation [46–49]. However, by binding EP receptors on the cell surface, PGE_2 not only can activate the downstream G-proteins but also can indirectly trigger Wnt signaling, peroxisome proliferator-activated receptor (PPAR)-δ, and epithelial growth factor receptor (EGFR) pathway. Moreover, PGE_2 can cause nuclear localization of β-catenin and increase the transcriptional targets, such as c-myc, c-jun, and cyclin D1, of Wnt signaling. The aberrant activation of Wnt pathway is thought to be relative to the initiation of various types of epithelial tumors, including CRC. In a study published in 2005 [47], it was demonstrated that PGE_2-mediated stimulation of EP2 receptors led to a direct association of the α-subunit of the regulator G-protein signaling and axin. Further, this binding causes inactivation of

GSK-3β, the downstream effector in Wnt pathway, and results in the accumulation of β-catenin in the nucleus [47].

The recent establishment of mice lacking the genes encoding EP receptors [50–52] has enhanced our understanding of the involvement of PGE_2 and its receptors in the development of colon cancer (Fig. 12.2a). Watanabe and colleagues [51] examined the development of ACFs in two strains in EP receptor (EP1 and EP3, respectively) KO mice, by treatment with the colon carcinogen AOM. The formation of ACFs was decreased only in the EP1 KO mice, and the administration of the selective EP1 antagonists, ONO-8711 [48] and ONO-8713 [51], to AOM-treated wild-type mice also resulted in a dose-dependent reduction of ACF formation. The same approach using EP3 KO mice indicated that the deficiency of EP3 receptor has no effect on colon carcinogenesis [49]. These results strongly suggest that PGE_2 contributes to colon carcinogenesis to some extent through its action at the EP1 receptor and EP1 antagonists may be good candidates as chemopreventive agents for colon cancer. In the study performed by Mutoh et al., the authors reported the development of ACFs in mice lacking EP2 or EP4 receptors and observed that only the deletion of EP4 reduced the formation of ACF lesions [49]. These data were confirmed by using the EP4-selective antagonist on the formation of colon ACFs induced by AOM in C57BL/6 mice and on the development of intestinal polyps in $APC^{Min/+}$ mice [49]. Recently, Ma and colleagues examined actions of PGE_2 in tumor microenvironment in colon tumorigenesis by using a model of colitis-associated cancer (CAC) in KO mice deficient for EP1, EP2, or EP3 receptors [52]. Among the different murine models, only EP2 KO mice showed significant suppression of colon tumor formation with reduced inflammatory responses and inflammatory cell infiltration. In fact, the authors found that EP2 in neutrophils and tumor-associated fibroblast promotes colon tumorigenesis by amplifying inflammation and shaping tumor microenvironment [52].

Involvement of TXA_2-TP Signaling in Tumorigenesis

TXA_2 is a potent vasoconstrictor, mitogen, and platelet activator [53–55], and it may be also implicated in cellular hypertrophy [56]. TXA_2 acts via the binding to the TXA_2 receptor (TP), a member of the seven transmembrane G-protein-coupled receptor superfamily. TPs are widely distributed in different organs, and they are localized on both cell membranes and intracellular structures. Two alternatively spliced variants of human TP have been described [57], and they differ in amino acids (aa) sequence at the C-terminal tail of the receptor. The original placenta-derived clone of 343 aa receptor has been designated as TPα, and a 407 aa splice variant cloned from endothelium is designated as TPβ. TP mRNAs are widely expressed in the lung, liver, kidney, heart, uterus, and vascular cells [58]. In these organs, TPα is the dominant isoform. Both TPα and TPβ couple via Gq, G11, and G12/13 to activate PLC-dependent inositol phosphate generation and elevate intracellular calcium [58], leading to vasoconstriction and platelet aggregation [59, 60]. In recent years, several studies have indicated functional roles for both TXS and TP in cancer progression in different organs such as the prostate, breast, lung, brain, bladder, and colon [61]. TXS and TXA_2 biosynthesis are increased in colon cancer and cause detrimental effects by promoting TP signaling [62] (Fig. 12.2b). The involvement of TXS in colon tumorigenesis was investigated by Pradono and colleagues [62]; in this study, the gene transfer of TXS increased colon cancer cell growth in vivo and enhanced angiogenesis. Strong evidence of a role for TXS in colorectal carcinoma was provided when its marked overexpression was observed in colorectal tumors of different grades compared to the paired normal tissues. The same study found increased expression of TXS in colon cancer cell lines and showed that abrogation of TXA_2 signaling with TXS inhibitors and TXS anti-sense as well as TP antagonists reduced proliferation of the CRC cell lines [63, 64]. The expression levels

of TP mRNA were assessed in 62 tumors and adjacent normal colon tissues: TP was expressed at higher levels in tumors compared to normal tissues but displayed lower levels in cultured CRC cell lines (HT-29 and HCA-7) [63]. The mechanism of TXA_2-induced cancer cell proliferation has been studied by Shimizu and collaborators [65] who found that a voltage-gated K+ channel, Kv7.1, is involved in the TXA_2-induced colon cancer cell proliferation and that it is upregulated by the TXA_2 receptor-mediated cAMP pathway [65] (Fig. 12.2b). In addition, in patients with FAP, which is an inherited disorder characterized by cancer of the large intestine (colon) and rectum, enhanced generation of TXB_2 in vivo was found by the assessment of its urinary enzymatic metabolite, 11-dehydro-TXB_2 [66]. In these patients, the levels of TXM were unaffected by the administration of celecoxib suggesting the involvement of a COX-1-dependent pathway, presumably from platelets. Furthermore, Sciulli et al. [67] have reported that enhanced systemic biosynthesis of TXA_2 was detected in patients with CRC and it is mainly from platelet COX-1 since it is reduced by the administration of low-dose aspirin.

NSAIDs and Cancer Prevention: Clinical Evidences

The central role of COX isozymes in human tumorigenesis is supported by the efficacy of tNSAIDs and coxibs in protecting against some cancers, particularly of the lower GI tract [1]. Epidemiologic (nonrandomized) studies have found that long-term users of aspirin or other NSAIDs have a lower risk of colorectal adenomatous polyps and CRC than nonusers, although one study has not [1]. Randomized clinical trials (RCTs) have confirmed that two NSAIDs, the prodrug sulindac [29, 68, 69] and the selective COX-2 inhibitor celecoxib [9], effectively inhibit the growth of adenomatous polyps and cause regression of existing polyps in patients with FAP. Recently, in a prospective, observational study, patients with stage III colon cancer that were enrolled in an adjuvant chemotherapy trial showed both significantly increased "event-free" and "overall" survival after consistent use of aspirin or COX-2 selective drugs (celecoxib or rofecoxib) during and after chemotherapy (according to questionnaires) [70]. Despite these positive findings, the main limit to the use of NSAIDs is that they share both beneficial and adverse effects due to the same mechanism of action, i.e., the inhibition of COX activity [71]. Their beneficial therapeutic use as anti-inflammatory and analgesic agents is associated with increased risk of clinically relevant GI side effects, i.e., GI bleeding, perforation and obstruction [31, 32], and CV side effects, including an increased risk of nonfatal myocardial infarction [32, 33, 72, 73]. NSAIDs injure the GI tract by causing topical injury to the mucosa and by systemic effects associated with mucosal prostanoid depletion derived from COX inhibition. CV toxicity is probably linked to the inhibition of COX-2-derived PGI_2 in vascular endothelial cells in the absence of an almost complete and persistent inhibition of platelet COX-1-derived TXA_2 [73]. Thus, the efficacy and safety of long-term NSAID prophylaxis against colorectal or other cancers remain unproven, and fundamental questions remain about their safety, efficacy, mechanisms of action, optimal treatment regimens, and contraindications for preventive therapy. Concerns about NSAID-associated CV toxicity have also refocused attention on the chemopreventive properties of aspirin.

Aspirin as Chemopreventive Agent

The recent results of the analysis of RCTs with aspirin by Rothwell and colleagues have fueled a renewed interest in performing studies to elucidate COX-dependent and COX-independent mechanisms of cancer prevention. The lines of evidence supporting the chemopreventive effect of aspirin against cancer mainly refer to GI tract tumors [1, 74, 75]. In particular, these evidences are derived from a large number of case-control and cohort studies reporting the association

between aspirin administration and reduced risk of different types of cancer, with largest effects on risk of GI cancers [76]. Moreover, four randomized, placebo-controlled trials in subjects with sporadic colorectal adenomas and their meta-analysis [77] demonstrated reduced risk of recurrence in aspirin-treated subjects, and a placebo-controlled, RCT in patients with Lynch Syndrome (LS, hereditary nonpolyposis colon cancer) showed that aspirin reduced cancer incidence during long-term follow-up [78] but not during the scheduled treatment phase of the trial [79]. Finally, a post hoc, individual patient data meta-analysis of 51 randomized controlled trials of daily aspirin in prevention of vascular events reported a 25 % reduction in overall cancer incidence from 3 years onward [80]. Interestingly, a reduced risk of developing CRC was also detected during long-term follow-up of healthy women treated with alternate-day 100-mg aspirin dosing versus placebo [81] and of high-risk men treated with a 75-mg controlled-release formulation of aspirin [82] (with negligible systemic bioavailability). Finally, a recent population-based, case-control study including 10,280 adults with an initial diagnosis of CRC and 102,800 adult control participants without CRC showed that the continuous use of low-dose aspirin for 5 or more years was associated with reduced risk for CRC, but overall long-term use that was possibly discontinuous was not [83].

Obesity is associated with a substantial increase of CRC risk in patients with LS; thus, in a prospective study, participants with LS were recruited to the CAPP2 study (in which they were randomly assigned to receive aspirin 600 mg per day or aspirin placebo) to evaluate the association between body mass index and cancer risk. Interestingly, this study showed that this risk is abrogated in those taking aspirin, suggesting that such patients are likely to benefit from obesity prevention and/or regular aspirin [84].

However, from a mechanistic point of view, the most interesting results came from the meta-analyses of CV trials [80, 82, 85], because they suggested that aspirin preventive effect is detectable at daily low doses of 75 mg [84], used for CV prevention [86], and it is saturable at low doses. In fact, at much higher doses (e.g., 1200 mg daily), this effect is not further improved [82].

Several mechanisms of action could explain a chemopreventive effect of high-dose aspirin [74]; among them, the COX-2 inhibition in GI mucosa and its effects on cellular proliferation, apoptosis, and angiogenesis have been proposed [80]. Differently, it seems unlikely that the targeting of nucleated cell could explain the chemopreventive effect of low-dose aspirin due to its pharmacokinetics and pharmacodynamics.

Pharmacodynamics and Pharmacokinetics of Aspirin

Aspirin, as other NSAIDs, reduces prostanoid generation by inhibiting the COX activity [71, 87]. However, aspirin, but not nonaspirin NSAIDs, causes an irreversible inactivation of COX isozymes [86]. Recent evidences have displayed a functional crosstalk between the two monomers of each COX enzyme: both monomers bind the substrate AA, but a monomer acts as an allosteric subunit (regulatory) which transforms the partner monomer into the catalytic one transforming AA into PGG_2; then PGG_2 is transformed to PGH_2 by the peroxidase activity of COX-1 and COX-2. Aspirin binds to one monomer of COX-1 and COX-2 by the interaction with Arg120 residue and modifies covalently COX isozymes by the acetylation of Ser529 and Ser516 on COX-1 and COX-2, respectively; the acetylated monomer becomes the allosteric subunit, and the partner monomer becomes the catalytic monomer. Acetylation of the allosteric subunit of COX-1 and COX-2 by aspirin causes an irreversible inactivation of the COX activity [88]. The acetylated COX-2 has a significantly compromised ability to form PGG_2 but produces an alternative product, 15R-hydroxyeicosatetraenoic acid (15R-HETE) from AA [89]. Studies performed by Smith's group [96] showed that aspirin acetylation of the regulatory monomer of COX-2 is associated with an irreversible inhibition of the catalytic monomer to form PGG_2. In contrast, the

acetylated monomer is able to transform AA into 15R-HETE. Several studies in vitro have shown that 15R-HETE is then metabolized to the epi-lipoxins (LXs) in leukocytes through the action of 5-lipoxygenase (5-LO) [90, 91]; this enzyme is also responsible for initiation of leukotriene biosynthesis. The epi-LXs may cause antiproliferative and anti-inflammatory responses [92–95]. However, convincing evidence that these lipid mediators triggered by aspirin are generated in vivo in humans is lacking.

When orally administered, aspirin is rapidly absorbed in the stomach and upper intestine by passive diffusion across GI membranes [89, 96], peak plasma levels occur 30–40 min after aspirin ingestion, and the functional inhibition of platelets is evident by 1 h. In contrast, it can take up to 6–7 h to reach peak plasma levels after administration of enteric-coated aspirin (100 mg) in healthy subjects [96]. Aspirin has a half-life of 15–20 min [87, 88]. However, despite the rapid clearance of aspirin from the circulation, the inhibitory effect of COX-1 and COX-2 is long lasting because of the irreversible inactivation of the COX isozymes. Thus, in a nucleated cell treated with aspirin, the biosynthesis of prostanoids recovers because a *de novo* protein synthesis of COXs occurs within 3–4 h. In platelets, with a limited capacity of protein synthesis [97], the irreversible inhibition of COX-1 by aspirin persists for the life-span of the platelet [98], and this effect can be reversed only through the generation of new platelets, which in humans have a mean life-span of 8–10 days. Thus, approximately 10–12 % of circulating platelets are replaced within 24 h [87]. This explains the use of aspirin at low doses (75–100 mg/daily) once daily in the antithrombotic therapy, where the target is platelet COX-1. The antiplatelet effect of aspirin is largely independent of systemic bioavailability [99–101] due to the fact that platelet COX-1 is acetylated in the presystemic circulation.

Differently, the use of high dose of aspirin (325–600 mg, given every 4–6 h, and 1.2 g, given every 4–6 h, respectively) is required to obtain an analgesic and anti-inflammatory effect, because these effects mainly occur by inhibiting COX-2 in spinal cord and inflammatory cells [19]. The necessity to use higher doses of aspirin can be explained by the reduced capability of aspirin to inhibit COX-2 than COX-1 and by the reduced plasma concentrations of aspirin, detected in the systemic circulation compared to presystemic compartment, due to its first-pass metabolism [13, 89]. Moreover, the administration of multiple doses is necessary to obtain persistent COX-2 acetylation in nucleated cells that have the capacity to resynthesize the acetylated enzyme within 3–6 days.

Clinical Pharmacology of Aspirin

In vitro experiments show that aspirin is 60-fold more potent to inhibit platelet COX-1 than monocyte COX-2 [13]. When administered in vivo to healthy subjects once daily, aspirin causes a dose-dependent inhibition of platelet COX-1 activity ex vivo, as assessed by the measurement of the generation of TXB_2 [102] in whole blood that is allowed to clot for 1 h at 37 °C (serum TXB_2 is a capacity index of platelet COX-1 activity). However, the maximal inhibition of platelet COX-1 activity is obtained at a low dose of 75–100 mg. At these doses, aspirin inhibits platelet COX-1 activity >95 %, at 1 h after dosing, and this effect persists up to 24 h [102]. The almost complete inhibition of platelet capacity to generate TXA_2 by low-dose aspirin is associated with a profound inhibition of TXA_2-dependent platelet function which persists throughout dosing interval (i.e., 24 h) [98] and represents a fundamental requisite to obtain an antithrombotic effect [87, 88]. In fact, even tiny concentrations of TXA_2 can activate platelets, and they can synergize with low concentrations of other agonists to cause a complete platelet aggregation [103].

The pharmacological effect of aspirin on systemic biosynthesis of TXA_2 is evaluated by measuring the urinary levels of major enzymatic metabolites of TXB_2, such as 11-dehydro-TXB_2, that represent indexes of actual systemic biosynthesis of TXA_2 in vivo, derived mainly from

platelet COX-1 activity [104–106]. However, the assessments of platelet COX-1 activity ex vivo and systemic TXA_2 biosynthesis in vivo are considered indirect biomarkers of aspirin action on COX-1. Between them, a nonlinear relationship has been described; in fact, >97 % inhibition of platelet COX-1 activity ex vivo is required to obtain a reduction of 70–80 % in TXA_2 biosynthesis in vivo [107]. In a recent study performed in healthy subjects treated with enteric-coated low-dose aspirin (EC-aspirin, 100 mg/day) for 7 days, we evaluated the effect of the drug on the extent and duration of platelet COX-1 acetylation at Ser529 by using a novel stable isotope dilution LC-MS/MS (liquid chromatography-mass spectrometry) technique [96]. In this study, the maximal degree of acetylated COX-1 after the seventh dose of the drug averaged 76% and was associated with an average inhibition of platelet COX-1 activity in whole blood of 99% [96]. Thus, in this study, the assessment of platelet COX-1 acetylation at Ser529 has been proposed as direct biomarker of aspirin action on COX-1.

The oral administration of low-dose aspirin once daily is associated with a maximal systemic drug concentration (approximately 7 μM) [99] which may affect only marginally COX-2 activity expressed in nucleated cells. In addition, *de novo* synthesis of the acetylated COX-2 in a nucleated cell may cause a rapid recovery of prostanoid biosynthesis. Thus, the administration of low-dose aspirin did not significantly affect whole blood COX-2 activity ex vivo [108]. Moreover, systemic biosynthesis of vascular PGI_2 (as assessed by the measurement of a major enzymatic urinary metabolite, 2,3-dino-6-keto-$PGF_{1\alpha}$, PGI-M), mainly derived from the activity of COX-2 [33], was only partially affected by low-dose aspirin [109]. However, at higher doses of aspirin, a profound inhibitory effect on the biosynthesis in vivo of PGI_2 was found [109], which might contribute to the apparent reduced antithrombotic benefit detected at high doses of the drug.

The administration of low-dose aspirin doubles the relative risks (RR) of upper GI bleeding (UGIB) in comparison to aspirin nonusers. Since, after oral dosing with low-dose aspirin once daily, the levels of the drug in the systemic circulation are insufficient to cause a substantial inhibition of the biosynthesis of cytoprotective prostanoids in the GI tract, it is plausible that the antiplatelet effect of low-dose aspirin contributes to enhanced incidence of UGIB.

Role of COX-1 in Intestinal Tumorigenesis

Platelet COX-1-Related Mechanisms

As reported above, the implication of platelet activation in cancer is sustained by the analysis of data from long-term follow-up of RCTs of daily aspirin versus control [83, 86]. These trials were designed to determine the efficacy of aspirin in the prevention of vascular occlusive events. However, it was found that regular use of aspirin, even at low doses (which targeted selectively platelets), reduces cancer incidence and mortality, in particular in the GI tract. In addition, one of the CV RCTs in which the chemopreventive effect of aspirin was detected on long-term follow-up (i.e., Thrombosis Prevention Trial, TPT) [110] involved the administration of a controlled-release formulation of aspirin (75 mg) (with negligible systemic bioavailability). Altogether these findings, even if indirectly, support the hypothesis that the inhibitory effect of platelet COX-1 by aspirin is the central mechanism in the anticancer effect of aspirin. As reported above, platelet activation has been found in patients with intestinal cancer [66], and enhanced systemic biosynthesis of TXA_2 is detected in patients with CRC, and it is mainly from platelet COX-1 since it is reduced by low-dose aspirin [67]. Platelets may contribute to tumor development through different molecular mechanisms as reported and explained below.

Inhibition of EGFR and COX-2

Platelets may participate in the early phases of intestinal tumorigenesis through the induction of phenotypic changes in stromal and epithelial cells [111] (Fig. 12.3). In fact, platelets by releasing a

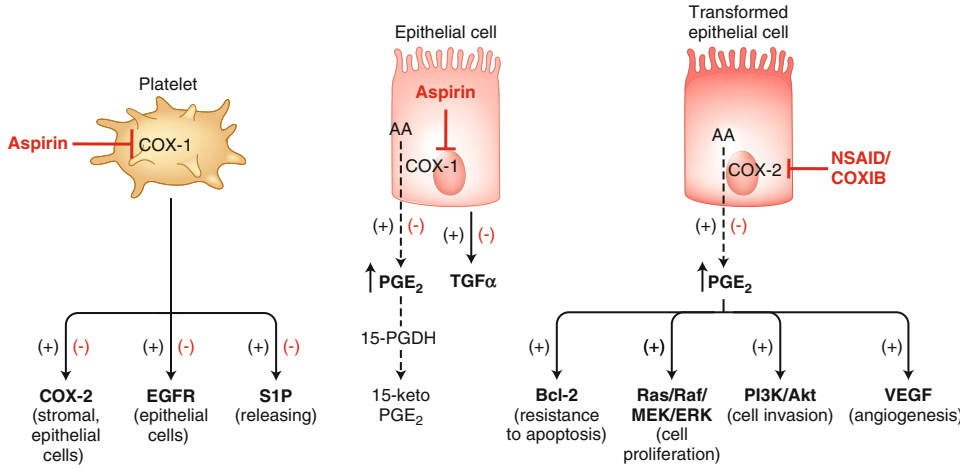


Fig. 12.3 COX-1- and COX-2-related mechanisms in intestinal tumorigenesis. Cyclooxygenase isozymes expressed in different cells (such as platelets, intestinal epithelial cells, and transformed epithelial cells) regulate several pathways involved in tumorigenesis. The chemopreventive effect of aspirin and other NSAIDs (both traditional and selective for COX-2, coxibs) may be achieved by the inhibition of these molecular events. *AA*, arachidonic acid, *PG* prostaglandin, *COX* cyclooxygenase, *NSAIDs* nonsteroidal anti-inflammatory drugs, *coxibs* COX-2 selective inhibitors, *EGFR* epithelial growth factor receptor, *S1p* sphingosine-1-phosphate, *TGF*-α transforming growth factor-α, *15-PGDH* hydroxyprostaglandin dehydrogenase 15-(NAD), *VEGF* vascular endothelial growth factor

plethora of mediators can regulate the expression of COX-2 in stromal cells; the activated stroma, in turn, can release prostanoids and protein mediators, which induce the overexpression of COX-2 in the epithelial cells [95]. The overexpression of COX-2 in the epithelium, through the generation of PGE_2, contributed to the induction of proliferative capacity, migration, invasion, and inhibition of apoptosis of epithelial cells [23]. In this scenario, low-dose aspirin, by the inhibition of platelet activation, may counteract the induction of phenotypic changes in stromal and epithelial cells induced by platelets (Fig. 12.3). In addition novel insights into the mechanism of action of aspirin in preventing CRC, are recently provided. Li and collaborators [112] addressed the hypothesis that the drug normalizes the expression of epidermal growth factor receptor (EGFR), a transmembrane receptor tyrosine kinase of the ErbB family implicated in the etiology of CRC [113] (Fig. 12.3). In FAP patients, the expression levels of EGFR and COX-2 in intestinal epithelial cells resulted to be more abundant with respect to healthy individuals [112]. They found that EGFR and COX-2 proteins were overexpressed in premalignant and malignant lesions and were colocalized. FAP patients, classified as aspirin regular users [two or more standard (325 mg) tablets per week within the previous 12 months], showed lower levels of EGFR and also COX-2 [112]. Based on clinical pharmacology data, the administration of two or more standard (325 mg) aspirin tablets per week used in the study by Li et al. seems to be incompatible with an inhibitory effect of the drug on COX-2-dependent prostanoids produced by nucleated intestinal epithelial cells. In contrast, this aspirin administration schedule might have indirectly downregulated COX-2 expression in colonic epithelial cells through the inhibition of platelet function [1].

Inhibition of Sphingosine-1-Phosphate (S1P) Release

Another mechanism underlying the chemopreventive effect of aspirin in tumorigenesis involved the inhibition of sphingosine-1-phosphate (S1P) release from platelets (Fig. 12.3). Recently, Ulrych and collaborators [114] have

shown that aspirin, both in vitro (at micromolar concentrations) and ex vivo [after dosing with a single analgesic dose (500 mg) or after the administration of an antiplatelet dose of 100 mg day, for 3 days], inhibits the release of S1P from human platelets even after stimulation with the potent peptide agonist of the thrombin receptor protease-activated receptor-1 (PAR-1) [114]. The effect of aspirin was mediated by the inhibition of platelet COX-1-dependent TXA_2 generation. In fact, formation and release of S1P from platelets are dependent on the activation of the TP receptor. S1P plays key roles as regulatory molecule in cancer development [115, 116] by the promotion of cell proliferation, survival, and regulation of angiogenesis, thus suggesting its implication in tumorigenesis. In humans, S1P is a natural constituent of plasma and is generated from sphingosine (SPH) via sphingosine kinase (SPHK), of which two isoforms (SPHK1 and SPHK2) are known [117]. Platelets generate and store high amounts of S1P released upon stimulation with activators of protein kinase C (PKC), such as thrombin and TXA_2 [118]. SPHK is highly active in platelets, which, however, lack the ability to synthesize the substrate SPH [117]. Thus, uptake of extracellular SPH and subsequent phosphorylation to S1P has been proposed as the primary mechanism of S1P formation in platelets [118]. As platelets lack the S1P-degrading enzyme S1P lyase, S1P accumulates intracellularly, and large amounts are released upon platelet activation [118].

Intestinal COX-1-Related Mechanisms

COX-1 is the only isoform constitutively expressed in the normal GI mucosa [126]. The endogenous metabolites of AA formed via COX-1, mainly PGE_2, are involved as local physiological mediator or modulator of mucosal function of the GI tract. In fact, PGE_2 can inhibit acid secretion, stimulate bicarbonate and mucus secretion, as well as affect sodium and chloride ionic flux across the injured mucosa [119].

COX-2 is not detectable in GI epithelial cells under physiological conditions, but it is induced in response to injury and inflammation [19, 120, 121]. COX-2 has been detected in the epithelial cells of colon adenomas and sporadic human colon cancers [113, 122–124], as well as in the stroma of polyps isolated from $APC^{Min/+}$ mice [125].

In serum and tissues, PGE_2 is rapidly metabolized to 15-keto PGE_2 by 15-PGDH, an enzyme which can metabolize a variety of PGs in an NAD+-dependent fashion [126]. 15-keto PGE_2 is further altered by additional enzymatic and nonenzymatic processes to produce 13,14-dihydro-15-keto-PGE_2 and tetranor PGEM. Reduced expression of 15-PGDH leads to prolonged availability and action of PGE_2 and has been linked to several cancers, including colorectal, bladder, pancreatic, and gastric adenocarcinomas [127].

It has been proposed that 15-PGDH downregulation is a very early event in intestinal tumorigenesis occurring even before COX-2 induction [128, 129]. Thus, enhanced PGE_2 might be produced early in colorectal neoplasia through the activity of COX-1 and PGDH downregulation. Recently, it has been shown that the activation of β-catenin signaling, which is deregulated early in colorectal neoplasia, represses PGDH expression [129], leading to increased PGE_2 levels, possibly even before COX-2 upregulation. Enhanced PGE_2 may also contribute to the activation of β-catenin in CRC cells, thus indicating a potential role of PGE_2 in a positive feedback loop [130, 47]. Altogether these results might explain the efficacy of low-dose aspirin to affect early steps in colorectal neoplasia through the inhibition of enhanced COX-1-dependent PGE_2 in colorectal epithelial cells associated with suppression of PGDH expression (Fig. 12.3). Despite this is an interesting hypothesis, it remains to be supported by clinical data. In particular, this hypothesis involves that aspirin, even at low-dose, may affect COX-1 activity by acetylating the protein expressed in the colorectum. Until now this information is missing.

Recently, the relation between the chemopreventive effect of aspirin and the expression of 15-PGDH in the CRC has been described. By analyzing data and samples from Nurses'

Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS), Fink and collaborators found that regular aspirin use was associated with lower risk of CRC that developed within a background of colonic mucosa with high 15-PGDH expression (multivariable HR = 0.49; 95 % CI, 0.34–0.71), but not with low 15-PGDH expression (multivariable HR = 0.90; 95 % CI, 0.63–1.27) (P for heterogeneity = 0.018). Regular aspirin use was associated with lower incidence of CRC arising in association with high 15-PGDH expression, but not with low 15-PGDH expression in normal colon mucosa [131]. This data suggested that 15-PGDH expression level in normal colon mucosa may serve as a biomarker which may predict stronger benefit from aspirin chemoprevention.

Interestingly, the results of these studies suggest the possibility to identify individuals who will get stronger benefit from aspirin chemoprevention. However, the study suffers from several significant limitations that reduce the clinical importance and usefulness of these results. The first limitation is the dose of aspirin. In the NHS, they defined regular aspirin users as women who consumed two or more standard-dose (325 mg) aspirin tablets per week and nonusers as those who reported an intake of a lower number of aspirin tablets per week. In the HPFS, the regular users of aspirin were men who reported consumption of standard-dose (325 mg) aspirin at least two times per week, while men who reported less frequent aspirin consumption were defined as nonusers [131]. Considering the data of pharmacodynamics and pharmacokinetics of aspirin, this dosage regimen and frequency schedule is compatible with the inhibition of platelet COX-1 activity and platelet function, but not with a systemic effect of the drug on COX isozymes expressed in nucleated cells. In addition, the study did not assess direct (acetylation of COX isozymes) or indirect (prostanoid levels in colonic tissue or enzymatic urinary metabolites) markers of aspirin action.

An important issue to address is whether aspirin administration could affect the activity of COX isozymes in the intestinal mucosa. Sample and colleagues [132], using rectal PGE_2 levels as a mucosal biomarker, showed that the administration of different doses of aspirin (81, 325, and 650 mg) for 4 weeks in subjects with prior sporadic colorectal adenomas significantly suppressed PGE_2 levels. In particular, aspirin at 81-mg dose significantly suppressed PGE_2 levels compared to the placebo, as well as at higher doses. Another study performer by Barnes and colleagues [133] showed that aspirin administration reduced two putative surrogate end point biomarkers of chemoprevention of CRC: mucosal PGE_2 formation and transforming growth factor-α (TGF-α) expression (Fig. 12.3). The treatment with aspirin 81 mg daily for 3 months significantly reduced rectal mucosal PGE_2 formation and TGF-α expression in patients with a history of adenomatous polyps. The data obtained in these works indirectly demonstrated that aspirin, administered even at low dose, acts systemically by inhibiting the activity of COX isozymes in the intestinal mucosa. These data should be confirmed in studies where the direct aspirin target will be evaluated, such as the acetylation of serine residues of COXs.

COX-2-Related Mechanisms in Intestinal Tumorigenesis

As reported above, the canonical Wnt pathway activation in the colonic epithelium is a key event in polyp formation and it is associated with the upregulation of several genes involved in tumor development and progression [30], including COX-2 (Fig. 12.3).

Alterations of genetic, epigenetic, and inflammatory pathways involved in intestinal carcinogenesis may influence COX-2 expression leading to elevated prostanoid biosynthesis in tumor microenvironment during the early phases on carcinogenesis. Then, an aberrant expression of COX-2 occurs in epithelial cells and may contribute to the different steps of intestinal tumorigenesis, i.e., hyperplasia and dysplasia to carcinoma and metastasis [134–136]. It is generally well accepted that transcriptional activation of COX-2 can occur early during tumorigenesis.

Due to the complexity of combined genetic alterations and inflammatory signaling occurring in the tumor microenvironment, identifying a single transcriptional pathway which plays a decisive role in promoting constitutive COX-2 expression in colon cancer has been limiting [38].

In normal cells, COX-2 expression levels are largely regulated at the posttranscriptional level through various RNA sequence elements present within the mRNA 3′ untranslated region (3′UTR) of COX-2 mRNA. A well-established mechanism controlling the expression of many inflammatory cytokines, growth factors, and proto-oncogenes is their inherent ability to be targeted for rapid mRNA decay. These cancer-associated gene transcripts are unstable due to the presence of a common *cis*-acting element known as the adenylate- and uridylate (AU)-rich element [137]. AREs mediate their regulatory function through the association of transacting RNA-binding proteins that display high affinity for AREs. It has previously reported that a loss of ARE-mediated regulation is lost early during tumor development. With regard to COX-2 regulation, similar findings have been observed in human colon carcinoma cells [38]. As result of the inability of the COX-2 ARE to function properly in CRC cells, enhanced mRNA stability was detected, and increased expression of a reporter gene containing the COX-2 3′UTR was also observed. To date, at least 16 different RNA-binding proteins have been reported to bind the COX-2 3′UTR [138].

Among them, the mRNA stability factor human antigen R (HuR) [139, 140] overexpression and its cytoplasmatic localization are associated to elevated COX-2 expression that is correlated with advancing stages of malignancy and poor clinical outcome [141, 142].

More recently, small noncoding RNAs called microRNAs (miRNAs) have emerged as global mediators of posttranscriptional gene regulation through their ability to control mRNA stability and translation by imperfect base-pairing to the 3′UTR of its target mRNA [143].

In CRC, differential expression of several miRNAs has been observed, and the loss or overexpression of specific miRNAs can impact various cellular pathways associated with colon tumorigenesis [144, 145]. Currently, 5 miRNAs have been reported to target COX-2 mRNA and control its expression, i.e., miR-16, miR-101, miR-199, miR-143, and miR-542-3p. Since miRNAs can bind imperfectly to the 3′UTR of targeted transcripts to attenuate target gene expression [146], a single miRNA can potentially control a number of putative mRNA targets and impact the expression of a large number of proteins with varying cellular functions. Thus, it is of considerable interest how alterations of miRNA expression in cancer can contribute to tumorigenesis.

COX-Independent Mechanisms of Aspirin

In addition to COX-dependent mechanism (both in platelets and intestinal mucosa), there are several evidences that aspirin may exert a chemopreventive effect through the interference with molecular pathways independent from COX activity. It is noteworthy that most of these antineoplastic effects were found using concentrations of aspirin higher than those detected after dosing with therapeutic doses of the drug.

AMPK and mTOR Signaling

AMP-activated protein kinase (AMPK) is a key energy sensor which regulates cellular metabolism to maintain energy homeostasis. AMPK acts as a metabolic master switch regulating several intracellular systems including the cellular uptake of glucose, the β-oxidation of fatty acids, and the biogenesis of glucose transporter 4 (GLUT4) and mitochondria. The energy-sensing capability of AMPK can be attributed to its ability to detect and react to fluctuations in the AMP/ATP ratio that take place during rest and exercise (muscle stimulation) [147].

Phosphorylated AMPK suppresses the downstream target mTOR (the mammalian target of

rapamycin) which functions as an intracellular nutrient sensor to control protein synthesis, cell growth, and metabolism [148].

Recently, two published works showed that aspirin activates AMPK/mTOR signaling in vitro and in vivo. Hawley and colleagues [149] demonstrated in vitro that at concentrations (millimolar) reached in plasma following administration of salsalate, or aspirin at high doses, salicylate activates AMPK. Salicylate can directly bind AMPK at the same site as the synthetic activator A-769662, to cause allosteric activation and inhibition of dephosphorylation of the activating phosphorylation site, Thr172 [149]. At the same time, Din and coworkers [150] showed that high concentration of aspirin inhibits mTOR signaling in CRC cells as evidenced by inhibition of phosphorylation of downstream effectors of mTOR signaling [i.e., ribosomal protein S6, S6 kinase 1 (S6K1), and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)], through the activation of AMPK. Interestingly, they assessed the inhibition of mTOR signaling in healthy subjects treated with aspirin 600 mg/day. After 24 h and 7 days of aspirin intake, the phosphorylation levels of S6 and S6K1 significantly decreased in normal rectal mucosa [150].

NF-kB Signaling Pathway

Several studies have shown that modulation of the NF-kB signal transduction pathway is a key mechanism for the proapoptotic activity of aspirin and other NSAIDs [151, 152]. The NF-kB transcription factor generally exists as a heterodimer bound in the cytoplasm by the inhibitor protein IkB. Following cellular stimulation by specific inducers, IkB is phosphorylated by the IkB kinase (IKK) complex and then degraded by the ubiquitin-proteasome machinery [153]. Subsequently, NF-kB translocates to the nucleus where it regulates transcription of its target genes, including those controlling cell growth. It has been shown that aspirin, as well as sodium salicylate, inhibits IKK-β activity in vitro at millimolar concentration [154] by binding to IKK-β, thus competing with ATP for the binding to the kinase, an event necessary to phosphorylate IkB.

Wnt/β-Catenin Pathway

The aberrant activation of the Wnt/β-catenin pathway is the first step in almost all CRC. The consequence of this is the accumulation of the β-catenin in the cytoplasm which can translocate to the nucleus where it binds with members of the T-cell factor (Tcf)/lymphoid enhancer factor (Lef) family of transcription factors and activates the transcription of target genes such as cyclin D, c-Myc, and COX-2 [155]. Bos and colleagues [156] showed that aspirin caused a time- and concentration-dependent increase in β-catenin phosphorylation, thereby reducing Wnt/β-catenin pathway activity, in CRC cell lines. They found that aspirin acted through the inhibition of protein phosphatase activity, the enzyme involved in the regulation of the phosphorylation status of β-catenin.

ERK Signaling

The extracellular signal-regulated kinase (ERK) signaling pathway is a major determinant in the control of different cellular processes such as proliferation, survival, differentiation, and motility. This signaling resulted to be hyperactivated in a high percentage of tumors [157]. Because of its multiple roles in the acquisition of a complex malignant phenotype, specific blockade of the ERK pathway is expected to result in an antiproliferative effect but also in antimetastatic and antiangiogenic effects in tumor cells [157]. It has been shown that aspirin and other NSAIDs inhibit ERK signaling by preventing the binding of Ras oncogene to c-Raf kinase in vitro [158].

Inhibition of AP-1 Activity

AP-1 is an inducible transcription factor containing products of the *jun* and *fos* oncogene

families [159, 160]. AP-1 is activated in response to a number of stimulants including the tumor promoter phorbol esters, epidermal growth factor, tumor necrosis factor-α, and interleukin-1 [159]. Some of the genes known to be regulated by AP-1 are involved in the immune and inflammatory responses, tumor promotion, and tumor progression. Dong and colleagues [161] evaluated the antitumor effect of aspirin in JB6 cells, a well-developed cell culture model for studying tumor promotion. They found that aspirin and salicylate, at millimolar concentration, inhibit transcription factor AP-1 activity and tumor promoter-induced transformation through a mechanism independent of PG synthesis and of the inhibition of Erk1 and Erk2 pathways, but probably involving the intracellular H^+ concentration.

Acetylation of Non-COX Proteins by Aspirin

Studies over the past decades suggest that, besides COXs, aspirin acetylates other cellular proteins. Experiments with radiolabeled ^3H or ^{14}C aspirin demonstrated that aspirin acetylates several proteins in vitro and in vivo through a transacetylation reaction [162, 163]. Aspirin acetylates human serum albumin and fibrinogen in vitro and in vivo [164, 165]. It can also acetylates several other proteins and biomolecules, such as hemoglobin, DNA, RNA, histones, and transglutaminase, as well as other plasma constituents including hormones and enzymes [162, 166]. Recently, it has been shown that aspirin, at micromolar concentration, acetylates the tumor suppressor protein p53, a known regulator of apoptosis, in human breast cancer cells. This event was associated with the induction of p21CIP, a protein involved in cell cycle arrest, and Bax, a proapoptotic protein [167]. Thus, aspirin could exert its anticancer effects by involving the acetylation of the tumor suppressor p53 and the induction of p21CIP.

Other Antiplatelet Therapies in Cancer

Several evidences support the hypothesis that platelet activation is involved in the development of cancer, particularly CRC and in facilitating metastasis [168]. In this setting, the development of platelet inhibitors that influence malignancy progression and clinical testing of currently available antiplatelet drugs represents a promising area of targeted cancer therapy.

At this time, a limited number of mechanistic studies and clinical trial data support the use of currently available antiplatelet agents in cancer therapy and the combination of antiplatelet therapy with existing tumor-targeted therapy. In contrast, the laboratory data using antiplatelet therapy continue to accumulate.

Blockage of Platelet GPIIb/IIIa Receptors

The importance of platelet receptor GPIIb/IIIa in the tumor mechanisms was showed by Boukerche and collaborators who demonstrated that human malignant melanoma cells directly interact with platelets through the GPIIb/IIIa receptor and cause platelet aggregation [169]. In this study, Fab fragments of a monoclonal antibody MoAb (LYP18), directed against the platelet GPIIb-IIIa complex, inhibited platelet-melanoma interactions and platelet-platelet aggregation. In a murine model of metastasis, Nierodzik and colleagues founded that the blockage of the platelet GPIIb/IIIa receptor using the monoclonal antibody 10E5 decreased lung colonization of cancer cells [170]. A challenging aspect of the administration of GPIIb/IIIa antagonists in the clinical setting has been the need for intravenous administration of these agents, which are now widely used in high-risk acute coronary syndromes. However, an oral inhibitor of GPIIb/IIIa, XV454, has halted experimental metastasis formation in a murine model of lung cancer [171].

Blockage of Platelet GPVI Receptor

Glycoprotein (GP)VI is a key receptor for collagen on the platelet surface. It is a member of the immunoglobulin superfamily and is uniquely expressed on the surface of platelets, where it is assembled with the immunoreceptor tyrosine activation motif subunit, FcR-γ. Jain and colleagues have shown that in a murine model of metastasis, using either a Lewis lung carcinoma (D121) or melanoma (B16F10.1) cell line, an approximately 50 % reduction in the number of visible tumor foci in GPVI-deficient mice as compared with control C57BL/6 J mice was observed [172].

In vitro data suggest that also a GPVI antagonist (revacept) may affect metastasis by inhibiting platelet-tumor cell crosstalk [173]. Revacept can bind to atherosclerotic endothelium both with and without plaque rupture; it binds vascular collagen, and thus it might interfere with other collagen-dependent pathways including α2/β1 integrins or vWF-mediated GPIb activation [174]. Revacept reduces platelet adhesion and aggregation without increasing the risk of bleeding complications and without affecting the general hemostasis [174].

Dovizio et al. have recently shown a novel pharmacological effect of revacept in platelet-cancer cell crosstalk [173]. In fact, this drug was able to interfere with the interaction of platelet collagen receptors with galectin-3 expressed in colon cancer cells HT-29. Thus, the drug, at clinically relevant concentrations [174], completely prevented the platelet-induced upregulation of COX-2 in HT-29 cells and the induction of epithelial-mesenchymal transition (EMT) markers which occurred in tumor cells by platelet interaction [173].

P-Selectin Antagonists

P-selectin is a cell surface adhesion molecule that has a central role in mediating interactions between platelets and both leukocytes and the endothelium [175]. When P-selectin is expressed on activated platelets and endothelial cells, its primary ligand, P-selectin glycoprotein ligand type 1 (PSGL-1), mediates the initial tethering and rolling process that precedes leukocyte transmigration through the vessel wall [176]. It has been proposed that P-selectin facilitated the interaction between tumor cells with both platelets and endothelial cells via sialylated fucosylated carbohydrates [177]. In addition, it has been shown that P-selectin facilitates human carcinoma metastasis in immunodeficient mice by mediating early interactions of platelets with blood-borne tumor cells via their cell surface mucins, and this process can be blocked by heparin [178].

PAR Antagonists

Thrombin, a serine protease generated by the coagulation cascade, is responsible for the generation of fibrin and in addition is a potent activator of human platelets via actions on two platelet surface G-protein-coupled receptors, PAR-1 and PAR-4 [179]. Italiano and collaborators showed that distinct populations of platelet α-granules, containing different angiogenesis influencing proteins, can be differentially released. The release of a different set of α-granules from platelets is regulated by PAR-1 and PAR-4 activation [180]. PAR-1 and PAR-4 have been shown to regulate the release of endostatin and VEGF from human platelets. These protease-activated receptors could therefore play a crucial role in regulating angiogenesis and in turn could regulate the processes of wound healing and tumor growth [181]. In a murine model of hematogenous metastasis, melanoma cells were intravenously injected in PAR-4 KO mice, and protection from lung metastases was observed. Thus, this study suggests that thrombin-induced platelet activation makes an important contribution to hematogenous metastasis [181].

P2Y12 Receptor Antagonists

Once released, ADP causes the coordinate activation of G-protein-coupled receptors, the purinergic receptors [88, 182, 183]. There are 3 types of receptors for ADP on platelets: a P2X-type ion channel-linked receptor and 2 P2Y-type G-protein-coupled receptors, P2Y1 and P2Y12 [182]. Activation of P2Y1 receptors induces a phospholipase C-mediated increase of intracellular calcium leading to platelet shape change and initial reversible aggregation via Gαq. P2Y12 receptors in their turn complete the aggregation response initiated by P2Y1receptors via Gαi-mediated inhibition of adenylyl cyclase and through a less well-defined activation of PI3K [184]. The effects of other antiplatelet agents, such as the antagonists of P2Y12 receptor, in the prevention of cancer and tumor metastasis remain to be characterized in humans. However, several experimental evidences sustain a possible anticancer effect of these agents. It has been reported that activated platelets promote metastasis through the release of small molecules such as ATP and ADP [185]. As platelet activation is largely mediated through ADP engagement of the purinergic receptor P2Y12 on platelets, P2Y12 represents an attractive target for inhibiting tumor metastases. It has been shown that the thienopyridine SR 25989, an enantiomer of the anti-aggregant clopidogrel (Plavix) lacking anti-aggregant activity, inhibits endothelial cell proliferation in vitro by increasing the expression of endogenous thrombospondin-1, a natural potent inhibitor of angiogenesis. The antiangiogenic effect of SR 25989 was further assessed in vitro in a quantitative assay of angiogenesis using a fragment of rat aorta embedded in a fibrin gel and in vivo, using a pulmonary metastatic model in C57BL/6 mice inoculated in the foot pad with the highly metastatic melanoma cell line B16 F10 [186]. Recently, Wang et al. demonstrated that tumor metastases are reduced in P2Y12-deficient mice [187]. The coadministration of the antiplatelet drugs aspirin and clopidogrel (an antagonist of P2Y12 receptor) prevents or delays hepatocarcinoma and improves survival in a mouse model of chronic immune-mediated hepatitis B [188].

Conclusive Remarks

Several evidences support the anticarcinogenic effect of aspirin and other NSAIDs [1, 120]. The findings that the protective effect of aspirin against cancer, particularly CRC, does not appear to be dose dependent and the maximal effect is detected at low doses—which are the same recommended for the prevention of CV disease—strongly support the hypothesis that the inhibition of platelet function is an important determinant [1, 13, 120, 189]. Platelets are considered inflammatory cells [190], and when activated they release a massive quantity of a wide spectrum of growth and angiogenic factors, inflammatory proteins, lipids, and vesicles containing also genetic material, including miRNAs. Platelets may be activated as a consequence of vascular and epithelial damage, as it may occur in response to lifestyle and environment factors. Altogether these events alter the normal functions of epithelial cells, thus leading to cellular transformation through the overexpression of COX-2 [95, 191]. Thus, antiplatelet agents may play a role in the prevention of CRC by modifying epigenetic mechanisms involved in colorectal tumorigenesis. Platelets may also contribute to the progression of cancer through the development of metastasis [168, 173, 192, 193]. Several mechanisms have been described, including the formation of platelet aggregates surrounding tumor cells which may support tumor cell survival and protection from immune elimination and enhancement of the adhesion of tumor cells to the endothelium, thus leading to tumor cell arrest and extravasation. The recent findings showing that platelet-derived signals induce the activation of programs [173, 192] provide new insights into the molecular mechanisms which modulate the plasticity of cellular phenotypes and open the way to novel

therapeutic interventions using antiplatelet agents to restrain and possibly prevent the development of cancer metastasis. Additional mechanistic studies to test the "platelet hypothesis" should be performed in animal models of intestinal cancer and, ideally, in different stages of the human disease. These could help to address the current uncertainty concerning the optimal chemopreventive dose and dosing regimen of aspirin. If this hypothesis would be confirmed by ongoing studies, this would provide a rationale for targeting other pathways of platelet activation and assessing the efficacy and safety of combined antiplatelet strategies for cancer prevention.

An important field of clinical research is focused on the discovery of biomarkers to select the individuals who will respond better to the antineoplastic effect of aspirin. They include plasma inflammatory markers, such as soluble tumor necrosis factor receptor-2 (TNF-R2), as well as the tumor expression levels of genes involved in prostanoid biosynthesis, including COX-2, or signaling pathways implicated in the aberrant expression of COX-2, such as PI3K. In particular, Liao and colleagues have highlighted the benefit of aspirin use in a molecular-defined subgroup of patients affected by metastatic CRC who carried activating mutations in PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) [194]. Most of these studies have the limitation of being large cohorts of participants who provided data on aspirin use from a questionnaire. Thus, these results should be confirmed in large RCTs. In these clinical studies, the use the innovative systems biology approach for the analysis of heterogeneous data sets (genomics, epigenomics, proteomics, lipidomics, and clinical) would allow to perform dynamic systems modeling of candidate pathways involved in the antineoplastic efficacy of aspirin. This strategy will also allow to identify CRC susceptibility profiles and to use them to develop new biomarkers to predict the occurrence/recurrence of CRC.

References

1. Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst. 2002;94:252–66.
2. Rigas B, Goldman IS, Levine L. Altered eicosanoid levels in human colon cancer. J Lab Clin Med. 1993;122:518–23.
3. Wang D, Dubois RN. Cyclooxygenase-2: a potential target in breast cancer. Semin Oncol. 2004;31:64–73.
4. McLemore TL, Hubbard WC, Litterst CL, Liu MC, Miller S, McMahon NA, Eggleston JC, Boyd MR. Profiles of prostaglandin biosynthesis in normal lung and tumor tissue from lung cancer patients. Cancer Res. 1988;48:3140–7.
5. Hambek M, Baghi M, Wagenblast J, Schmitt J, Baumann H, Knecht R. Inverse correlation between serum PGE2 and T classification in head and neck cancer. Head Neck. 2007;29:244–8.
6. Menter DG, Dubois RN. Prostaglandins in cancer cell adhesion, migration, and invasion. Int J Cell Biol. 2012;2012:723419.
7. Wang D, Fu L, Sun H, Guo L, DuBois RN. Prostaglandin E2 promotes colorectal cancer stem cell expansion and metastasis in mice. Gastroenterology. 2015;149:1884–95.
8. Wang D, DuBois RN. The role of prostaglandin E2 in tumor-associated immunosuppression. Trends Mol Med. 2016;22:1–3.
9. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK, Levin B, Godio L, Patterson S, Rodriguez-Bigas MA, Jester SL, King KL, Schumacher M, Abbruzzese J, DuBois RN, Hittelman WN, Zimmerman S, Sherman JW, Kelloff G. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med. 2000;342:1946–52.
10. Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med. 2006;355:885–95.
11. Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim KM, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med. 2006;355:873–84.
12. Baron JA, Sandler RS, Bresalier RS, Quan H, Riddell R, Lanas A, et al. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterology. 2006;131:1674–82.
13. Dovizio M, Bruno A, Tacconelli S, Patrignani P. Mode of action of aspirin as a chemopreventive agent. Recent Results Cancer Res. 2013;191:39–65.
14. Simmons L, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev. 2004;56:387–437.

15. Gijón MA, Leslie CC. Regulation of arachidonic acid release and cytosolic phospholipase A2 activation. J Leukoc Biol. 1999;65:330–6.
16. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. FASEB J. 1998;12:1063–73.
17. Smyth EM, Grosser T, Wang M, Yu Y, FitzGerald GA. Prostanoids in health and disease. J Lipid Res. 2009;50:S423–8.
18. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science. 2001;294:1871–5.
19. Patrignani P, Patrono C. Cyclooxygenase inhibitors: from pharmacology to clinical read-outs. Biochim Biophys Acta. 2015;1851:422–32.
20. Kudo I, Murakami M. Prostaglandin e synthase, a terminal enzyme for prostaglandin E2 biosynthesis. J Biochem Mol Biol. 2005;38:633–8.
21. Joo M, Sadikot RT. PGD synthase and PGD2 in immune response. Mediators Inflamm. 2012;2012:503128.
22. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, Ichimasa M, Sugamura K, Nakamura M, Takano S, Nagata K. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. J Exp Med. 2001;193:255–61.
23. Wang D, Dubois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. Oncogene. 2010;29:781–8.
24. Backlund MG, Mann JR, Holla VR, Buchanan FG, Tai HH, Musiek ES, Milne GL, Katkuri S, DuBois RN. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. J Biol Chem. 2005;280:3217–23.
25. Wolf I, O'Kelly J, Rubinek T, Tong M, Nguyen A, Lin BT, Tai HH, Karlan BY, Koeffler HP. 15-hydroxyprostaglandin dehydrogenase is a tumor suppressor of human breast cancer. Cancer Res. 2006;66:7818–23.
26. Hughes D, Otani T, Yang P, Newman RA, Yantiss RK, Altorki NK, Port JL, Yan M, Markowitz SD, Mazumdar M, Tai HH, Subbaramaiah K, Dannenberg AJ. NAD+-dependent 15-hydroxyprostaglandin dehydrogenase regulates levels of bioactive lipids in non-small cell lung cancer. Cancer Prev Res. 2008;1:241–9.
27. Thiel A, Ganesan A, Mrena J, Junnila S, Nykänen A, Hemmes A, Tai HH, Monni O, Kokkola A, Haglund C, Petrova TV, Ristimäki A. 15-hydroxyprostaglandin dehydrogenase is down-regulated in gastric cancer. Clin Cancer Res. 2009;15:4572–80.
28. Moser AR, Luongo C, Gould KA, McNeley MK, Shoemaker AR, Dove WF. ApcMin: a mouse model for intestinal and mammary tumorigenesis. Eur J Cancer. 1995;31A:1061–4.
29. Giardiello FM, Casero Jr RA, Hamilton SR, Hylind LM, Trimbath JD, Geiman DE, Judge KR, Hubbard W, Offerhaus GJ, Yang VW. Prostanoids, ornithine decarboxylase, and polyamines in primary chemoprevention of familial adenomatous polyposis. Gastroenterology. 2004;126:425–31.
30. Buchanan FG, DuBois RN. Connecting COX-2 and wnt in cancer. Cancer Cell. 2006;9:6–8.
31. Massó González EL, Patrignani P, Tacconelli S, García Rodríguez LA. Variability among nonsteroidal antiinflammatory drugs in risk of upper gastrointestinal bleeding. Arthritis Rheum. 2010;62:1592–601.
32. Garcia Rodriguez LA, Tacconelli S, Patrignani P. Role of dose potency in the prediction of risk of myocardial infarction associated with nonsteroidal anti-inflammatory drugs in the general population. J Am Coll Cardiol. 2008;52:1628–36.
33. Grosser T, Fries S, FitzGerald GA. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. J Clin Invest. 2006;116:4–15.
34. Wang D, DuBois RN. Pro-inflammatory prostaglandins and progression of colorectal cancer. Cancer Lett. 2008;267:197–203.
35. Samuelsson B, Morgenstern R, Jakobsson PJ. Membrane prostaglandin E synthase-1: a novel therapeutic target. Pharmacol Rev. 2007;59:207–24.
36. Jakobsson PJ, Thoren S, Morgenstern R, Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. Proc Natl Acad Sci U S A. 1999;96:7220–55.
37. Sampey AV, Monrad S, Crofford LJ. Microsomal prostaglandin E synthase-1: the inducible synthase for prostaglandin E2. Arthritis Res Ther. 2005;7:114–7.
38. Dixon DA, Blanco FF, Bruno A, Patrignani P. Mechanistic aspects of COX-2 expression in colorectal neoplasia. Recent Results Cancer Res. 2013;191:7–37.
39. Nakanishi M, Montrose DC, Clark P, Nambiar PR, Belinsky GS, Claffey KP, Xu D, Rosenberg DW. Genetic deletion of mPGES-1 suppresses intestinal tumorigenesis. Cancer Res. 2008;68:3251–9.
40. Elander N, Ungerbäck J, Olsson H, Uematsu S, Akira S, Söderkvist P. Genetic deletion of mPGES-1 accelerates intestinal tumorigenesis in APC(Min/+) mice. Biochem Biophys Res Commun. 2008;372:249–53.
41. Nakanishi M, Gokhale V, Meuillet EJ, Rosenberg DW. mPGES-1 as a target for cancer suppression: a comprehensive invited review "Phospholipase A2 and lipid mediators". Biochimie. 2010;92:660–4.
42. Howe LR, Subbaramaiah K, Chung WJ, Dannenberg AJ, Brown AM. Transcriptional activation of cyclooxygenase-2 in Wnt-1-transformed mouse mammary epithelial cells. Cancer Res. 1999;59:1572–7.
43. Takahashi R, Amano H, Satoh T, Tabata K, Ikeda M, Kitasato H, Akira S, Iwamura M, Majima M. Roles of microsomal prostaglandin E synthase-1 in lung

metastasis formation in prostate cancer RM9 cells. Biomed Pharmacother. 2014;68:71–7.
44. Hanaka H, Pawelzik SC, Johnsen JI, Rakonjac M, Terawaki K, Rasmuson A, Sveinbjörnsson B, Schumacher MC, Hamberg M, Samuelsson B, Jakobsson PJ, Kogner P, Rådmark O. Microsomal prostaglandin E synthase 1 determines tumor growth in vivo of prostate and lung cancer cells. Proc Natl Acad Sci U S A. 2009;106:18757–62.
45. Sonoshita M, Takaku K, Oshima M, Sugihara K, Taketo MM. Cyclooxygenase-2 expression in fibroblasts and endothelial cells of intestinal polyps. Cancer Res. 2002;62:6846–9.
46. Chell SD, Witherden IR, Dobson RR, Moorghen M, Herman AA, Qualtrough D, Williams AC, Paraskeva C. Increased EP4 receptor expression in colorectal cancer progression promotes cell growth and anchorage independence. Cancer Res. 2006;66:3106–13.
47. Castellone MD, Teramoto H, Williams BO, Druey KM, Gutkind JS. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. Science. 2005;310:1504–10.
48. Watanabe K, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H, Maruyama T, Kondo K, Ushikubi F, Narumiya S, Sugimura T, Wakabayashi K. Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. Cancer Res. 1999;59:5093–6.
49. Mutoh M, Watanabe K, Kitamura T, Shoji Y, Takahashi M, Kawamori T, Tani K, Kobayashi M, Maruyama T, Kobayashi K, Ohuchida S, Sugimoto Y, Narumiya S, Sugimura T, Wakabayashi K. Involvement of prostaglandin E receptor subtype EP(4) in colon carcinogenesis. Cancer Res. 2002;62:28–32.
50. Kawamori T, Kitamura T, Watanabe K, Uchiya N, Maruyama T, Narumiya S, Sugimura T, Wakabayashi K. Prostaglandin E receptor subtype EP(1) deficiency inhibits colon cancer development. Carcinogenesis. 2005;26:353–7.
51. Watanabe K, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H, Maruyama T, Kondo K, Narumiya S, Sugimura T, Wakabayashi K. Inhibitory effect of a prostaglandin E receptor subtype EP (1) selective antagonist, ONO-8713, on development of azoxymethane-induced aberrant crypt foci in mice. Cancer Lett. 2000;156:57–61.
52. Ma X, Aoki T, Tsuruyama T, Narumiya S. Definition of prostaglandin E2-EP2 signals in the colon tumor microenvironment that amplify inflammation and tumor growth. Cancer Res. 2015;75:2822–32.
53. Dorn GW, Sens D, Chaikhouni A, Mais D, Halushka PV. Cultured human vascular smooth muscle cells with functional thromboxane A2 receptors: measurement of U46619-induced 45calcium efflux. Circ Res. 1987;60:952–6.
54. Pakala R, Willerson JT, Benedict CR. Effect of serotonin, thromboxane A2, and specific receptor antagonists on vascular smooth muscle cell proliferation. Circulation. 1997;96:2280–6.
55. FitzGerald GA. Mechanisms of platelet activation: thromboxane A2 as an amplifying signal for other agonists. Am J Cardiol. 1991;68:11B–5B.
56. Ali S, Davis MG, Becker MW, Dorn 2nd GW. Thromboxane A2 stimulates vascular smooth muscle hypertrophy by up-regulating the synthesis and release of endogenous basic fibroblast growth factor. J Biol Chem. 1993;268:17397–403.
57. Raychowdhury MK, Yukawa M, Collins LJ, McGrail SH, Kent KC, Ware JA. Alternative splicing produces a divergent cytoplasmic tail in the human endothelial thromboxane A2 receptor. J Biol Chem. 1995;270:7011.
58. Breyer RM, Bagdassarian CK, Myers SA, Breyer MD. Prostanoid receptors: subtypes and signaling. Annu Rev Pharmacol Toxicol. 2001;41:661–90.
59. Needleman P, Wyche A, Raz A. Platelet and blood vessel arachidonate metabolism and interactions. J Clin Invest. 1979;63:345–9.
60. Cogolludo A, Moreno L, Bosca L, Tamargo J, Perez-Vizcaino F. Thromboxane A2-induced inhibition of voltage-gated K+ channels and pulmonary vasoconstriction: role of protein kinase C zeta. Circ Res. 2003;93:656–63.
61. Ekambaram P, Lambiv W, Cazzolli R, Ashton AW, Honn KV. The thromboxane synthase and receptor signaling pathway in cancer: an emerging paradigm in cancer progression and metastasis. Cancer Metastasis Rev. 2011;30:397–408.
62. Pradono P, Tazawa R, Maemondo M, Tanaka M, Usui K, Saijo Y, Hagiwara K, Nukiwa T. Gene transfer of thromboxane A(2) synthase and prostaglandin I(2) synthase antithetically altered tumor angiogenesis and tumor growth. Cancer Res. 2002;62:63–6.
63. Gustafsson A, Hansson E, Kressner U, Nordgren S, Andersson M, Lönnroth C, Lundholm K. Prostanoid receptor expression in colorectal cancer related to tumor stage, differentiation and progression. Acta Oncol. 2007;46:1107–12.
64. Sakai H, Suzuki T, Takahashi Y, Ukai M, Tauchi K, Fujii T, et al. Upregulation of thromboxane synthase in human colorectal carcinoma and the cancer cell proliferation by thromboxane A2. FEBS Lett. 2006;580:3368–74.
65. Shimizu T, Fujii T, Takahashi Y, Takahashi Y, Suzuki T, Ukai M, Tauchi K, Horikawa N, Tsukada K, Sakai H. Up-regulation of Kv7.1 channels in thromboxane A2-induced colonic cancer cell proliferation. Pflugers Arch. 2014;466:541–8.
66. Dovizio M, Tacconelli S, Ricciotti E, Bruno A, Maier TJ, Anzellotti P, Di Francesco L, Sala P, Signoroni S, Bertario L, Dixon DA, Lawson JA, Steinhilber D, FitzGerald GA, Patrignani P. Effects of celecoxib on prostanoid biosynthesis and circulating angiogenesis proteins in familial adenomatous polyposis. J Pharmacol Exp Ther. 2012;341:242–50.
67. Sciulli MG, Filabozzi P, Tacconelli S, Padovano R, Ricciotti E, Capone ML, Grana M, Carnevale V, Patrignani P. Platelet activation in patients with

colorectal cancer. Prostaglandins Leukot Essent Fatty Acids. 2005;72:79–83.
68. Labayle D, Fischer D, Vielh P, Drouhin F, Pariente A, Bories C, Duhamel O, Trousset M, Attali P. Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology. 1991;101:635–9.
69. Nugent KP, Farmer KC, Spigelman AD, Williams CB, Phillips RK. Randomized controlled trial of the effect of sulindac on duodenal and rectal polyposis and cell proliferation in patients with familial adenomatous polyposis. Br J Surg. 1993;80:1618–9.
70. Ng K, Meyerhardt JA, Chan AT, Sato K, Chan JA, Niedzwiecki D, Saltz LB, Mayer RJ, Benson 3rd AB, Schaefer PL, Whittom R, Hantel A, Goldberg RM, Venook AP, Ogino S, Giovannucci EL, Fuchs CS. Aspirin and COX-2 inhibitor use in patients with stage III colon cancer. J Natl Cancer Inst. 2015;107:345.
71. FitzGerald GA, Cheng Y, Austin S. COX-2 inhibitors and the cardiovascular system. Clin Exp Rheumatol. 2001;19:S31–6.
72. Bueno H, Bardají A, Patrignani P, Martín-Merino E, García-Rodríguez LA. Spanish case–control study to assess NSAID-Associated ACS risk investigators use of non-steroidal antiinflammatory drugs and type-specific risk of acute coronary syndrome. Am J Cardiol. 2010;105:1102–6.
73. Patrignani P, Tacconelli S, Bruno A, Sostres C, Lanas A. Managing the adverse effects of nonsteroidal anti-inflammatory drugs. Expert Rev Clin Pharmacol. 2011;4:605–21.
74. Chan AT. Aspirin and chemoprevention of cancer: reaching beyond the colon. Gastroenterology. 2012;143:1110–2.
75. Patrono C. The multifaceted clinical readouts of platelet inhibition by low-dose aspirin. J Am Coll Cardiol. 2015;66:74–85.
76. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol. 2012;13:518–27.
77. Cole BF, Logan RF, Halabi S, Benamouzig R, Sandler RS, Grainge MJ, Chaussade S, Baron JA. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. J Natl Cancer Inst. 2009;101:256–66.
78. Burn J, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet. 2011;378:2081–7.
79. Burn J, Bishop DT, Mecklin JP, Macrae F, Möslein G, Olschwang S, et al. Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome. N Engl J Med. 2008;359:2567–78.
80. Rothwell PM, Price JF, Fowkes FG, Zanchetti A, Roncaglioni MC, Tognoni G, Lee R, Belch JF, Wilson M, Mehta Z, Meade TW. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet. 2012;379:1602–12.
81. Cook NR, Lee IM, Zhang SM, Moorthy MV, Buring JE. Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. Ann Intern Med. 2013;159:77–85.
82. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, Meade TW. Long term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet. 2010;376:1741–50.
83. Friis S, Riis AH, Erichsen R, Baron JA, Sørensen HT. Low-dose aspirin or nonsteroidal anti-inflammatory drug use and colorectal cancer risk: a population-based, case-control study. Ann Intern Med. 2015;163:347–55.
84. Movahedi M, Bishop DT, Macrae F, Mecklin JP, Moeslein G, Olschwang S, Eccles D, Evans DG, Maher ER, Bertario L, Bisgaard ML, Dunlop MG, Ho JW, Hodgson SV, Lindblom A, Lubinski J, Morrison PJ, Murday V, Ramesar RS, Side L, Scott RJ, Thomas HJ, Vasen HF, Burn J, Mathers JC. Obesity, aspirin, and risk of colorectal cancer in carriers of hereditary colorectal cancer: a prospective investigation in the CAPP2 study. J Clin Oncol. 2015;33:3591–7.
85. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet. 2011;377:31–41.
86. Patrono C, García Rodríguez LA, Landolfi R, Baigent C. Low-dose aspirin for the prevention of atherothrombosis. N Engl J Med. 2005;353:2373–83.
87. Capone ML, Tacconelli S, Di Francesco L, Sacchetti A, Sciulli MG, Patrignani P. Pharmacodynamic of cyclooxygenase inhibitors in humans. Prostaglandins Other Lipid Mediat. 2007;82:85–94.
88. Patrono C, Baigent C, Hirsh J, Roth G, American College of Chest Physicians. Antiplatelet drugs: American college of chest physicians evidence-based clinical practice guidelines (8th edition). Chest. 2008;133:199S–233S.
89. Lecomte M, Laneuville O, Ji C, DeWitt DL, Smith WL. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. J Biol Chem. 1994;269:13207–15.
90. Patrignani P, Tacconelli S, Piazuelo E, Di Francesco L, Dovizio M, Sostres C, Marcantoni E, Guillem-Llobat P, Del Boccio P, Zucchelli M, Patrono C, Lanas A. Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and traditional indirect biomarkers of drug action. J Thromb Haemost. 2014;12:1320–30.
91. Sharma NP, Dong L, Yuan C, Noon KR, Smith WL. Asymmetric acetylation of the cyclooxygenase-2 homodimer by aspirin and its effects on the oxygenation of arachidonic, eicosapentaenoic, and

docosahexaenoic acids. Mol Pharmacol. 2010;77:979–86.
92. Gilroy DW. The role of aspirin-triggered lipoxins in the mechanism of action of aspirin. Prostaglandins Leukot Essent Fatty Acids. 2005;73:203–10.
93. Serhan CN. Lipoxins and aspirin-triggered 15-epi-lipoxins are the first lipid mediators of endogenous anti-inflammation and resolution. Prostaglandins Leukot Essent Fatty Acids. 2005;73:141–62.
94. Romano M. Lipoxin and aspirin-triggered lipoxins. Scientific World Journal. 2010;10:1048–64.
95. Fierro IM, Kutok JL, Serhan CN. Novel lipid mediator regulators of endothelial cell proliferation and migration: aspirin-triggered-15R-lipoxin A(4) and lipoxin A(4). J Pharmacol Exp Ther. 2002;300:385–92.
96. Di Francesco L, López Contreras LA, Sacco A, Patrignani P. New insights into the mechanism of action of aspirin in the prevention of colorectal neoplasia. Curr Pharm Des. 2015;21:5116–26.
97. Evangelista V, Manarini S, Di Santo A, Capone ML, Ricciotti E, Di Francesco L, Tacconelli S, Sacchetti A, D'Angelo S, Scilimati A, Sciulli MG, Patrignani P. De novo synthesis of cyclooxygenase-1 counteracts the suppression of platelet thromboxane biosynthesis by aspirin. Circ Res. 2006;98:593–5.
98. Patrignani P, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. J Clin Invest. 1982;69:1366–72.
99. Charman WN, Charman SA, Monkhouse DC, Frisbee SE, Lockhart EA, Weisman S, Fitzgerald GA. Biopharmaceutical characterization of a low-dose (75 mg) controlled-release aspirin formulation. Br J Clin Pharmacol. 1993;36:470–3.
100. Pedersen AK, FitzGerald GA. Dose-related kinetics of aspirin. Presystemic acetylation of platelet cyclooxygenase. N Engl J Med. 1984;311:1206–11.
101. Altman R, Bosch B, Brune K, Patrignani P, Young C. Advances in NSAID development: evolution of diclofenac products using pharmaceutical technology. Drugs. 2015;75:859–77.
102. Patrono C, Ciabattoni G, Pinca E, Pugliese F, Castrucci G, De Salvo A, et al. Low dose aspirin and inhibition of thromboxane B2 production in healthy subjects. Thromb Res. 1980;17:317–27.
103. Minuz P, Fumagalli L, Gaino S, Tommasoli RM, Degan M, Cavallini C, Lecchi A, Cattaneo M, Lechi Santonastaso C, Berton G. Rapid stimulation of tyrosine phosphorylation signals downstream of G protein-coupled receptors for thromboxane A2 in human platelets. Biochem J. 2006;400:127–34.
104. Ciabattoni G, Maclouf J, Catella F, FitzGerald GA, Patrono C. Radioimmunoassay of 11-dehydrothromboxane B2 in human plasma and urine. Biochim Biophys Acta. 1987;918:293–7.
105. Ciabattoni G, Pugliese F, Davì G, Pierucci A, Simonetti BM, Patrono C. Fractional conversion of thromboxane B2 to urinary 11-dehydrothromboxane B2 in man. Biochim Biophys Acta. 1989;992:66–70.
106. Catella F, FitzGerald GA. Paired analysis of urinary thromboxane B2 metabolites in humans. Thromb Res. 1987;47:647–56.
107. Reilly IA, FitzGerald GA. Inhibition of thromboxane formation in vivo and ex vivo: implications for therapy with platelet inhibitory drugs. Blood. 1987;69:180–6.
108. Capone ML, Tacconelli S, Sciulli MG, Grana M, Ricciotti E, Minuz P, Di Gregorio P, Merciaro G, Patrono C, Patrignani P. Clinical pharmacology of platelet, monocyte, and vascular cyclooxygenase inhibition by naproxen and low-dose aspirin in healthy subjects. Circulation. 2004;109:1468–71.
109. FitzGerald GA, Maas RL, Lawson JA, Oates JA, Roberts LJ, Brash AR. Aspirin inhibits endogenous prostacyclin and thromboxane biosynthesis in man. Adv Prostaglandin Thromboxane Leukot Res. 1983;11:265–6.
110. Meade TW. Thrombosis prevention trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in the primary prevention of ischaemic heart disease in men at increased risk. The Medical Research Council's General Practice Research Framework. Lancet. 1998;351:233–41.
111. Dovizio M, Alberti S, Guillem-Llobat P, Patrignani P. Role of platelets in inflammation and cancer: novel therapeutic strategies. Basic Clin Pharmacol Toxicol. 2014;114:118–27.
112. Li H, Zhu F, Boardman LA, Wang L, Oi N, Liu K, Li X, Fu Y, Limburg PJ, Bode AM, Dong Z. Aspirin prevents colorectal cancer by normalizing EGFR expression. EBioMedicine. 2015;2:447–55.
113. Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, Carotenuto A, De Feo G, Caponigro F, Salomon DS. Epidermal growth factor receptor (EGFR) signaling in cancer. Gene. 2006;366:2–16.
114. Ulrych T, Böhm A, Polzin A, Daum G, Nüsing RM, Geisslinger G, Hohlfeld T, Schrör K, Rauch BH. Release of sphingosine-1-phosphate from human platelets is dependent on thromboxane formation. J Thromb Haemost. 2011;9:790–8.
115. Kawamori T, Kaneshiro T, Okumura M, Maalouf S, Uflacker A, Bielawski J, Hannun YA, Obeid LM. Role for sphingosine kinase 1 in colon carcinogenesis. FASEB J. 2009;23:405–14.
116. Ogretmen B, Hannun YA. Biologically active sphingolipids in cancer pathogenesis and treatment. Nat Rev Cancer. 2004;4:604–16.
117. Tani M, Sano T, Ito M, Igarashi Y. Mechanisms of sphingosine and sphingosine 1-phosphate generation in human platelets. J Lipid Res. 2005;46:2458–67.
118. Yatomi Y. Sphingosine 1-phosphate in vascular biology: possible therapeutic strategies to control vascular diseases. Curr Pharm Des. 2006;12:575–87.
119. Tai HH. Prostaglandin catabolic enzymes as tumor suppressors. Cancer Metastasis Rev. 2011;30:409–17.

120. Whittle BJ. COX-1 and COX-2 products in the gut: therapeutic impact of COX-2 inhibitors. Gut. 2000;47:320–5.
121. Patrono C, Patrignani P, García Rodríguez LA. Cyclooxygenase-selective inhibition of prostanoid formation: transducing biochemical selectivity into clinical read-outs. J Clin Invest. 2001;108:7–13.
122. Kargman S, Charleson S, Cartwright M, Frank J, Riendeau D, Mancini J, Evans J, O'Neill G. Characterization of Prostaglandin G/H Synthase 1 and 2 in rat, dog, monkey, and human gastrointestinal tracts. Gastroenterology. 1996;111:445–54.
123. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994;107:1183–8.
124. Kargman SL, O'Neill GP, Vickers PJ, Evans JF, Mancini JA, Jothy S. Expression of prostaglandin G/H synthase-1 and −2 protein in human colon cancer. Cancer Res. 1995;55:2556–9.
125. Sano H, Kawahito Y, Wilder RL, Hashiramoto A, Mukai S, Asai K, Kimura S, Kato H, Kondo M, Hla T. Expression of cyclooxygenase-1 and −2 in human colorectal cancer. Cancer Res. 1995;55:3785–9.
126. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell. 1996;87:803–9.
127. Lim SC, Cho H, Lee TB, Choi CH, Min YD, Kim SS, Kim KJ. Impacts of cytosolic phospholipase A2, 15-prostaglandin dehydrogenase, and cyclooxygenase-2 expressions on tumor progression in colorectal cancer. Yonsei Med J. 2010;51:692–99.
128. Myung SJ, Rerko RM, Yan M, Platzer P, Guda K, Dotson A, Lawrence E, Dannenberg AJ, Lovgren AK, Luo G, Pretlow TP, Newman RA, Willis J, Dawson D, Markowitz SD. 15-Hydroxyprostaglandin dehydrogenase is an in vivo suppressor of colon tumorigenesis. Proc Natl Acad Sci U S A. 2006;103:12098–102.
129. Smartt HJ, Greenhough A, Ordóñez-Morán P, Talero E, Cherry CA, Wallam CA, Parry L, Al Kharusi M, Roberts HR, Mariadason JM, Clarke AR, Huelsken J, Williams AC, Paraskeva C. β-catenin represses expression of the tumour suppressor 15-prostaglandin dehydrogenase in the normal intestinal epithelium and colorectal tumour cells. Gut. 2012;61:1306–14.
130. Shao J, Jung C, Liu C, Sheng H. Prostaglandin E2 Stimulates the beta-catenin/T cell factor-dependent transcription in colon cancer. J Biol Chem. 2005;280:26565–72.
131. Fink SP, Yamauchi M, Nishihara R, Jung S, Kuchiba A, Wu K, et al. Aspirin and the risk of colorectal cancer in relation to the expression of 15-hydroxyprostaglandin dehydrogenase (HPGD). Sci Transl Med. 2014;6:233re2.
132. Sample D, Wargovich M, Fischer SM, Inamdar N, Schwartz P, Wang X, Do KA, Sinicrope FA. A dose-finding study of aspirin for chemoprevention utilizing rectal mucosal prostaglandin E(2) levels as a biomarker. Cancer Epidemiol Biomarkers Prev. 2002;11:275–9.
133. Barnes CJ, Hamby-Mason RL, Hardman WE, Cameron IL, Speeg KV, Lee M. Effect of aspirin on prostaglandin E2 formation and transforming growth factor alpha expression in human rectal mucosa from individuals with a history of adenomatous polyps of the colon. Cancer Epidemiol Biomarkers Prev. 1999;8:311–5.
134. DuBois RN, Radhika A, Reddy BS, Entingh AJ. Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. Gastroenterology. 1996;110:1259–62.
135. Hao Y, Gu X, Zhao Y, Greene S, Sha W, Smoot DT, et al. Enforced expression of miR-101 inhibits prostate cancer cell growth by modulating the COX-2 pathway in vivo. Cancer Prev Res (Phila). 2011;4:1073–83.
136. Shiff SJ, Rigas B. The role of cyclooxygenase inhibition in the antineoplastic effects of nonsteroidal antiinflammatory drugs (NSAIDs). J Exp Med. 1999;190:445–50.
137. de Silanes Lopez I, Quesada MP, Esteller M. Aberrant regulation of messenger RNA 3'-untranslated region in human cancer. Cell Oncol. 2007;29:1–17.
138. Young LE, Dixon DA. Posttranscriptional regulation of cyclooxygenase 2 expression in colorectal cancer. Curr Colorectal Cancer Rep. 2010;6:60–7.
139. Dixon DA, Tolley ND, Bemis-Standoli K, Martinez ML, Weyrich AS, Morrow JD, Prescott SM, Zimmerman GA. Expression of COX-2 in platelet-monocyte interactions occurs via combinatorial regulation involving adhesion and cytokine signaling. J Clin Invest. 2006;116:2727–38.
140. Young LE, Sanduja S, Bemis-Standoli K, Pena EA, Price RL, Dixon DA. The mRNA binding proteins HuR and tristetraprolin regulate cyclooxygenase 2 expression during colon carcinogenesis. Gastroenterology. 2009;136:1669–79.
141. Dixon DA, Tolley ND, King PH, Nabors LB, McIntyre TM, Zimmerman GA, Prescott SM. Altered expression of the mRNA stability factor HuR promotes cyclooxygenase-2 expression in colon cancer cells. J Clin Invest. 2001;108:1657–65.
142. Dixon DA, Balch GC, Kedersha N, Anderson P, Zimmerman GA, Beauchamp RD, Prescott SM. Regulation of cyclooxygenase-2 expression by the translational silencer TIA-1. J Exp Med. 2003;198:475–81.
143. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem. 2010;79:351–79.
144. O'Hara SP, Mott JL, Splinter PL, Gores GJ, LaRusso NF. MicroRNAs: key modulators of

posttranscriptional gene expression. Gastroenterology. 2009;136:17–25.
145. Wiemer EA. The role of microRNAs in cancer: no small matter. Eur J Cancer. 2007;43:1529–44.
146. Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet. 2008;9:102–14.
147. Winder WW. Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. J Appl Physiol. 1985;91:1017–28.
148. Inoki K, Kim J, Guan KL. AMPK and mTOR in cellular energy homeostasis and drug targets. Annu Rev Pharmacol Toxicol. 2012;52:381–400.
149. Hawley SA, Fullerton MD, Ross FA, Schertzer JD, Chevtzoff C, Walker KJ, Peggie MW, Zibrova D, Green KA, Mustard KJ, Kemp BE, Sakamoto K, Steinberg GR, Hardie DG. The ancient drug salicylate directly activates AMP-activated protein kinase. Science. 2012;336:918–22.
150. Din FV, Valanciute A, Houde VP, Zibrova D, Green KA, Sakamoto K, Alessi DR, Dunlop MG. Aspirin inhibits mTOR signaling, activates AMP-activated protein kinase, and induces autophagy in colorectal cancer cells. Gastroenterology. 2012;142:1504–15.
151. Shao J, Fujiwara T, Kadowaki Y, Fukazawa T, Waku T, Itoshima T, Yamatsuji T, Nishizaki M, Roth JA, Tanaka N. Overexpression of the wild-type p53 gene inhibits NF-kappaB activity and synergizes with aspirin to induce apoptosis in human colon cancer cells. Oncogene. 2000;19:726–36.
152. Takada Y, Singh S, Aggarwal BB. Identification of a p65 peptide that selectively inhibits NF-kappa B activation induced by various inflammatory stimuli and its role in down-regulation of NF-kappaB-mediated gene expression and up-regulation of apoptosis. J Biol Chem. 2004;279:15096–104.
153. Chen Z, Hagler J, Palombella VJ, Melandri F, Scherer D, Ballard D, Maniatis T. Signal-induced site-specific phosphorylation targets I kappa B alpha to the ubiquitin-proteasome pathway. Genes Dev. 1995;9:1586–97.
154. Yin MJ, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I(kappa)B kinase-beta. Nature. 1998;396:77–80.
155. Mann B, Gelos M, Siedow A, Hanski ML, Gratchev A, Ilyas M, Bodmer WF, Moyer MP, Riecken EO, Buhr HJ, Hanski C. Target genes of beta-catenin-T cell-factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. Proc Natl Acad Sci U S A. 1999;96:1603–8.
156. Bos CL, Kodach LL, van den Brink GR, Diks SH, van Santen MM, Richel DJ, Peppelenbosch MP, Hardwick JC. Effect of aspirin on the Wnt/beta-catenin pathway is mediated via protein phosphatase 2A. Oncogene. 2006;25:6447–56.
157. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene. 2007;26:3291–310.
158. Pan MR, Chang HC, Hung WC. Non-steroidal anti-inflammatory drugs suppress the ERK signaling pathway via block of Ras/c-Raf interaction and activation of MAP kinase phosphatases. Cell Signal. 2008;20:1134–41.
159. Angel P, Karin M. The role of Jun, Fos and the AP-1 complex in cell-proliferation and transformation. Biochim Biophys Acta. 1991;1072:129–57.
160. Curran T, Franza Jr BR. Fos and Jun: the AP-1 connection. Cell. 1988;55:395–7.
161. Dong Z, Huang C, Brown RE, Ma WY. Inhibition of activator protein 1 activity and neoplastic transformation by aspirin. J Biol Chem. 1997;272:9962–70.
162. Pinckard RN, Hawkins D, Farr RS. In vitro acetylation of plasma proteins, enzymes and DNA by aspirin. Nature. 1968;219:68–9.
163. Rainsford K, Schweitzer A, Brune K. Distribution of the acetyl compared with the salicyl moiety of acetylsalicylic acid: Acetylation of macromolecules in organs wherein side-effects are manifest. Biochem Pharmacol. 1983;32:1301–8.
164. Hawkins D, Pinckard RN, Farr RS. Acetylation of human serum albumin by acetylsalicylic acid. Science. 1968;160:780–1.
165. Bjornsson T, Schneider D, Berger H. Aspirin acetylates fibrinogen and enhances fibrinolysis. Fibrinolytic effect is independent of changes in plasminogen activator levels. J Pharmacol Exp Ther. 1989;250:154–61.
166. Lai TS, Davies C, Greenberg CS. Human tissue transglutaminase is inhibited by pharmacologic and chemical acetylation. Protein Sci. 2010;19:229–35.
167. Alfonso LF, Srivenugopal KS, Arumugam TV, Abbruscato TJ, Weidanz JA, Bhat GJ. Aspirin inhibits camptothecin-induced p21CIP1 levels and potentiates apoptosis in human breast cancer cells. Int J Oncol. 2009;34:597–608.
168. Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis. Nat Rev Cancer. 2011;11:123–34.
169. Boukerche H, Berthier-Vergnes O, Tabone E, Doré JF, Leung LL, McGregor JL. Platelet-melanoma cell interaction is mediated by the glycoprotein IIb-IIIa complex. Blood. 1989;74:658–63.
170. Nierodzik ML, Klepfish A, Karpatkin S. Role of platelets, thrombin, integrin IIb-IIIa, fibronectin and von Willebrand factor on tumor adhesion in vitro and metastasis in vivo. Thromb Haemost. 1995;74:282–90.
171. Amirkhosravi A, Amaya M, Siddiqui F, Biggerstaff JP, Meyer TV, Francis JL. Blockade of GpIIb/IIIa inhibits the release of vascular endothelial growth factor (VEGF) from tumor cell-activated platelets and experimental metastasis. Platelets. 1999;10:285–92.
172. Jain S, Russell S, Ware J. Platelet glycoprotein VI facilitates experimental lung metastasis in syngenic mouse models. J Thromb Haemost. 2009;7:1713–7.
173. Dovizio M, Maier TJ, Alberti S, Di Francesco L, Marcantoni E, Münch G, John CM, Suess B, Sgambato A, Steinhilber D, Patrignani P.

Pharmacological inhibition of platelet-tumor cell cross-talk prevents platelet-induced overexpression of cyclooxygenase-2 in HT29 human colon carcinoma cells. Mol Pharmacol. 2013;84:25–40.
174. Ungerer M, Rosport K, Bültmann A, Piechatzek R, Uhland K, Schlieper P, Gawaz M, Münch G. Novel antiplatelet drug revacept (Dimeric Glycoprotein VI-Fc) specifically and efficiently inhibited collagen-induced platelet aggregation without affecting general hemostasis in humans. Circulation. 2011;123:1891–9.
175. Blann AD, Nadar SK, Lip GY. The adhesion molecule P-selectin and cardiovascular disease. Eur Heart J. 2003;24:2166–79.
176. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell. 1994;76:301–14.
177. Mannori G, Crottet P, Cecconi O, Hanasaki K, Aruffo A, Nelson RM, Varki A, Bevilacqua MP. Differential colon cancer cell adhesion to E-, P-, and L-selectin: role of mucin-type glycoproteins. Cancer Res. 1995;55:4425–31.
178. Borsig L, Wong R, Hynes RO, Varki NM, Varki A. Synergistic effects of L- and P-selectin in facilitating tumor metastasis can involve non-mucin ligands and implicate leukocytes as enhancers of metastasis. Proc Natl Acad Sci U S A. 2002;99:2193–8.
179. Leger AJ, Covic L, Kuliopulos A. Protease-activated receptors in cardiovascular diseases. Circulation. 2006;114:1070–7.
180. Italiano Jr JE, Richardson JL, Patel-Hett S, Battinelli E, Zaslavsky A, Short S, Ryeom S, Folkman J, Klement GL. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. Blood. 2008;111:1227–33.
181. Ma L, Perini R, McKnight W, Dicay M, Klein A, Hollenberg MD, Wallace JL. Proteinase-activated receptors 1 and 4 counter-regulate endostatin and VEGF release from human platelets. Proc Natl Acad Sci U S A. 2005;102:216–20.
182. Fitzgerald DJ, Fitzgerald GA. Historical lessons in translational medicine: cyclooxygenase inhibition and P2Y12 antagonism. Circ Res. 2013;112:174–94.
183. Born G, Patrono C. Antiplatelet drugs. Br J Pharmacol. 2006;147 Suppl 1:S241–51.
184. Kahner BN, Shankar H, Murugappan S, Prasad GL, Kunapuli SP. Nucleotide receptor signaling in platelets. J Thromb Haemost. 2006;4:2317–26.
185. Jurasz P, Alonso-Escolano D, Radomski MW. Platelet–cancer interactions: mechanisms and pharmacology of tumour cell-induced platelet aggregation. Br J Pharmacol. 2004;143:819–26.
186. Mah-Becherel MC, Céraline J, Deplanque G, Chenard MP, Bergerat JP, Cazenave JP, Klein-Soyer C. Anti-angiogenic effects of the thienopyridine SR 25989 in vitro and in vivo in a murine pulmonary metastasis model. Br J Cancer. 2002;86:803–10.
187. Wang Y, Sun Y, Li D, Zhang L, Wang K, Zuo Y, Gartner TK, Liu J. Platelet P2Y12 is involved in murine pulmonary metastasis. PLoS One. 2013;8, e80780.
188. Sitia G, Aiolfi R, Di Lucia P, Mainetti M, Fiocchi A, Mingozzi F, et al. Antiplatelet therapy prevents hepatocellular carcinoma and improves survival in a mouse model of chronic hepatitis B. Proc Natl Acad Sci U S A. 2012;109:E2165–72.
189. Guillem-Llobat P, Dovizio M, Alberti S, Bruno A, Patrignani P. Platelets, cyclooxygenases, and colon cancer. Semin Oncol. 2014;41:385–96.
190. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest. 2005;115:3378–84.
191. Prescott SM. Is cyclooxygenase-2 the alpha and the omega in cancer? J Clin Invest. 2000;105:1511–3.
192. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell. 2011;20:576–90.
193. Mitrugno A, Williams D, Kerrigan SW, Moran N. A novel and essential role for FcγRIIa in cancer cell-induced platelet activation. Blood. 2014;123:249–60.
194. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT, Ogino S. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med. 2012;367:1596–606.

Clinical Effects of NSAIDs and COXIBs in Colon Cancer Prevention

13

Elena Piazuelo and Angel Lanas

Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide. It is estimated that around 5 % of world population will develop CRC throughout their lifespan [1]. In 2012, the incidence of this type of tumour was estimated in 1,360,602 new cases, and it caused 693,933 deaths, accounting for 8.5 % of all cancer deaths, making it the fourth most common cause of death from cancer. CRC is the third most common tumour (9.7 % of all neoplasms), behind breast and prostate cancer [2]. Far from diminishing, it is estimated that the incidence will continue increasing in the next decades to more than two million cases per year in 2030 (http://globocan.iarc.fr/Pages/burden_sel.aspx), with a consequent increase in mortality. Incidence rates vary substantially worldwide; the highest rates are in Australia/New Zealand, Europe and Northern America and the lowest in Africa and South-Central Asia. Rates are higher in men than in women in most parts of the world [3]. According to the Surveillance, Epidemiology, and End Results Program (SEER) data (2004–2010), 5-year survival for CRC is 64.7 %, varying from 89.8 % for local stage disease to 12.9 % for distant metastatic cancer [4]. The most important risk factor is age, since 90 % diagnoses occur from age 50 years. Other risk factors are type 2 diabetes, gender (male), race (African-Americans), chronic inflammation, lifestyle such as tobacco and alcohol consumption, physical activity and diet. Most CRC cases are sporadic, arising in individuals without any known familial predisposition. Around 10–30 % of cases have a positive family history of CRC, although the predisposing genetic factors involved in such a setting have not yet been characterised. Inherited CRC syndromes are less frequent, accounting for only 5 % of all CRC cases [5].

Sporadic CRC arise from the stepwise accumulation of multiple somatic mutations. Hereditary CCR results from specific, single germ line mutations. Several hereditary syndromes have been characterised and the genes involved in them identified. Thus, Lynch syndrome is caused by inherited mutations in mismatch repair genes; familial adenomatous polyposis (FAP) is caused by inherited mutations in the APC gene; and MYH-associated polyposis (MAP) is produced by biallelic mutations in the MUTYH gene.

E. Piazuelo, M.D., Ph.D.
Instituto Aragones de Ciencias de la Salud, Zaragoza, Spain

IIS Aragón, CIBER Enfermedades Hepáticas y Digestivas (CIBERehd), Universidad de Zaragoza, Zaragoza, Spain

A. Lanas, M.D., Ph.D., A.G.A.F, A.C.G.F (✉)
Universidad de Zaragoza, IIS Aragón, CIBER Enfermedades Hepáticas y Digestivas (CIBERehd), Universidad de Zaragoza, Zaragoza, Spain

Service of Digestive Diseases, University Hospital Lozano Blesa, Zaragoza, Spain
e-mail: alanas@unizar.es

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_13

Mutations in STK11 gene cause Peutz-Jeghers syndrome, and juvenile polyposis is due to mutations in SMAD4 or BMPR1A. All of these syndromes, except MAP, which is autosomal recessive, are characterised by autosomal dominant inheritance. People with FAP-associated mutations have a 90 % absolute risk of developing CRC by age 45, and people with mutations of mismatch repair proteins (Lynch syndrome) have a 40–80 % absolute risk of CRC by age 75 [6].

CRC development is a multistep process involving genetic and epigenetic changes that activate oncogenes or inactivate tumour suppressor genes or mutator genes. Different carcinogenesis pathways have been identified according to the type of genetic alterations and the order in which these alterations take place. In the majority of CRC, transformation of normal colonic epithelium to cancer is believed to follow the adenoma-carcinoma histological carcinogenesis sequence, which involves several steps: development of dysplasia in a single crypt; development of clusters that form adenomas; and changes in adenoma architecture from tubular to tubulovillous to villous increasing in size, adenoma cells showing more severe atypia, adenocarcinoma, local invasion and metastasis. It is estimated that this progression requires 10–40 years; however, most adenomas do not progress to cancer. A different histological sequence, the serrated pathway, has also been described, in which serrated polyps progress to cancer. Serrated polyps are characterised by sawtooth-like infolding of the crypt epithelium and associated with high levels of DNA methylation as the lesions progress to cancer [6].

Chemoprevention is defined as the use of natural, synthetic or biologic chemical agents to delay, prevent or reverse the development of adenomas in the large bowel and interfere with the progression from adenoma to carcinoma. Besides being effective, a chemopreventive agent should meet certain requirements to be considered as such. These requirements include the following: easily manageable, low cost and, above all, no or minimum side effects in the target population [7].

NSAIDs, especially aspirin and selective cyclooxygenase (COX-2) inhibitors (COXIBs), are one of the most studied classes of drugs in CRC chemoprevention, since a vast number of epidemiological and experimental studies have shown an inverse relationship between the consumption of these drugs and the risk of developing CRC. In this chapter, we summarise scientific evidence derived from clinical studies assessing the role of NSAIDs and COXIBs in the prevention of both sporadic and hereditary CRC. Since there is another chapter in this book dealing exclusively on aspirin, we will not include in this section those studies involving this drug and will focus on nonaspirin NSAIDs (NA-NSAIDs).

Mechanism of Action of NSAIDs/COXIBs

NSAIDs are a diverse group of drugs that are mainly used to reduce fever, pain and inflammation, being among the most frequently used classes of medications. This family of compounds exerts its pharmacological action by inhibiting the synthesis of prostanoids, a family of bioactive lipids which comprises prostaglandin (PG) E_2, $PGF_{2\alpha}$, PGD_2 and PGI_2 and thromboxane (TX) A_2. Prostanoids play important roles in many physiological processes, such as modulation of the inflammatory response, gastrointestinal cytoprotection, haemostasis and thrombosis, renal haemodynamics, atheroprotection, angiogenesis or cancer, among others [8]. Prostanoids are synthesised by the action of the enzymes PGG/H synthases 1 and 2, known as cyclooxygenases 1 and 2, homodimers of 576 and 581 amino acids, respectively [9]. Each subunit of the dimer contains the cyclooxygenase and peroxidase active sites. Both isoenzymes display the same activities and catalyse the rate-limiting step of prostanoid synthesis, which is the generation of PGH_2 from arachidonic acid, which is released from membrane phospholipids by the action of phospholipases following cellular activation. PGH_2 is transformed to the different prostanoids by different synthases. Thus, the synthesis of PGE_2 is carried out by PGE

synthase. There are three PGE synthases, one cytosolic, cPGES, and the other two bound to cell membrane, mPGES-1 and mPGES-2. Both cPGES and mPGES-2 are constitutive enzymes, whereas mPGES-1 is inducible. The latter is thought to be responsible for the increased levels of PGE_2 found in inflammation and cancer in coordination with COX-2 [10]. PGD_2 is generated by the action of two PGD synthases, lipocalin (L-PGDS) and haematopoietic (H-PGDS). The biosynthesis of thromboxane A_2 is performed by TX synthase (TS), and finally, PGI_2 is generated by PGI synthase. Despite COX-1 and COX-2 displaying the same catalytic activity and synthesising the same product, PGH_2, each of them supports different biological functions, which is explained by differences in regulation of gene expression, the requirement of different levels of substrate or distinct junction with downstream enzymes [8]. Thus, the role of COX-1 is to sustain a basal rate of prostanoid biosynthesis in the body and to enable a rapid, but brief, increase in the synthesis of prostanoids when the levels of free arachidonic acid are increased [11]. Among the most important roles of COX-1 are the constitutive synthesis of PGE_2 in the gastrointestinal tract to sustain gastrointestinal homeostasis and generation of thromboxane A_2 by activated platelets involved in haemostasis [11]. Conversely, COX-2 is induced in response to inflammatory stimuli and growth factors and is responsible for increased production of prostanoids in the presence of low levels of free arachidonic acid [12] (Fig. 13.1). In determined cells, such as endothelial cells, COX-2 is constitutively expressed, where it contributes to the continuous production of vasoprotective PGI_2.

In general, NSAIDs act by competitive and transient inhibition of arachidonic acid binding to the COX active site. Aspirin is an exception, since it causes an irreversible inactivation of COX-1 and COX-2. While therapeutic effects of NSAIDs are a consequence mainly of COX-2 inhibition, many of the side effects of NSAIDs, especially in the gastrointestinal tract, are caused by the knockdown of the protective effects of prostanoids synthesised by COX-1 [13].

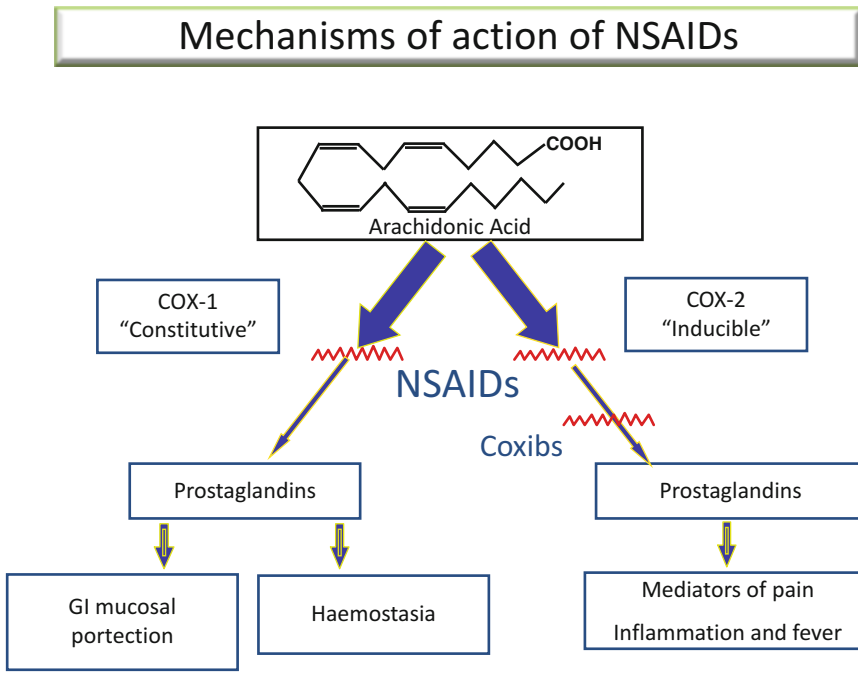


Fig. 13.1 Mechanisms of action of NSAIDs

Implication of COX-2 in CRC

Accumulating evidence has shown that COX-2 is involved in tumour promotion and progression. Most results derive from animal studies, especially in the APC^{Min} (multiple intestinal neoplasia) murine model, in which multiple intestinal polyps are formed as a consequence of a mutation in the APC gene, similar to human familial adenomatous polyposis [14]. In this model, COX-2 is expressed in dysplastic and neoplastic foci within polyps [15], and administration of COX inhibitors inhibits intestinal polyp formation [16]. Similar results were observed in other murine model of CRC such as APC Δ^{716} mice [17]. Another model of CRC induced by azoxymethane in rats is associated with an increase in COX-2 expression [18], and treatment with NS-398, a selective COX-2 inhibitor [19], or aspirin [20], inhibits carcinogenesis. COX-2 selective inhibitors prevent the growth of human CRC cell xenografts in nude mice too [21]. In humans, upregulation of COX-2 has been found in advanced colorectal adenomas and almost all CRCs [22]. Moreover, COX-2 expression has been found to increase parallel to tumour size and to be associated with more advance stage, more probability of developing lymph nodes and worse survival [23]. In addition, the role of COX-2 in human colorectal tumorigenesis is supported by the efficacy of COXIBs in reducing the risk of colorectal adenoma recurrence [24–26].

PGE_2 is the most abundant prostanoid detected in human CRC and is considered the most important downstream effector of carcinogenesis [27]. Thus, PGE_2 preserves small intestinal adenomas from NSAID-induced regression in $Apc^{Min/+}$ mice [28]. Recent studies showed that PGE_2 treatment dramatically increased intestinal adenoma cargo in the $Apc^{Min/+}$ model [29]. In addition, the increase of endogenous PGE_2 due to loss of 15-hydroxyprostaglandin dehydrogenase (15-PGDH), the enzyme responsible for PGE_2 degradation, augmented colon tumour growth in both $Apc^{Min/+}$ and azoxymethane models [30]. Even more, the leading role of PGE_2 in colorectal cancer has been corroborated by analysing the development of CCR in mice with homozygous deletion of PGE_2 receptors [31–33]. PGE_2 acts through different signal transduction pathways producing as a result the stimulation of angiogenesis, cell motility and invasion, proliferation and the inhibition of apoptosis. In addition, since many of the downstream pathways of PGE_2 upregulate COX-2 expression, such feedback loops may enhance the activity of the COX-2 pathway and as a consequence may boost the potency of COX-2 inhibitors [27].

Clinical Effects of NA-NSAIDs on Colorectal Cancer

The data supporting the use of NA-NSAIDs as chemopreventive agents in CRC come from observational, cohort and case-control studies. The first description of the inverse relationship between NSAID use and risk of colorectal cancer was reported in 1988 [34]. That study aimed to investigate the associations between colorectal cancer risk and several chronic diseases, operations and treatments. It included 715 colorectal cancer cases and 727 age/sex-matched controls obtained from "the Melbourne Colorectal Cancer Study", a large population-based study conducted in Australia. The authors found significant lower consumption of aspirin and aspirin-containing drugs among cases. This association was observed for both colon and rectal cancer and for both males and females. It must be noted that this association remained after adjustment was made for individuals with arthritis, who are frequent users of aspirin-containing compounds. The relationship between NA-NSAID intake and the risk of colorectal cancer has also been investigated. Thus, after that, other epidemiological studies showed that regular users of NA-NSAIDs, as well as aspirin, have a lower risk for CRC than non-users. Risk ratios reported for NA-NSAIDs ranged from 0.43 to 0.77, resulting in a summary RR of 0.63 (95 % CI = 0.57-0.70) derived from six studies [35]. A large population-based study was carried out

with the aim of determining the association between the use of aspirin and individual NA-NSAIDs and risk of CRC, focused especially on the role of dose and duration of drug consumption [35]. Using the General Practice Research Database, the world's largest computerised database of anonymised longitudinal clinical records from primary care, the authors studied a final cohort of 943,903 persons. In that population, the risk of CRC was decreased in users of NA-NSAIDs; adjusted relative risk was 0.5 (CI = 0.4-0.7). The protection was observed after 6 months of continuous treatment and disappeared 1 year after interrupting NSAID treatment (Fig. 13.2). Another interesting finding of this study is the observation that the protection conferred by NA-NSAIDs was independent of treatment indication. Regarding the dose of NA-NSAID necessary to prevent CRC, the authors found that highdose daily use was associated with a RR of 0.4 (95 % CI =0.3–0.7), in contrast to a RR of 0.7 (95 % CI=0.5–1.1) estimate for low-medium use. Concerning individual NA-NSAIDs, among the most frequently used NSAIDs, which were ibuprofen, diclofenac, naproxen, indomethacin and piroxicam, the latter was associated with the lowest estimate RR. More recently, a systematic review including 19 case-control studies with 20,815 cases and 11 cohort studies (1,136,110 individuals) concluded that regular use of aspirin or NSAID was consistently associated with a reduced risk of CRC, especially after use of 10 years or more, with no difference between aspirin and other NSAIDs [36]. In 2007, The US Preventive Services Task Force (USPSTF) published a new systematic review that included randomised trials, case-control and cohort studies, showing a relative risk reduction in CRC incidence of 30–40 % and in adenoma incidence of approximately 23–46 % with NA-NSAIDs [37].

Another large population-based study in Denmark assessing the associations between the use of low-dose aspirin or other NSAIDs and CRC risk has been very recently published [38]. The study population included 10,289 case patients with CRC and 102,800 control participants. In this population, use of NA-NSAIDs was associated with a minimal reduction of the risk of CRC, OR, 0.94 [CI, 0.90–0.98]. However, when duration and intensity of NA-NSAID consumption were analysed, the authors found a marked inverse association with CRC risk. Thus, 5 or more years of NA-NSAID use at an estimated average dose

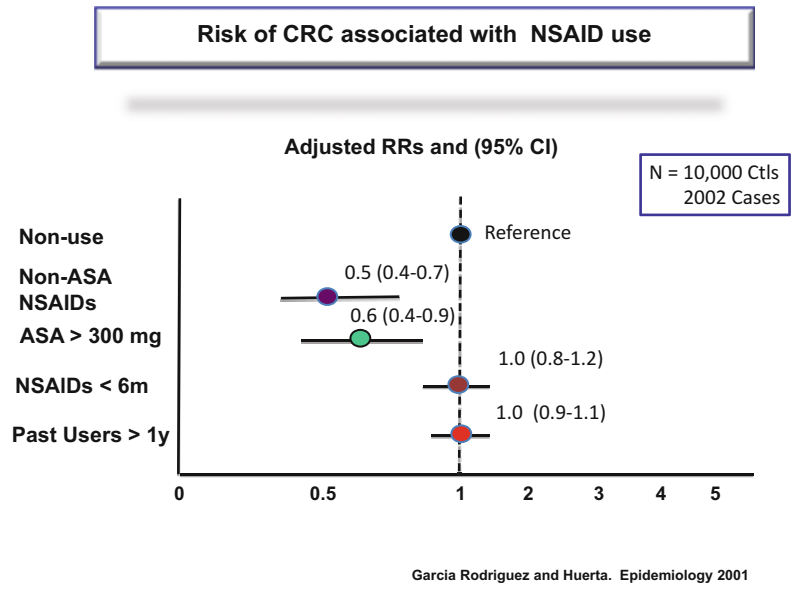


Fig. 13.2 Risk of CRC associated with NSAID use

per day of 0.3 DDD or higher was associated with a 30 % decrease in CRC risk, OR, 0.70 [CI, 0.62–0.78]. Similar results were observed for consistent use of 5 years or longer (≥2 prescriptions per year). Concerning the type of NA-NSAID, the greatest effect was seen for those with the highest COX-2 selectivity. Thereby, long-term, high-intensity use of COX-2 selective NSAIDs was associated with an OR for CRC of 0.57 [CI, 0.44–0.74], whereas for non-selective NSAIDs, the associated OR was of 0.73 [CI, 0.64–0.84].

The combination of different chemopreventive agents has been proposed to increase the effectiveness of NSAIDs and at the same time minimise their side effects [39]. The strategy consists in giving two or more drugs at low dose to decrease their side effects but obtaining the same benefit by the addition of their individual chemopreventive effects. In this manner, a phase III randomised, double-blind placebo-controlled clinical chemoprevention trial evaluating the combination of the polyamine synthesis inhibitor difluoromethylornithine (DFMO) and sulindac for the prevention of colon polyp recurrence was performed in the USA in 2008 [40]. In that trial, 375 patients with a history of resected (≥3 mm) adenomas were randomly assigned to receive oral DFMO 500 mg and sulindac 150 mg once daily or matched placebos for 36 months, stratified by use of low-dose aspirin (81 mg) at baseline and clinical site. Follow-up colonoscopy was done 3 years after randomisation. The results of that trial indicate that the combination of a low dose of DFMO plus the nonspecific NSAID sulindac at a dose one half the usual therapeutic dose markedly reduced the recurrence of all adenomas (70 % decrease), advanced adenomas (92 % decrease) and recurrence of more than one adenoma (95 % decrease) in a population of individuals at moderately high risk for sporadic adenomas (41 % of patients receiving placebos developed recurrent adenomas). No significant differences in the proportion of serious adverse effects were observed between the two arms. However, 1 year later, an analysis of the cardiovascular safety was published, reporting an increase in cardiovascular events in those subjects with cardiovascular risk factors [41].

FAP is characterised by the presence, at an early age, of multiple adenomatous polyps in the colon and rectum (hundreds or thousands), with a cumulative risk of CRC development of nearly 100 % in the fourth to fifth decade of life, if not detected and treated early. Treatment of these patients consists in colon removal (pancolectomy with ileal reservoir); however, it does not remove totally the risk of developing CRC, and, for this reason, patients have to be followed up after surgery. Since surgery has repercussion both on physical and psychological level on patients, there is a great interest on chemoprophylaxis that delay the time of surgery. In this syndrome, it is difficult to conduct studies with a large number of patients, and then, most scientific evidence is based on observational and small phase II/III trials. At the present time, the drugs with the most evidence as chemopreventive agents in FAP patients are sulindac among traditional NSAIDs and COXIBs [39]. Indeed, NSAIDs have been widely studied as chemopreventive agents in patients with FAP [42]. The first study suggesting a role for NSAIDs in chemoprevention in FAP patients was a non-randomised study of four members of a family with Gardner's syndrome; three of them had prophylactic surgery and the other conserved the colon intact. In all of them, the polyps almost completely disappeared when sulindac was administered [43]. This finding was later confirmed in a randomised, double-blind, placebo-controlled trial of 22 FAP patients and 18 of them without previous colectomy. Patients received sulindac at a dose of 150 mg twice a day or placebo during 9 months. When treatment was stopped after nine months, both the number and the size of polyps had diminished to 44 and 35 % of baseline values. Unfortunately, no patient had complete resolution of the polyps, and 3 months after the end of treatment, both the number and size of polyps had increased again although they remained significantly lower than baseline values. No side effects were observed in this study [44]. The effect of long-term sulindac has also been investigated. In a study involving

12 FAP patients who had undergone a colectomy with ileorectal anastomosis, sulindac (mean dosage, 158 mg/day) for a mean period of 63.4 ± 31.3 months induced a significant regression of polyp number in all patients. In addition, sulindac also prevents the recurrence of higher-grade adenomas (tubulovillous, villous adenomas). The most common side effect was rectal mucosal erosions [45]. The effect of sulindac in regression of adenomas has been shown in other randomised, placebo-controlled clinical trials. In one of them, 10 FAP patients who had been previously treated with colectomy and ileorectal anastomosis and had rectal polyps received sulindac, 300 mg/day, or placebo during two 4-month periods separated by a 1-month wash-out phase, showing that sulindac induced regression of rectal polyps [46]. In another trial in 24 FAP patients with a previous prophylactic colectomy and advanced duodenal polyposis, sulindac therapy during 6 months induced a significant regression of rectal polyps although the effect in duodenal polyps was much smaller and non-significant [47]. In addition, a large number of non-randomised clinical trials have assessed the efficacy of NA-NSAIDs in regression of polyps in FAP patients. In most of them, the NSAID used was sulindac given at doses ranging from 200 to 400 mg/day, the majority demonstrated a benefit by reducing the polyp burden [48–54]. Only a few studies used other NSAIDs. Thus, in two studies, indomethacin given as suppository or sustained-release formula decreased the number of polyps but increased after cessation of treatment [55, 56].

The sulfone derivate of sulindac (exisulind) has shown antineoplastic effects in colon cancer, which are attributed to the inhibition of cyclic guanosine monophosphate (cGMP) phosphodiesterase since this sulindac metabolite is not able to suppress COX activity [42]. This drug has been tested in a randomised, placebo-controlled study involving 281 patients with sporadic adenomatous polyps. Although exisulind at the highest dose tested (400 mg) caused significant regression of sporadic adenomatous polyps, it was associated with more toxicity [57]. In this sense, a phase I trial failed to show a decrease in the number of polyps and set the maximum safe dose of exisulind in 300 mg p.o. twice a day [58].

The capacity of sulindac in primary chemoprevention in FAP patients has been evaluated in a randomised, double-blind, placebo-controlled study of 41 young subjects who were genotypically affected with familial adenomatous polyposis but not phenotypically affected at that moment. After 4 years of treatment with sulindac at standard doses, no differences in the number and size of polyps were found between sulindac and placebo groups [59]. Finally, there is no evidence that sulindac prevents the development of CRC. Therefore, at the present time, sulindac can be given in FAP patients to delay the progression of polyposis but is not recommended as a primary chemopreventive agent [42].

Clinical Effects of COXIBs on Colorectal Cancer

COXIBs were developed in the 1990s with the objective of conserving the benefits of NSAIDs, such as the analgesic and anti-inflammatory effect attributed to COX-2 inhibition, but at the same time minimising the side effects derived from COX-1 inhibition, mainly gastrointestinal toxicity. The first marketed COXIBs were rofecoxib and celecoxib in 1999. During the following years, other COXIBs were introduced in the market. These included etoricoxib; valdecoxib; parecoxib, the water-soluble and injectable prodrug of valdecoxib; and finally lumiracoxib. Except lumiracoxib which is a phenyl acetic acid derivate of diclofenac, the rest of the COXIBs have a similar chemical structure, since all of them are diaryl heterocyclic derivates containing a phenylsulphone (rofecoxib and etoricoxib) or a phenylsulphonamide moiety (celecoxib and valdecoxib) [7]. From the point of view of cancer chemoprevention, the development of COXIBs raised great expectations, since indeed these drugs showed to be as effective as traditional NSAIDs but with less gastrointestinal side effects. Thus, a systematic review involving 17 trials and more than 25,000 participants revealed that serious gastrointestinal

complications and symptomatic ulcers were significantly decreased in patients allocated to COXIBs compared with non-selective NSAIDs [60]. These findings were confirmed by another systematic review of randomised controlled trials that found a 74 % reduction of the RR of gastroduodenal ulcers and 61 % reduction of the RR for relevant ulcer complications, with the use of COXIBs versus non-selective NSAIDs [61]. It must be noted that among NSAIDs, some differences exist, basically dependent on the dose but also on the type of NSAID. Thus, a case-control study showed that diclofenac, aceclofenac and ibuprofen exhibit the lowest RR of upper gastrointestinal bleeding (UGIB), whereas piroxicam and ketorolac were associated with the highest risk. In that study, the RR of UGIB associated to celecoxib was similar to that observed with paracetamol or the combination of NSAIDs with proton pump inhibitors [62]. The relevance of the dose was shown in the CLASS trial, a randomised comparison of high-dose regimens of celecoxib versus diclofenac and ibuprofen in patients with osteoarthritic pathologies, which failed to demonstrate a statistically significant difference in ulcer complications between them [63].

The first clinical trial using a COXIB with a preventive purpose in CRC was performed in FAP patients. This trial was a randomised, double-blind, placebo-controlled study where patients were allocated to two different doses of celecoxib (400 or 100 mg twice daily) or placebo for 6 months. A total of 77 patients were included in the study, who underwent endoscopy at the beginning and end of the study. Both the number and size of polyps were evaluated, and the response to treatment was expressed as the mean percent change from baseline. After six months, the patients receiving 400 mg of celecoxib twice a day showed a significant reduction both in the mean number of colorectal polyps (by 28 %) and in the polyp burden (by 30.7 %) compared with placebo group. By contrast, 100 mg of celecoxib induced a reduction of only 11.9 % and 14.6 %, respectively, which was not statistically significant [26]. The effect of celecoxib was also evaluated on duodenal polyposis showing that 400 mg induced a significant reduction in duodenal polyposis after 6 months, which was reported in a separate paper [64]. This effect was more pronounced in those patients with significant clinically disease (more than 5 % covered by polyps) at baseline. Results of this study were the basis for the preliminary FDA approval on the use of celecoxib at a dose of 800 mg/day for the treatment of patients with FAP [7]. Interestingly, a mechanistic study evaluated cell proliferation, apoptosis and PGE_2 levels in colorectal epithelia from FAP trial participants and found suppression of cell proliferation and an increased apoptotic ratio, as well as the ratio of apoptosis to cell proliferation, accompanying polyp regression, but any significant variation of PGE_2 levels was observed neither in normal mucosa nor in adenomas. Moreover, PGE_2 levels did not differ significantly among treatment arms [65]. The safety and efficacy of celecoxib was assessed in a phase I, dose-escalation trial in 18 children. That study concluded that celecoxib at a dose of 16 mg/kg/day, corresponding to the adult dose of 400 mg BID, is safe and well tolerated and significantly reduced the number of colorectal polyps in children with FAP [66]. The results of a clinical trial assessing the utility of combining two chemopreventive agents, celecoxib and DFMO (ClinicalTrials.gov number N01-CN95040), have just been published. In this study, celecoxib combined with DFMO yielded moderate synergy (40 % reduction in adenoma burden with the combination versus 27 % reduction with celecoxib), although the difference was not statistically significant. Importantly, there were no adverse cardiovascular outcomes in either trial arm [67]. Another COXIB, rofecoxib, was tested in a small group of FAP patients, showing a highly significant decrease in the rate of polyp formation (70–100 %) in all patients after a median follow-up of 16 months. In addition, no patient developed cancer or high-grade adenoma [68].

Data obtained from observational studies showed the efficacy of COXIBs as chemopreventive agents in patients with FAP, propitiated studies to examine the efficacy and

Table 13.1 Results of adenoma incidence and adverse events (cardiovascular and gastrointestinal) in COXIB trials in average-risk individuals

Study	Cohort	Subjects, *n*	Trial arms	RR for adenoma incidence	RR for CV adverse events	RR for GI adverse events
APPROVe	Prior adenoma	2587	Rofecoxib 25 mg once	0.76; 95 % CI, 0.69–0.83	1.89; 95 % CI, 1.18–3.04	4.9; 95 % CI, 1.9–14.5
			Placebo			
APC	Prior adenoma	2035	Celecoxib 200 mg b.i.d	0.67; 95 % CI, 0.59–0.77	1.5; 95 % CI, 0.9-2.3	1.0; 95 % CI, 0.8–1.4
			Celecoxib 400 mg b.i.d	0.55; 95 % CI, 0.48–0.64	1.8; 95 % CI, 1.2–2.8	0.9; 95 % CI, 0.7–1.3
			Placebo			
PreSAP	Prior adenoma	1561	Celecoxib 400 mg once	0.64; 95 % CI, 0.56–0.75	1.53; 95 % CI, 0.89–2.64	1.17; 95 % CI, 0.8–1.5
			Placebo			

Gastrointestinal adverse events included symptomatic upper-GI ulcers in APPROVe trial, or gastrointestinal bleeding, gastritis or duodenitis, upper- or lower-gastrointestinal ulceration, or other haemorrhages in PreSAP and APC trials

safety of these agents in preventing the recurrence of sporadic colorectal polyps. Thus, three randomised trials with similar designs (multicentre, randomised and placebo-controlled) were initiated between 1999 and 2000: the Adenomatous Polyp Prevention on Vioxx (APPROVe), the Adenoma Prevention with Celecoxib (APC) and the Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP) trials (Table 13.1). These studies examined the role of different COXIBs for 3 years in individuals with a recent history of adenomas and were followed up to 5 years in order to evaluate drug safety. Their primary objective was the incidence of adenomas, and secondary objectives were the incidence of advanced adenomas and the number and size of polyps [39]. The APPROVe trial included a total of 2587 subjects at high risk of developing adenomas, who were randomised to receive placebo or rofecoxib 25 mg daily. The authors found that rofecoxib reduced the risk of adenoma recurrence by 24 % compared with placebo and also the risk of advanced adenomas. It must be noted that the chemopreventive effect of rofecoxib was superior in the first year of the study than in the subsequent 2 years [25]. Regarding the safety analysis, the study was discontinued before the planned end of the trial following the advice of the External and Monitoring Board because an increased risk of cardiovascular events was observed in the rofecoxib arm [69]. In parallel, the APC trial was performed in a cohort of patients of high risk of CRC too. This trial included 2035 patients who had been recently removed an adenomatous polyp and were randomised to either placebo or celecoxib 200 mg or 400 mg twice daily. In this trial, a reduction of adenoma incidence was observed for the two doses of celecoxib tested (RR 0.55; 95 % CI, 0.48–0.64; $p < 0.001$ for 400 mg dose and RR 0.67; 95 % CI, 0.59–0.77; $p<0.001$ for 200 mg dose) as well as a reduction for advanced adenoma (RR 0.34; 95 % CI, 0.31–0.61, in the 200 mg group and RR 0.43; 95 % CI, 0.24–0.50, in the 400 mg group). After 5-year follow-up, the researchers reported that the chemopreventive action of both doses of celecoxib persisted [24]. The last trial, PRESAP, which was run parallel to ACP trial, confirmed the beneficial effect of celecoxib in preventing adenomas. This study compared the effect of celecoxib given daily in a single 400 mg dose with placebo. A total of 1561 patients were recruited. Celecoxib reduced by 36 % the risk of any adenoma and by 51 % the risk of advanced adenomas. The effect was apparent at the first year follow-up colonoscopy and continued at year 3. These results were not affected when low-dose aspirin intake at baseline was considered [70]. Subjects in the PRESAP trial were followed up 5 years from baseline and evaluated

again (the last 2 years off therapy). In the new analysis, celecoxib treatment was associated with a lower cumulative rate of adenomas and advanced adenomas compared to placebo when considering the whole period up to year 5, but when the first 3 years were omitted, the analysis showed that patients randomised to celecoxib were more prone to develop adenomas or advanced adenomas than those on placebo [71].

Expectations generated by positive results derived from these prevention trials with COXIBs were soon abrogated because of the parallel demonstration of their cardiovascular toxicity. In 2004, rofecoxib was withdrawn from the market due to the increased cardiovascular toxicity observed in the APPROVe trial. In that trial, the adverse cardiovascular effects were shown after 18 months of initiating treatment [25]. Follow-up of APPROVe participants was extended after treatment was stopped to evaluate the long-term cardiovascular toxic effects. This new analysis revealed that the increased cardiovascular risk persisted during the first year of treatment and probably was present early on therapy [72]. Cardiovascular toxicity appears to be a class effect; in fact, other trials with COXIBs have reported similar results. In the APC trial, a significant increase of serious cardiovascular events, including death from cardiovascular causes, myocardial infarction, stroke and heart failure, was observed, with a hazard ratio of 2.3 (95 % CI, 0.9–5.5) and 3.4 (95 % CI, 1.4–7.8) for the 200 mg dose and 400 mg dose of celecoxib, respectively [73]. As in the APPROVe trial, the study was ended early due to CV toxicity.

The problem of cardiovascular and gastrointestinal effects of COXIBs and traditional NSAIDs has been addressed in a large meta-analysis with a total sample over 300,000 patients from 639 trials [74]. This study has shown that high doses of diclofenac and ibuprofen are associated with similar vascular risks than COXIBs. Interestingly, this effect was not observed with high-dose naproxen. Thus, compared with placebo, COXIBs and diclofenac were associated with an increase of major vascular events, RR 1.37; CI 1.14–1.66 and RR 1.41, CI 1.12–1.78. Ibuprofen use was associated with an increase of major coronary events, RR 2.22; CI 1.10–4.48, but not with major vascular events. Conversely, naproxen did not significantly increase major vascular events. The most plausible mechanism to explain cardiovascular toxicity associated to the use of COXIBs is that they inhibit COX-2-dependent PGI_2 generation while not affecting platelet function [7]. Other non-selective NSAIDs, which are reversible inhibitors of COXs, produce a profound inhibition of COX-2-dependent PGI_2. Although they can also inhibit COX-1 and hence TXA_2 synthesis in platelets, their effect is short and incomplete because they have short half-lives. In contrast, naproxen has a long half-life, so at high doses it is the only NSAID with the ability to suppress almost completely platelet COX-1 in the interval between doses [7, 75].

Ongoing Clinical Trials with Coxibs or NA-NSAIDS

At this moment, there are only a few clinical trials assessing the role of NA-NSAIDs or COXIBs in colorectal cancer. Most of the ongoing trials are being performed in patients with established colorectal cancer. As we have previously commented, side effects of NSAIDs/COXIBs, especially their gastrointestinal and cardiovascular toxicity, have limited their use for CRC chemoprevention to high-risk populations such as hereditary CRC syndromes.

There is a phase 1 study exploring the safety of combining indomethacin with platinum-containing chemotherapy (ClinicalTrials.gov Identifier: NCT01719926) in patients with colorectal, oesophageal and ovarian neoplasms. The rationale to use indomethacin in this trial is to inhibit the synthesis of two platinum-induced fatty acids (PIFAs), the oxo-heptadecatetraenoic acid (KHT) and the omega-3 fatty acid hexadecatetraenoic acid, that are produced by mesenchymal stem cells via the COX-1 pathway, since they induce resistance to a broad spectrum of chemotherapies. Another trial, entitled "A Double Blind Placebo-Controlled Trial of

Eflornithine and Sulindac to Prevent Recurrence of High Risk Adenomas and Second Primary Colorectal Cancers in Patients With Stage 0-III Colon or Rectal Cancer, Phase III - Preventing Adenomas of the Colon With Eflornithine and Sulindac (PACES)" (ClinicalTrials.gov Identifier: NCT01349881), aims to assess whether eflornithine 500 mg or sulindac 150 mg is effective in reducing the 3-year event rate, defined as high-risk adenoma or 2nd primary colorectal cancer, in stages 0, I, II and III colon cancer patients. Sulindac is also being tested in a randomised, double-blind, phase III trial in FAP patients in order to determine if the combination of eflornithine plus sulindac is superior to sulindac or eflornithine as single agents in delaying time to the first occurrence of any FAP-related event, including FAP-related disease progression, indicating the need for excisional intervention involving the colon, rectum, pouch, duodenum and/or clinically important events which include progression to more advanced duodenal polyposis, cancer or death (ClinicalTrials.gov Identifier: NCT01483144). Another traditional NSAID, naproxen, is being evaluated in a randomised phase Ib trial to study the side effects and best dose in preventing deoxyribonucleic acid (DNA) mismatch repair-deficient colorectal cancer in patients with Lynch syndrome, "Naproxen in Preventing DNA Mismatch Repair Deficient Colorectal Cancer in Patients With Lynch Syndrome" (ClinicalTrials.gov identifier: NCT02052908).

Regarding COXIBs, there is a phase II trial studying how well capecitabine and celecoxib with or without radiation therapy work in treating patients with colorectal cancer that is newly diagnosed or has been previously treated with fluorouracil and has metastasized (Clinical Trials.gov identifier: NCT01729923). Celecoxib is being tested in another clinical trial as part of a new regimen of treatment (capecitabine, cyclophosphamide, methotrexate, celecoxib administered orally at low daily doses and without planed brakes) for patients with metastatic colorectal carcinoma (ClinicalTrials.gov Identifier: NCT02280694). A randomised phase III trial is giving oxaliplatin, leucovorin, calcium and fluorouracil together to compare how well they work when given together with or without celecoxib in treating patients with stage III colon cancer previously treated with surgery (ClinicalTrials.gov Identifier: NCT01150045). In the setting of chemoprevention, a placebo-controlled RCT to test whether celecoxib is effective in preventing colon polyp formation in children with FAP (ClinicalTrials.gov Identifier: NCT00585312) was terminated early (31 Oct 2013) due to low enrolment and low endpoint accumulation rate.

With respect to cardiovascular safety of COXIBs, there is an ongoing randomised clinical trial "Prospective Randomized Evaluation Of Celecoxib Integrated Safety Vs Ibuprofen Or Naproxen (PRECISION)" (ClinicalTrials.gov Identifier: NCT00346216), which will compare the risk of celecoxib with respect to the two most commonly prescribe traditional non-selective NSAIDs in the treatment of arthritis pain, ibuprofen and naproxen, in patients with high cardiovascular risk. The cardiovascular, gastrointestinal and renal safety and symptomatic benefit in each treatment group will be assessed accordingly. One potential limitation of this ambitious study is the fact that patients receiving low-dose ASA ($\leq$325 mg/day) are allowed to participate in the trial. Since it has been shown that both ibuprofen and naproxen, but not COXIBs, may interfere with the inhibition of platelet COX-1 by ASA, this fact can distort results obtained in the study [7].

Development of New NSAIDs

In an attempt to improve the potency and safety of NSAIDs, different chemical modifications have been introduced in some conventional NSAIDs in the last years to obtain new chemical entities that can be used in chemoprevention. One of these modifications consists in the incorporation of a part of $-ONO_2$, which releases nitric oxide (NO). This is achieved by covalent union through the carboxylic fraction of the NSAID since most NSAIDS are carboxylic acids [76]. The rationale to create nitric

oxide-releasing NSAIDs was to counteract the ulcerogenic properties of NSAIDs. Since the damage of gastroduodenal mucosa by NSAIDs is due to the inhibition of the synthesis of cytoprotective prostaglandins, and NO acts in a similar way as prostaglandins at this level, it was assumed that damage would be prevented if the NSAID could release locally NO. This hypothesis has been confirmed by several animal and human studies where NO-NSAIDs have shown to be able to confer gastric mucosa protection against the damage that the original NSAID would have caused [77]. In addition to their safer profile at gastroduodenal level, NO-NSAIDs are supposed to have a safer cardiovascular profile than traditional NSAIDs, since the well-known vasodilatory effect of NO can prevent the increase in blood pressure caused by NSAIDs. On the other hand, there is substantial evidence at preclinical level of the efficacy of the anticancer effect of NO-NSAIDs. Thus, NO-ASA, NO-sulindac and NO-ibuprofen inhibit the growth of the human colon adenocarcinoma cell line, HT-29, much more potently than their parent NSAIDs [78]. This effect has been observed with other NO-NSAIDs and other cell lines. The antitumoural action of NO-NSAIDs has been confirmed in animal models of cancer of colon cancer. Thus, tumour incidence and multiplicity were reduced in both *Min* mice and the azoxymethane model of colon cancer. In addition, the growth of colon cancer xenografts was significantly reduced with NO-ASA [79–81]. A large number of mechanistic studies have been developed to reveal the anticancer action of NO-NSAIDs. NO-NSAIDs have a strong cell growth inhibitory effect, which results from inhibition of cell proliferation, induction of apoptosis and the slowness of cell cycle phase transitions. Most mechanistic studies have been performed with NO-ASA, which have underlined the importance of the induction of apoptosis for the chemopreventive effect of the drug. Behind this pro-apoptotic effect are the induction of oxidative stress followed by activation of the intrinsic apoptosis pathway and also the inhibition of the Wnt signalling pathway. In addition to these effects, NO-ASA has shown to modulate other molecular targets such as mitogen-activated protein kinase (MAPK), the inhibition of NF-kB, inducible nitric oxide synthase, drug metabolising enzymes such as NDA(P)H:quinone oxidoreductase (NQO) or glutathione S-transferase (GST) and translocation of Nrf2 into the nucleus [77]. Recently, it has been shown that NO-ASA was more effective at suppressing microsatellite instability in mismatch repair-deficient cells than the parent ASA, which suggest a potential role of NO-ASA in chemoprevention for HNPCC patients. Unfortunately, a clinical trial of NO-ASA for the prevention of colon cancer was ended before the appointed time due to potential genotoxicity [77]. This question must be investigated before NO-NSAIDs can be considered in the area of colon cancer prevention.

Phospho-NSAIDs consist of an NSAID molecule that is connected to dialkylphosphate via a linker. Structurally, phospho-NSAIDs can be considered as diethylphosphate analogs of nitrate NO-NSAIDs [82]. The antitumoural activity of these compounds has been extensively assessed both in vitro and in animal studies. Thus, phospho-sulindac (OXT-328), phospho-ibuprofen (MDC-917), phospho-flurbiprofen (MCD-813), phospho-aspirin (MCD-118) and phospho-deoxysulindac (MCD-922) have been shown to inhibit tumour growth by decreasing cell proliferation and inducing apoptosis. Phospho-aspirin and phospho-sulindac were also found to display anticancer activity in vivo without showing detectable toxicity [83]. In addition, phospho-sulindac synergised with DFMO to prevent CRC, decreasing tumour multiplicity in the APC/*Min* mice by 90 % [84]. It has been proposed that the chemopreventive effect of phospho-NSAIDs is mediated by a COX-independent mechanism. Among the mechanisms involved, the increase of intracellular levels of reactive oxygen species (ROS), the inhibition of the thioredoxin system and a redox-sensitive transcription factor NF-kB or the induction of spermidine/spermine acetyltransferase activity has been reported [76, 82]. Another group of NSAID derivates which have been shown to possess chemoprevention action in

experimental models of CRC is NSAIDs associated with phosphatidylcholine (PC-NSAIDs). Thus, PC-aspirin and PC-ibuprofen have been reported to inhibit the growth of colon cancer cells and also the development of colonic aberrant crypt foci in azoxymethane-treated rats. These compounds have been demonstrated in rodents and in pilot clinical trials that protect against GI side effects but maintain their capacity to inhibit cyclooxygenase activity [76].

Anticholinergic NSAIDs were designed with the aim of conferring local anticholinergic activity in the gastrointestinal tract and hence protection against gastric ulcers since anticholinergic agents through the block of M1, M2 and M3 muscarinic receptors generate an optimal blood flow and oxygen supply. On the other hand, other NSAID prodrugs with acetylcholinesterase inhibitory activity (AChEI-NSAIDs) have been designed to display an anti-inflammatory activity through the increase in the levels of acetylcholine for receptor binding [82].

Tetramethyl-1-piperidinyloxy (TEMPO) and 4-hydroxy-TEMPO (TEMPOL) can play an antioxidant role. Two TEMPO-NSAIDs, TEMPO-ASA and TEMPO-indomethacin, have been synthesised. These two compounds have been shown to scavenge superoxide and also inhibit PGE_2 synthesis. Interestingly, both of them also inhibited leukotriene B_4 (LTB_4) synthesis, which is a very potent activator of leukocytes. Regarding their safety, TEMPO-INDO was shown to be about 10 times less ulcerogenic than the parent drug. Finally, hydrogen sulphide-releasing NSAIDs (HS-NSAIDs) have been synthesised. The anticancer activity of these compounds is substantially increased when combined with an NO donor. One of these NOSH-NSAID, the salicylic ester NBS-1120, has shown potent in vitro and in vivo anticancer activity [82].

References

1. National Cancer Institute. DevCan: Probability of Developing or Dying of Cancer Software, Version 6.7.0; Statistical Research and Applications Branch, National Cancer Institute, 2005.
2. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer;2013. http://globocan.iarc.fr, accessed on 27.5.2016
3. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.
4. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. 2014;64(1):9–29.
5. Ferrández A, Piazuelo E, Castells A. Aspirin and the prevention of colorectal cancer. Best Pract Res Clin Gastroenterol. 2012;26(2):185–95.
6. Chubak J, Kamineni A, Buist DS, Anderson ML, Whitlock EP. Aspirin Use for the Prevention of Colorectal Cancer: An Updated Systematic Evidence Review for the U.S. Preventive Services Task Force. Evidence Synthesis No. 133. AHRQ Publication No.15-05228-EF-1. Agency for Healthcare Research and Quality: Rockville, MD; 2015.
7. Garcia Rodriguez LA, Cea-Soriano L, Tacconelli S, Patrignani P. Coxibs: pharmacology, toxicity and efficacy in cancer clinical trials. Recent Results Cancer Res. 2013;191:67–93.
8. Patrignani P, Patrono C. Cyclooxygenase inhibitors: From pharmacology to clinical read-outs. Biochim Biophys Acta. 2015;1851(4):422–32.
9. Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacol Rev. 2004;56:387–437.
10. Kudo I, Murakami M. Prostaglandin E synthase, a terminal enzyme for prostaglandin E2 biosynthesis. J Biochem Mol Biol. 2005;38(6):633–8.
11. Capone ML, Tacconelli S, Di Francesco L, Sacchetti A, Sciulli MG, Patrignani P. Pharmacodynamic of cyclooxygenase inhibitors in humans. Prostaglandins Other Lipid Mediat. 2007;82(1-4):85–94.
12. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem. 2000;69:145–82.
13. Brune K, Patrignani P. New insights into the use of currently available non-steroidal anti-inflammatory drugs. J Pain Res. 2015;8:105–18.
14. Doherty GA, Murray FE. Cyclooxygenase as a target for chemoprevention in colorectal cancer: lost cause or a concept coming of age? Expert Opin Ther Targets. 2009;13(2):209–18.
15. Williams CS, Luongo C, Radhika A, Zhang T, Lamps LW, Nanney LB, et al. Elevated cyclooxygenase-2-levels in Min mouse adenomas. Gastroenterology. 1996;111(4):1134–40.
16. Jacoby RF, Marshall DJ, Newton MA, Novakovic K, Tutsch K, Cole CE, et al. Chemoprevention of spontaneous intestinal adenomas in the Apc Min mouse

model by the nonsteroidal anti-inflammatory drug piroxicam. Cancer Res. 1996;56(4):710–4.
17. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, et al. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell. 1996;87 (5):803–9.
18. DuBois RN, Radhika A, Reddy BS, Entingh AJ. Increased cyclooxygenase-2 levels in carcinogen-induced rat colonic tumors. Gastroenterology. 1996;110(4):1259–62.
19. Yoshimi N, Shimizu M, Matsunaga K, Yamada Y, Fujii K, Hara A, et al. Chemopreventive effect of N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide (NS-398), a selective cyclooxygenase-2-inhibitor, in rat colon carcinogenesis induced by azoxymethane. Jpn J Cancer Res. 1999;90(4):406–12.
20. Reddy BS, Rao CV, Rivenson A, Kelloff G. Inhibitory effect of aspirin on azoxymethane-induced colon carcinogenesis in F344 rats. Carcinogenesis. 1993;14 (8):1493–7.
21. Sheng H, Shao J, Kirkland SC, Isakson P, Coffey RJ, Morrow J, et al. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. J Clin Invest. 1997;99(9):2254–9.
22. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994;107(4):1183–8.
23. Sheehan KM, Sheahan K, O'Donoghue DP, MacSweeney F, Conroy RM, Fitzgerald DJ, et al. The relationship between cyclooxygenase-2-expression and colorectal cancer. JAMA. 1999;282 (13):1254–7.
24. Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, et al. APC Study Investigators. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med. 2006;355(9):873–84.
25. Baron JA, Sandler RS, Bresalier RS, Quan H, Riddell R, Lanas A, APPROVe Trial Investigators, et al. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. Gastroenterology. 2006;131(6):1674–82.
26. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med. 2000;342(26):1946–52.
27. Wang D, DuBois RN. The role of COX-2 in intestinal inflammation and colorectal cancer. Oncogene. 2010;29(6):781–8.
28. Hansen-Petrik MB, McEntee MF, Jull B, Shi H, Zemel MB, Whelan J. Prostaglandin E(2) protects intestinal tumors from nonsteroidal anti-inflammatory drug-induced regression in Apc(Min/+) mice. Cancer Res. 2002;62(2):403–8.
29. Kawamori T, Uchiya N, Sugimura T, Wakabayashi K. Enhancement of colon carcinogenesis by prostaglandin E_2 administration. Carcinogenesis. 2003;24(5):985–90.
30. Myung SJ, Rerko RM, Yan M, Platzer P, Guda K, Dotson A, et al. 15-Hydroxyprostaglandin dehydrogenase is an in vivo suppressor of colon tumorigenesis. Proc Natl Acad Sci U S A. 2006;103(32):12098–102.
31. Mutoh M, Watanabe K, Kitamura T, Shoji Y, Takahashi M, Kawamori T, et al. Involvement of prostaglandin E receptor subtype EP(4) in colon carcinogenesis. Cancer Res. 2002;62(1):28–32.
32. Sonoshita M, Takaku K, Sasaki N, Sugimoto Y, Ushikubi F, Narumiya S, et al. Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc (Delta 716) knockout mice. Nat Med. 2001;7 (9):1048–51.
33. Watanabe K, Kawamori T, Nakatsugi S, Ohta T, Ohuchida S, Yamamoto H, et al. Role of the prostaglandin E receptor subtype EP1 in colon carcinogenesis. Cancer Res. 1999;59(20):5093–6.
34. Kune GA, Kune S, Watson LF. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Res. 1988;48(15):4399–404.
35. García-Rodríguez LA, Huerta-Alvarez C. Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. Epidemiology. 2001;12(1):88–93.
36. Flossmann E. Rothwell PM; British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet. 2007;369(9573):1603–13.
37. Rostom A, Dube C, Lewin G. Use of Aspirin and NSAIDs to prevent colorectal cancer: an evidence synthesis. Prepared for the Agency for Healthcare Research and Quality by the University of Ottawa Evidence-based Practice Center at the University of Ottawa, Ottawa Canada, under Contract No. 290-02-0021. AHRQ Publication No. 07-0596-EF-1. Rockville: Agency for Healthcare Research and Quality; 2007.
38. Friis S, Riis AH, Erichsen R, Baron JA, Sørensen HT. Low-dose aspirin or nonsteroidal anti-inflammatory drug use and colorectal cancer risk: a population-based, case-control study. Ann Intern Med. 2015;163(5):347–55.
39. Manzano A, Pérez-Segura P. Colorectal cancer chemoprevention: is this the future of colorectal cancer prevention? Scientific World Journal. 2012;2012:327341.
40. Meyskens Jr FL, McLaren CE, Pelot D, Fujikawa-Brooks S, Carpenter PM, Hawk E, et al. Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: a randomized placebo-controlled, double-blind trial. Cancer Prev Res (Phila). 2008;1(1):32–8.
41. Zell JA, Pelot D, Chen WP, McLaren CE, Gerner EW, Meyskens FL. Risk of cardiovascular events in a randomized placebo-controlled, double-blind trial of

difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas. Cancer Prev Res (Phila). 2009;2(3):209–12.
42. Kim B, Giardiello FM. Chemoprevention in familial adenomatous polyposis. Best Pract Res Clin Gastroenterol. 2011;25(4-5):607–22.
43. Waddell WR, Loughry RW. Sulindac for polyposis of the colon. J Surg Oncol. 1983;24(1):83–7.
44. Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hylind LM, Celano P, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med. 1993;328(18):1313–6.
45. Cruz-Correa M, Hylind LM, Romans KE, Booker SV, Giardiello FM. Long-term treatment with sulindac in familial adenomatous polyposis: a prospective cohort study. Gastroenterology. 2002;122(3):641–5.
46. Labayle D, Fischer D, Vielh P, Drouhin F, Pariente A, Bories C, et al. Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology. 1991;101(3):635–9.
47. Nugent KP, Farmer KC, Spigelman AD, Williams CB, Phillips RK. Randomized controlled trial of the effect of sulindac on duodenal and rectal polyposis and cell proliferation in patients with familial adenomatous polyposis. Br J Surg. 1993;80(12):1618–9.
48. Waddell WR, Ganser GF, Cerise EJ, Loughry RW. Sulindac for polyposis of the colon. Am J Surg. 1989;157(1):175–9.
49. Rigau J, Piqué JM, Rubio E, Planas R, Tarrech JM, Bordas JM. Effects of long-term sulindac therapy on colonic polyposis. Ann Intern Med. 1991;115 (12):952–4.
50. Spagnesi MT, Tonelli F, Dolara P, Caderni G, Valanzano R, Anastasi A, et al. Rectal proliferation and polyp occurrence in patients with familial adenomatous polyposis after sulindac treatment. Gastroenterology. 1994;106(2):362–6.
51. Winde G, Schmid KW, Brandt B, Müller O, Osswald H. Clinical and genomic influence of sulindac on rectal mucosa in familial adenomatous polyposis. Dis Colon Rectum. 1997;40(10):1156–69.
52. Tonelli F, Valanzano R, Messerini L, Ficari F. Long-term treatment with sulindac in familial adenomatous polyposis: is there an actual efficacy in prevention of rectal cancer? J Surg Oncol. 2000;74(1):15–20.
53. Fernández-López F, Conde-Freire R, Cadarso-Suárez C, García-Iglesias J, Puente-Domínguez JL, Potel-Lesquereux J. Sulindac in familial adenomatous polyposis: evaluation by nuclear morphometry. Eur J Surg. 2001;167(5):375–81.
54. Guldenschuh I, Hurlimann R, Muller A, Ammann R, Mullhaupt B, Dobbie Z, et al. Relationship between APC genotype, polyp distribution, and oral sulindac treatment in the colon and rectum of patients with familial adenomatous polyposis. Dis Colon Rectum. 2001;44(8):1090–9.
55. Hirota C, Iida M, Aoyagi K, Matsumoto T, Tada S, Yao T, et al. Effect of indomethacin suppositories on rectal polyposis in patients with familial adenomatous polyposis. Cancer. 1996;78(8):1660–5.
56. Akasu T, Yokoyama T, Sugihara K, Fujita S, Moriya Y, Kakizoe T. Peroral sustained-release indomethacin treatment for rectal adenomas in familial adenomatous polyposis: a pilot study. Hepatogastroenterology. 2002;49(47):1259–61.
57. Arber N, Kuwada S, Leshno M, Sjodahl R, Hultcrantz R, Rex D, Exisulind Study Group. Sporadic adenomatous polyp regression with exisulind is effective but toxic: a randomised, double blind, placebo controlled, dose-response study. Gut. 2006;55(3):367–73.
58. van Stolk R, Stoner G, Hayton WL, Chan K, DeYoung B, Kresty L, et al. Phase I trial of exisulind (sulindac sulfone, FGN-1) as a chemopreventive agent in patients with familial adenomatous polyposis. Clin Cancer Res. 2000;6(1):78–89.
59. Giardiello FM, Yang VW, Hylind LM, Krush AJ, Petersen GM, Trimbath JD, et al. Primary chemoprevention of familial adenomatous polyposis with sulindac. N Engl J Med. 2002;346 (14):1054–9.
60. Hooper L, Brown TJ, Elliott R, Payne K, Roberts C, Symmons D. The effectiveness of five strategies for the prevention of gastrointestinal toxicity induced by non-steroidal anti-inflammatory drugs: systematic review. BMJ. 2004;329(7472):948.
61. Rostom A, Muir K, Dubé C, Jolicoeur E, Boucher M, Joyce J, et al. Gastrointestinal safety of cyclooxygenase-2 inhibitors: a Cochrane Collaboration systematic review. Clin Gastroenterol Hepatol. 2007;5(7):818–28.
62. Lanas A, García-Rodríguez LA, Arroyo MT, Gomollón F, Feu F, González-Pérez A, et al. Asociación Española de Gastroenterología. Risk of upper gastrointestinal ulcer bleeding associated with selective cyclo-oxygenase-2 inhibitors, traditional non-aspirin non-steroidal anti-inflammatory drugs, aspirin and combinations. Gut. 2006;55(12):1731–8.
63. Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. JAMA. 2000;284(10):1247–55.
64. Phillips RK, Wallace MH, Lynch PM, Hawk E, Gordon GB, Saunders BP, et al. FAP Study Group. A randomised, double blind, placebo controlled study of celecoxib, a selective cyclooxygenase 2 inhibitor, on duodenal polyposis in familial adenomatous polyposis. Gut. 2002;50(6):857–60.
65. Sinicrope FA, Half E, Morris JS, Lynch PM, Morrow JD, Levin B, et al. Familial Adenomatous Polyposis Study Group. Cell proliferation and apoptotic indices predict adenoma regression in a placebo-controlled

trial of celecoxib in familial adenomatous polyposis patients. Cancer Epidemiol Biomarkers Prev. 2004;13 (6):920–7.
66. Lynch PM, Ayers GD, Hawk E, Richmond E, Eagle C, Woloj M, et al. The safety and efficacy of celecoxib in children with familial adenomatous polyposis. Am J Gastroenterol. 2010;105(6):1437–43.
67. Lynch PM, Burke CA, Phillips R, Morris JS, Slack R, Wang X, et al. An international randomised trial of celecoxib versus celecoxib plus difluoromethylornithine in patients with familial adenomatous polyposis. Gut. 2016;65(2):286–95.
68. Hallak A, Alon-Baron L, Shamir R, Moshkowitz M, Bulvik B, Brazowski E, et al. Rofecoxib reduces polyp recurrence in familial polyposis. Dig Dis Sci. 2003;48(10):1998–2002.
69. Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, et al. Adenomatous Polyp Prevention on Vioxx (APPROVe) Trial Investigators. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med. 2005;352(11):1092–102.
70. Arber N, Eagle CJ, Spicak J, Rácz I, Dite P, Hajer J, et al. PreSAP Trial Investigators. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med. 2006;355(9):885–95.
71. Arber N, Spicak J, Rácz I, Zavoral M, Breazna A, Gerletti P, et al. Five-year analysis of the prevention of colorectal sporadic adenomatous polyps trial. Am J Gastroenterol. 2011;106(6):1135–46.
72. Baron JA, Sandler RS, Bresalier RS, Lanas A, Morton DG, Riddell R, et al. Cardiovascular events associated with rofecoxib: final analysis of the APPROVe trial. Lancet. 2008;372(9651):1756–64.
73. Solomon SD, McMurray JJ, Pfeffer MA, Wittes J, Fowler R, Finn P, et al. Adenoma Prevention with Celecoxib (APC) Study Investigators. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med. 2005;352(11):1071–80.
74. Coxib and traditional NSAID Trialists' (CNT) Collaboration, Bhala N, Emberson J, Merhi A, Abramson S, Arber N, Baron JA, et al. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. Lancet. 2013;382 (9894):769–79.
75. Capone ML, Tacconelli S, Sciulli MG, Grana M, Ricciotti E, Minuz P, et al. Clinical pharmacology of platelet, monocyte, and vascular cyclooxygenase inhibition by naproxen and low-dose aspirin in healthy subjects. Circulation. 2004;109(12):1468–71.
76. Rigas B, Tsioulias GJ. The evolving role of nonsteroidal anti-inflammatory drugs in colon cancer prevention: a cause for optimism. J Pharmacol Exp Ther. 2015;353:2–8.
77. Rigas B, Williams JL. NO-donating NSAIDs and cancer: an overview with a note on whether NO is required for their action. Nitric Oxide. 2008;19: 199–204.
78. Williams JL, Borgo S, Hasan I, Castillo E, Traganos F, Rigas B. Nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NSAIDs) alter the kinetics of human colon cancer cell lines more effectively than traditional NSAIDs: implications for colon cancer chemoprevention. Cancer Res. 2001;61(8): 3285–9.
79. Williams JL, Kashfi K, Ouyang N, del Soldato P, Kopelovich L, Rigas B. NO-donating aspirin inhibits intestinal carcinogenesis in Min (APC(Min/+)) mice. Biochem Biophys Res Commun. 2004;313(3):784–8.
80. Rao CV, Reddy BS, Steele VE, Wang CX, Liu X, Ouyang N, et al. Nitric oxide-releasing aspirin and indomethacin are potent inhibitors against colon cancer in azoxymethane-treated rats: effects on molecular targets. Mol Cancer Ther. 2006;5(6):1530–8.
81. Leonetti C, Scarsella M, Zupi G, Zoli W, Amadori D, Medri L, et al. Efficacy of a nitric oxide-releasing nonsteroidal anti-inflammatory drug and cytotoxic drugs in human colon cancer cell lines in vitro and xenografts. Mol Cancer Ther. 2006;5(4):919–26.
82. Qandil AM. Prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs), more than meets the eye: a critical review. Int J Mol Sci. 2012;13: 17244–74.
83. Huang L, Mackenzie GG, Sun Y, Ouyang N, Xie G, Vrankova K, et al. Chemotherapeutic properties of phospho-nonsteroidal anti-inflammatory drugs, a new class of anticancer compounds. Cancer Res. 2011;71(24):7617–27.
84. Mackenzie GG, Sun Y, Huang L, Xie G, Ouyang N, Gupta RC, et al. Phospho-sulindac (OXT-328), a novel sulindac derivative, is safe and effective in colon cancer prevention in mice. Gastroenterology. 2010;139(4):1320–32.

Aspirin and the Prevention of Colorectal Cancer

14

Paul J. Lochhead and Andrew T. Chan

Introduction

Despite the efforts of population-based screening programs, colorectal cancer continues to rank as one of the leading causes of cancer-related mortality in economically developed countries and is responsible for over 690,000 deaths each year globally [1]. The majority of colorectal cancers arise from adenomatous polyps via the so-called traditional adenoma-carcinoma sequence [2], although the contribution of the serrated neoplasia pathway has been increasingly appreciated over the past decade [3]. Although there is robust evidence to support the efficacy of lower endoscopy and polypectomy in the reducing colorectal cancer incidence and mortality [4–6], the success of endoscopic screening has been limited by poor uptake, patient inconvenience, high cost, and some risk of morbidity [7, 8]. Furthermore, no currently available screening modality offers absolute protection against colorectal tumor development or colorectal cancer-related death. The concept of colorectal cancer chemoprevention, the use of natural or synthetic agents to prevent, suppress, or reverse carcinogenic progression to invasive cancer, has therefore gained popularity as an attractive preventive strategy [9, 10].

There is compelling evidence that aspirin (acetylsalicylic acid, ASA) and other nonsteroidal anti-inflammatory drugs (NSAIDs) prevent the development and progression of colorectal neoplasia [9, 10]. Aspirin has been marketed as an analgesic and antipyretic for over a century, and the pharmacologic use of salicylates spans millennia, with the use of willow leaves to alleviate fever described in the Ebers Papyrus of 1550 BCE [11]. With proven effectiveness in the prevention of cardiovascular events and worldwide aspirin production and consumption running at some 40,000 metric tonnes annually [12], aspirin has garnered an unparalleled clinical pedigree. Clinicians are eminently familiar with aspirin's toxicity and side effect profile, an asset that stands aspirin in good stead as chemoprevention candidate, particularly in light of adverse events encountered during clinical experience with more novel agents, such as the selective COX-2 inhibitors [13]. In this chapter we present a summary of the evidence that underpins the

P.J. Lochhead, M.B.Ch.B., Ph.D. (✉)
Clinical and Translational Epidemiology Unit, Division of Medicine, and Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA, USA.

Harvard Medical School, Boston, MA, USA.
e-mail: plochhead@mgh.harvard.edu

A.T. Chan, M.D., M.P.H.
Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

Division of Gastroenterology, Massachusetts General Hospital, Boston, MA, USA

Channing Division of Network Medicine, Department of Medicine, Harvard Medical School, Boston, MA, USA

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_14

case for aspirin in the prevention of colorectal neoplasia, highlight putative mechanisms of action, and explore the factors that have thus far limited the widespread adoption of aspirin as a chemopreventive agent.

Aspirin and Sporadic Colorectal Cancer: Evidence from Observational Studies

Although initial reports on the effect of the NSAID, sulindac, on polyp burden in patients with familial adenomatous polyposis (FAP) had begun to emerge during the early 1980s [14], it was not until 1988 that evidence for an association between aspirin and sporadic colorectal cancer was demonstrated [15]. In a case-control analysis of data from the Melbourne Colorectal Cancer Study, which examined associations between medications, chronic illnesses and operations, and colorectal cancer risk, a lower frequency of colorectal cancer was observed in those who used aspirin or aspirin-containing medications (odds ratio [OR], 0.53; 95 % confidence interval [CI], 0.40–0.71). This finding was unexpected, and the authors urged early replication given the potential implications for cancer chemoprevention [15]. However, results of the next major study of aspirin and cancer risk, published in 1989, were conflicting. In a prospective cohort of 13, 987 elderly residents of a retirement community in California, daily aspirin use was associated with a modestly increased risk of incident colon cancer (relative risk [RR], 1.5; 95 % CI, 1.1–2.2) over six and a half years of follow-up [16]. Large-scale, population-based, prospective data for aspirin and colon cancer mortality were first published in 1991, derived from the US Cancer Prevention Study II (CPSII) [17]. In this analysis of 662,424 men and women, the use of aspirin 16 or more times per month for at least 1 year was associated with a 40 % reduction in colon cancer mortality over 6 years of follow-up (hazard ratio [HR], 0.60; 95 % CI, 0.40–0.89) [17]. In a subsequent cancer incidence analysis, conducted within a subset of CPSII participants [18], daily use of standard-dose aspirin ($\geq$325 mg) for at least 5 years was associated with a RR for colorectal cancer of 0.68 (95 % CI, 0.52–0.90). Similar associations have been observed in other large population-based cohort studies. In an analysis of 47,363 male US health professionals in the Health Professionals Follow-Up Study (HPFS), who were followed up over 18 years, regular use of aspirin (at least twice per week) was associated with a 21 % reduction in colorectal cancer risk (RR, 0.79; 95 % CI, 0.69–0.90) [19]. A similar magnitude of risk reduction was obtained for women in an analysis of 82,911 participants of the Nurses' Health Study (NHS) [20]. During 20 years of follow-up, the use of two or more standard-dose aspirin tablets per week was associated with a 23 % reduction in colorectal cancer risk (RR, 0.77; 95 % CI, 0.67–0.88) [20]. In a separate analysis of 79,439 women enrolled in the NHS, current aspirin use was associated with a 28 % reduction in colorectal cancer mortality and a 25 % reduction in all-cause mortality [21]. In an additional large US cohort of older men and women, the NIH-AARP Diet and Health Study ($N = 301{,}240$), compared to no aspirin use, a reduction in incident colorectal cancer was observed in association with daily or weekly use of aspirin over the preceding 12 months (HR, 0.88 and 95 % CI, 0.80–0.97, and HR 0.86 and 95 % CI, 0.79–0.94, respectively) [22].

An inverse association between aspirin use and colorectal cancer risk has been observed in several smaller cohort studies and in a number of case-control analyses [23]. A meta-analysis of 26 case-control studies generated a pooled risk estimate of 0.67 (95 % CI, 0.60–0.74) for any aspirin use and 0.62 (95 % CI, 0.58–0.67) for the maximum category of aspirin intake across 17 studies that stratified by aspirin intake [23].

Finally, in addition to an association between aspirin use and risk of incident or fatal colorectal cancer, observational data also suggest that pre-diagnostic aspirin use may be associated with disease stage at presentation. In a meta-analysis of five cohort studies that included information on stage, regular aspirin use was associated with a reduced risk of cancers with distant metastases (OR, 0.69; 95 % CI, 0.57–0.83), but not with the likelihood of regional spread [23].

Data from Randomized Trials of Aspirin in the Prevention of Cardiovascular Events

Linking aspirin exposure during cardiovascular trials, where treatment was assigned rather than self-selected, to long-term outcomes represents a valuable research approach. Details of the major cardiovascular trials of aspirin are summarized in Table 14.1. Rothwell and colleagues evaluated cancer incidence and mortality in randomized trials of aspirin from the UK and Sweden, where post-trial outcome data could be reliably obtained from death and cancer registries [24]. Two primary prevention studies fulfilled the inclusion criteria of minimum recruitment of 1000 participants and treatment duration of least 2.5 years: the British Doctors Aspirin Trial (BDAT) [25], which recruited apparently healthy male physicians, and the Thrombosis Prevention Trial (TPT) [26], which identified men with high cardiovascular risk scores through their primary care physicians. Two secondary prevention trials were included: the Swedish Aspirin Low-Dose Trial (SALT) [27] and the UK-TIA Aspirin Trial [28], which examined women and men with a history of cerebrovascular disease or retinal artery occlusion. Aspirin dose in the treatment arms of these trials varied from 75 mg to 1200 mg daily, and the median duration of scheduled treatment ranged from 2.6 to 6.9 years [24]. Among a total of 14,033 participants randomized to aspirin or control, there were 397 documented colon and rectal cancers in 391 individuals, including 240 fatal cases. In a pooled analysis of individual patient data from the four trials, allocation to aspirin reduced colorectal cancer incidence by 24 % and colorectal cancer-specific mortality by 35 %, over a median of 18.3 years of follow-up [24]. Rothwell and colleagues subsequently conducted a further pooled analysis, incorporating individual patient data from eight trials where the mean scheduled aspirin treatment was at least 4 years (BDAT, UK-TIA, TPT, Early Treatment Diabetic Retinopathy Study [ETDRS] [29], Swedish Angina Pectoris Aspirin Trial [SAPAT] [30], Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes [JPAD] study [31], Prevention of Progression of Arterial Disease and Diabetes [POPADAD] study [32], and Aspirin for Asymptomatic Atherosclerosis [AAA] trial [33]). Among 25,570 participants, there were 674 within-trial cancer-related deaths. Assignment to aspirin at doses ranging from 75 mg to 1200 mg per day was associated with a statistically significant 21 % reduction in cancer-related mortality [34]. In an analysis restricted to data from the six trials that included site-specific cancer data, the HR for risk of death from colorectal cancer ($N = 54$) among those assigned to aspirin was 0.41 (95 %, CI, 0.17–1.00) after at least five years of follow-up. For BDAT, UK-TIA, and TPT, up to 20 years of extended posttrial follow-up was obtained; for an aspirin treatment duration of 5 years or longer, the pooled colorectal cancer mortality HR over 20 years was 0.69 (95 % CI, 0.45–0.81) [34].

The effect of aspirin on the risk of cancer metastases has also been examined by exploiting data from five UK cardiovascular randomized trials of daily aspirin [35]. Among 17,285 participants, there were 775 in-trial incident solid cancers for which the metastasis status was known. Compared to the control groups, those randomized to aspirin had an OR of 0.59 (95 % CI, 0.44–0.78) for metastases from all solid tumors and an OR of 0.36 (95 % CI, 0.18–0.74) for colorectal cancer metastases [35]. These data are consistent with observational data for regular aspirin use [23] and suggest that an effect of aspirin on cancer metastasis may partly explain the greater reduction in CRC fatality relative to CRC incidence observed in the meta-analysis of long-term effects of aspirin in randomized trials [24].

While these data on aspirin and cancer are certainly persuasive, it should be remembered that these were secondary analyses of cardiovascular prevention trials. Thus, the capture of within-trial cancer and cancer-related deaths may be less reliable compared to studies where cancer outcomes were primary endpoints. Furthermore, where post-trial follow-up was possible, ascertainment of outcomes was dependent on linkage with registry entries, and data on exposure to aspirin, NSAIDs, or cancer

Table 14.1 Cancer outcomes in major cardiovascular trials of aspirin[a]

Trial	Participants (active/control)	Placebo controlled, double blind	Population	Aspirin dose	Median treatment duration (years)	Result (95 % CI)		
						CRC incidence	CRC mortality	Mortality from any cancer
BDAT	3429/1710	No	Primary prevention of CVD in male physicians	500 mg daily vs. control	6.0	HR 0·70 (0·51–0·97)	OR 0·73 (0·49–1·10)	OR 0.79 (0.55–1.14)
TPT	2545/2540	Yes	Primary prevention of CVD in men at increased risk of vascular events	75 mg daily vs. placebo	6.9	HR 0.75 (0.56–0.97)[b]	OR 0·61 (0·40–0·94)	OR 0.83 (0.62–1.11)
SALT	676/684	Yes	Secondary prevention of CVD following TIA or minor ischemic stroke	75 mg daily vs. placebo	2.7		OR 0·71 (0·27–1·86)	OR 0.71 (0.27–1.86)
UK-TIA	811/821/817	Yes	Secondary prevention of CVD following TIA or minor ischemic stroke	300 mg vs. 1200 mg daily vs. placebo	4.4	HR 0·82 (0·49–1·38)	OR 0·50 (0·21–1·17)	OR 0.45 (0.25–0.82)
ETDRS	1856/1855	Yes	Primary prevention of CVD and renal disease in patients with diabetes	650 mg daily vs. placebo	5.0	–	–	OR 1.14 (0.56–2.35)
SAPAT	1009/1026	Yes	Primary prevention of myocardial infarction in patients with stable angina	75 mg daily vs. placebo	4.2	–	–	OR 0.53 (0.25–1.15)
JPAD	1262/1277	Yes	Primary prevention of CVD in patients with type 2 diabetes	81 or 100 mg daily vs. placebo	4.4		HR 0·41 (0·17–1·00)[c]	OR 0.80 (0.40–1.57)
POPADAD	638/638	Yes	Primary prevention of CV events in patients with type 1 or 2 diabetes and asymptomatic arterial disease	100 mg daily vs. placebo	6.7			OR 0.80 (0.47–1.37)
AAA	1675/1675	Yes	Primary prevention of CVD in individuals at increased CV risk	100 mg daily vs. placebo	8.2			OR 0.86 (0.63–1.17)
PHS	11037/11034	Yes	Primary prevention of CVD and cancer in male physicians	325 mg alternate days vs. placebo	5.0	HR 1.03 (0.83–1.28)	–	–
WHS	19934/19942	Yes	Primary prevention of CVD and cancer in women	100 mg alternate days vs. placebo	9.0	HR 0.80 (0.67–0.97)	–	HR 0.97 (0.88–1.07)

BDAT British Doctors Aspirin Trial, *TPT* Thrombosis Prevention Trial, *SALT* Swedish Aspirin Low-Dose Trial, *UK-TIA* United Kingdom-Transient Ischaemic Attack Aspirin trial, *ETDRS* Early Treatment Diabetic Retinopathy Study, *SAPAT* Swedish Angina Pectoris Aspirin Trial, *JPAD* Japanese Primary Prevention of Atherosclerosis with Aspirin for Diabetes study, *POPADAD* Prevention of Progression of Arterial Disease and Diabetes study, *AAA* Aspirin for Asymptomatic Atherosclerosis trial, *PHS* Physicians' Health Study, *WHS* Women's Health Study, *HR* hazard ratio, *OR* odds ratio

[a]References [25–33]

[b,c]Pooled risk estimates from meta-analyses: [b]reference [24] and [c]reference [34]

surveillance and screening were not available. Thus, bias could have arisen through higher rates of endoscopy and polypectomy for the investigation of bleeding in those taking aspirin. Importantly, because of differences in aspirin-dosing schedule, these meta-analyses did not include the two largest aspirin primary prevention trials to date, the Physicians' Health Study (PHS) [36] and the Women's Health Study (WHS) [37]. The PHS, which randomized 22,071 male physicians to alternate-day aspirin 325 mg or placebo, reported no difference in incident colorectal cancer between groups after the 5-year scheduled treatment period [38], nor after extended follow-up to 12 years [39]. The WHS, which used an alternate-day regimen with an aspirin dose of 100 mg, was the only clinical trial specifically designed to examine the effect of aspirin on the primary prevention of cancer as well as cardiovascular disease [37]. After an average of 10 years of follow-up, randomization to aspirin was not associated with a reduction in the risk of total cancer or colorectal cancer [37]; however, in a subsequent analysis, which included post-trial follow-up through a median of 18 years, a 20 % reduction in incident colorectal cancer was observed in the aspirin group (HR, 0. 80; 95 % CI, 0.67–0.97) [40]. It remains unclear why the PHS and WHS, which both used alternate-day dosing, generated disparate results. The equivalent daily dose of aspirin in WHS is lower than that used in the PHS and some aspirin cardiovascular trials included in the Rothwell meta-analyses. It is of note that the latency period for the development of colorectal cancer has been estimated to be at least 10 years, and it remains possible that the post-trial follow-up in the PHS was still too brief to detect the effect of aspirin on colorectal carcinogenesis in this particular population.

Randomized Controlled Trials of Aspirin for the Prevention of Colorectal Adenomas

Adenomatous polyp occurrence or recurrence is a marker of colorectal cancer risk and is a widely accepted shorter-term intermediate or surrogate outcome measure that can be exploited in chemoprevention studies [41]. In 2009, Cole and colleagues published a meta-analysis of all known trials that had evaluated aspirin's effectiveness in the secondary prevention of colorectal adenomas [42]. The four studies that were included (summarized in Table 14.2) randomized a total of almost 3000 participants, with a recent

Table 14.2 Trials of aspirin in the prevention of colorectal adenomas[a]

Trial	Participants initially randomized	Inclusion criteria	Aspirin dose	Median follow-up (months)	Risk ratio (95 % CI)	
					Any adenoma	Advanced adenoma
APACC	272	Recent history of sporadic colorectal adenomas	160 mg or 200 mg daily vs. placebo	47.2	0.95 (0.75–1.21)	0.91 (0.51–1.60)
ukCAP	939	Recent history of sporadic colorectal adenomas	300 mg daily vs. placebo	37.5	0.79 (0.63–0.99)	0.63 (0.43–0.91)
AFPPS	1121	Recent history of sporadic colorectal adenomas	81 mg daily vs. 325 mg daily vs. placebo	32.2	0.88 (0.77–1.02)	0.74 (0.52–1.06)
CALGB 9270	635	Previous history of resected colorectal cancer	325 mg daily vs. placebo	31.3	0.61 (0.44–0.86)	0.77 (0.29–2.05)
J-CAPP	311	Previous history or sporadic colorectal adenomas	100 mg daily vs. placebo	24.0	0.60 (0.36–0.98)	–

APACC Association pour la Prévention par l'Aspirine du Cancer Colorectal, *ukCAP* United Kingdom Colorectal Adenoma Prevention, *AFPPS* Aspirin/Folate Polyp Prevention Study, *CALGB* Cancer and Leukemia Group B, *J-CAPP* Japan Colorectal Tumor Prevention Study: Randomized Controlled Trial by Low-Dose Aspirin

[a]References [44–48]

history of sporadic adenomas or previous colorectal cancer, to aspirin doses of between 81 mg and 325 mg per day or placebo [42–46]. The analysis was based on 2698 participants who had completed colonoscopic follow-up. The primary endpoint was adenoma occurrence after randomization, while incidence of advanced lesions (adenomas that were $\geq$1 cm in size, contained high-grade epithelial dysplasia or invasive cancer, or featured villous or tubulovillous morphology) served as a secondary endpoint. After median follow-up of 33 months, compared to placebo, the pooled risk estimate for any adenoma at any aspirin dose was 0.83 (95 % CI, 0.72–0.96) and 0.72 (95 % CI, 0.57–0.90) for any advanced lesion [42]. Interestingly, the greatest benefit from aspirin was apparent during the first year after randomization, suggesting that aspirin may exert an effect on early stages of adenomagenesis. In one of the component studies, the Association pour la Prévention par l'Aspirine du Cancer Colorectal (APACC) trial [47], in contrast to the findings at 1 year, no ongoing benefit in adenoma prevention was seen for daily low-dose aspirin after 4 years [43]. It should be noted, however, that the APACC trial was the smallest of the studies included in the meta-analysis and suffered a substantial attrition rate; only 185 of the initial 272 randomized participants underwent colonoscopy at 4 years [43].

All four of adenoma prevention studies included in the meta-analysis by Cole and colleagues were conducted in European or North American populations. Subsequently, however, a multicenter, randomized controlled trial involving 311 Japanese subjects with a history of single or multiple adenomas also reported a reduction in the risk of recurrent adenomas over 2 years in those assigned to aspirin 100 mg daily, compared to placebo (OR, 0.60; 95 % CI, 0.36–0.98) [48]. Interestingly, a greater magnitude of risk reduction was reported for nonsmokers (OR, 0.37; 95 % CI, 0.21–0.68), with an apparent increase in the risk of recurrent adenomas observed among smokers (OR, 3.44; 95 % CI, 1.12–10.64), although the estimates for this stratified analysis are based on relatively small participant and event numbers [48]. In a recent adenoma prevention trial, which found no benefit from aspirin 75 mg daily in combination with calcitriol and calcium, a borderline statistically significant interaction ($P = 0.046$) was observed between smoking and treatment in subgroup analyses according to smoking status [49]. Since smoking has been implicated in "resistance" to the antiplatelet effects of aspirin [50], the possibility of aspirin effect modification by smoking status deserves scrutiny in future studies.

Aspirin Trials in Familial Cancer Syndromes

Further evidence for the antitumor activity of aspirin in humans comes from clinical trials conducted in individuals with the two most common familial colorectal cancer syndromes, FAP and Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer, HNPCC). Classic FAP, which arises due to dominantly inherited mutations in the adenomatous polyposis coli (*APC*) tumor suppressor gene, is characterized by the development of hundreds or thousands of colorectal polyps starting in the second decade of life. Progression to colorectal cancer is inevitable, if not treated by prophylactic colectomy or proctocolectomy, with the average age at colorectal cancer diagnosis being 39 years [51]. Chemoprevention could have a role in delaying the time to prophylactic surgery in some patients with FAP or reducing polyp growth in residual rectal mucosa or in the small intestine. Somatic mutation of *APC* is a common early event in sporadic adenomas [52]. Thus, the results of chemoprevention studies among individuals with FAP may have broader relevance to sporadic colorectal neoplasia.

Early clinical studies in FAP using sulindac [53], and later trials involving the selective COX-2 inhibitors, celecoxib and rofecoxib [54, 55], indicate that these agents are effective in reducing polyp burden. Aspirin was first evaluated in the setting of FAP in the Colorectal Adenoma/Carcinoma Prevention Programme

1 (CaPP1) study, an international, randomized, placebo-controlled trial of aspirin (600 mg/day) and/or resistant starch (30 g/day) over 1–12 years in a two-by-two factorial design [56]. The primary endpoint was polyp number in the rectum and sigmoid colon. Of the 206 patients, aged 10–21 years, who were randomized, 133 had at least one follow-up lower endoscopy. Compared to placebo, individuals in the aspirin intervention arm experienced a statistically nonsignificant reduction in polyp number (RR, 0.77; 95 % CI, 0.54–1.10) and a significant reduction in average maximum polyp size when the analysis was restricted to those who had received treatment for more than 1 year (6.0 mm vs. 3.0 mm, $P = 0.02$) [57]. A Japanese randomized controlled trial has investigated the effect of low-dose aspirin (100 mg/day) compared to placebo in subjects with FAP. Although the target sample size determined by power calculations was 100, only 51 eligible patients were initially identified, of whom only 34 patients went on to complete the trial. Adverse events in three patients receiving aspirin lead the monitoring committee to suspend further recruitment. Since all subjects were already undergoing frequent surveillance and polypectomy, the average size of polyps assessed in the study was around 1.7 mm. After 6–10 months, there was no difference in the primary endpoint of reduction in polyp diameter between treatment and control groups, except in a subgroup analysis of subjects with polyps $\leq$2 mm at the baseline ($P = 0.046$) [58]. The implications of these results are limited by the small sample size and the diminutive size of the polyps, which were evaluated on a submillimetric scale.

The efficacy of aspirin as a chemopreventive agent has also been studied in Lynch syndrome, where autosomal dominantly inherited mutations in genes encoding components of the mismatch repair (MMR) system confer greatly elevated risk of colorectal cancer as well as risk for a spectrum of tumors at extracolonic sites, including the endometrium, stomach, ovaries, small intestine, and urological tract [51, 59]. Microsatellite instability (MSI) is the hallmark of colorectal cancers arising in the context of Lynch syndrome, which accounts for an estimated 3–5 % of all colorectal cancers. The effect of aspirin on tumorigenesis in Lynch syndrome may be relevant to the roughly 15 % of sporadic colorectal cancers that display MSI, commonly as a result of acquired epigenetic silencing of the MMR gene, *MLH1* [60]. The CaPP2 study employed a factorial design similar to that of CaPP1, with a daily aspirin dose of 600 mg. Of 1009 eligible patients, 746 completed the trial and were included in the analysis [61]. A genetically confirmed diagnosis was present in 83 % of participants, with the remainder having a clinical diagnosis of Lynch syndrome. After an average of 29 months of follow-up (27 months of mean treatment duration), there was no difference in colorectal cancer or adenoma incidence between the aspirin and placebo groups [61]. The CaPP2 study included a preplanned double-blind post-intervention follow-up period, and a further analysis was conducted when the earliest recruited participants reached 10 years post-randomization [62]. At this point, the average follow-up was 55.7 months, and 48 participants had developed a first colorectal cancer despite standard surveillance (18 of 427 assigned aspirin and 27 of 329 assigned aspirin placebo). In an intention-to-treat analysis, a nonsignificant trend toward reduced cancer incidence in the aspirin group was observed (HR, 0.63; 95 % CI, 0.35–1.13) [62]. Since the original CaPP2 protocol had specified an intervention of two years of duration [56], an analysis was performed including only those who had consumed a minimum of 1400 aspirin tablets (rounded down from the equivalent of two 300 mg tablets per day for two years). In this per-protocol analysis, aspirin treatment significantly reduced colorectal cancer incidence (HR, 0.41; 95 % CI, 0.19–0.86) and, in a planned secondary analysis, also reduced the risk of any Lynch-associated cancer (HR, 0.45; 95 % CI, 0.26–0.79) [62].

The CaPP3 study, for which recruitment has already started in the UK, is a dose inferiority trial that plans to randomize 3000 participants with Lynch syndrome to 100 mg, 300 mg, or 600 mg of aspirin per day for two years, followed by 100 mg daily for all. The study will collect

participant blood samples to evaluate potential biomarkers of aspirin response and is expected to run until at least 2021 [63].

Aspirin Use Following Colorectal Cancer Diagnosis

One adenoma prevention study, the Cancer and Leukemia Group B (CALGB) 9270 trial, demonstrated that, compared to placebo, 325 mg of aspirin daily over median follow-up of 30.9 months lead to a 35 % reduction in incident adenomas in patients with a history of colorectal cancer resection [45]. Although these data suggest that aspirin prevents recurrent colorectal neoplasia after colon cancer diagnosis, and data from cardiovascular trials have demonstrated a reduced risk of colorectal cancer metastasis among those assigned to aspirin, there are currently no randomized trial data on adjuvant aspirin and recurrence or survival following colorectal cancer diagnosis. Several observational studies have, however, explored this question. In an analysis of 1279 men and women with nonmetastatic colorectal cancer enrolled in the NHS and HPFS cohorts, regular aspirin use after diagnosis, compared to nonuse, was associated with a 29 % reduction in the risk of colorectal cancer-related death over median follow-up of 11.8 years (HR, 0.71; 95 % CI, 0.53–0.95) [64]. In a subgroup analysis of NHS and HPFS participants with tumor tissue available for immunohistochemical assessment, aspirin use was associated specifically with reduced mortality in individuals whose primary tumors overexpressed COX-2 (HR 0.39 and 95 % CI, 0.20–0.76, compared to HR 1.22 and 95 % CI, 0.36–4.18, for COX-2 negative tumors; $P_{\text{interaction}} = 0.04$) [64]. A later analysis, also conducted using data from the NHS and HPFS cohorts, suggested that the reduction in mortality associated with aspirin was restricted to the 10–20 % of individuals whose colorectal cancers harbored a mutation in the gene encoding phosphatidylinositol-4,5-bisphosphonate 3-kinase, catalytic subunit alpha (*PIK3CA*), which leads to upregulation of PI3K activity [65]. Among 964 all-stage colorectal cancer cases, compared to no aspirin use, post-diagnostic regular use of standard-dose aspirin was associated with a mortality HR of 0.18 (95 % CI, 0.06–0.61) for *PIK3CA*-mutated cancers, but was not associated with colorectal cancer mortality among those with *PIK3CA* wild-type cancers (HR, 0.96; 95 % CI, 0.69–1.32) [65]. Similar results were obtained from a post hoc analysis of data from 896 participants in the Vioxx in Colorectal Cancer Therapy: Definition of Optimal Regime (VICTOR) trial of adjuvant rofecoxib compared to placebo [66]. Among individuals with *PIK3CA*-mutated tumors, compared to no aspirin use, the use of low-dose aspirin was associated with a reduction in the risk of colorectal cancer recurrence 0.11 (95 % CI, 0.001–0.832) [66]. In both studies, the number of aspirin users with *PIK3CA*-mutated tumors was limited (66 and 14, respectively), and event numbers were small. In a survival study of 999 colorectal cancer patients from the Eindhoven Cancer Registry, no interaction was observed between post-diagnostic aspirin use and *PIK3CA* mutation [67]. Similarly, a lack of association between *PIK3CA* mutation and survival according to aspirin use was reported in an analysis of 1487 colorectal cancer patients from two Australian hospital-based cohorts [68]. A major limitation of this analysis is the fact that aspirin use was defined as exposure at the time of diagnosis, rather than after diagnosis.

In a recent meta-analysis of aspirin use and colorectal cancer survival, which included seven cohort studies of pre-diagnostic aspirin use and a similar number of cohort studies of post-diagnostic aspirin use, an overall survival benefit was observed for post-diagnostic aspirin use (HR, 0.84; 95 % CI, 0.75–0.94), but not for aspirin use before diagnosis (HR, 1.01; 95 % CI, 0.96–1.06) [69]. No association was observed between pre- or post-diagnostic aspirin use and colorectal cancer-specific mortality; however, only three of the seven post-diagnostic aspirin use studies included this as an endpoint [69].

A meta-analysis of studies that included stratification by tumor *PIK3CA* status reported that the association between aspirin use and colorectal cancer survival was restricted to individuals with *PIK3CA*-mutated tumors, although the authors of the analysis conceded that the number of available studies remains too small to make any definitive conclusions [70].

A number of imminent or ongoing clinical trials will investigate the benefit of adjuvant aspirin among colorectal cancer patients. The ADD-Aspirin double-blind, randomized controlled trial aims to recruit around 11,000 patients from the UK and India who have had potentially curative treatment for breast, colorectal, esophageal, gastric, or prostate cancer [71]. After an 8-week active run-in period on aspirin 100 mg daily, participants will be randomized to continue on aspirin at a dose of 100 mg or 300 mg daily or receive placebo for 5 years [71]. The primary outcome measure for colorectal cancer will be disease-free survival, and follow-up beyond 5 years will be possible using registry data. The Aspirin for Dukes C and High Risk Dukes B Colorectal Cancers (ASCOLT) trial is an ongoing study based in Singapore that anticipates eventual enrollment of 1200 predominantly Asian colorectal cancer patients [72]. Participants are randomized to aspirin 200 mg daily or placebo for 3 years, with disease-free survival as the primary endpoint and overall mortality at 5 years serving as a secondary endpoint. The estimated trial completion date is late 2021. Given the conflicting results from observational studies, prospective evaluation of the predictive capacity of *PIK3CA* mutation is essential. A Swiss multicenter clinical trial, which is due to commence recruitment in October 2015, plans to randomize 185 eligible patients with *PIK3CA*-mutated stage II or III colorectal cancers to aspirin, 100 mg daily, or placebo for 3 years. The completion date for the primary endpoint of disease-free survival is late 2018. Subject to funding, the ADD-Aspirin study also plans to assess tumor *PIK3CA* mutation status during the run-in period and use this as a stratification factor during randomization [71].

Mechanisms of Action of Aspirin in Cancer Chemoprevention

In common with traditional NSAIDs, the central mechanism responsible for the anti-inflammatory effect of aspirin involves inhibition of the prostaglandin-endoperoxide synthase (PTGS) enzyme, more commonly referred to as cyclooxygenase (COX). There are two COX isoforms, and both are selectively acetylated and irreversibly inactivated by aspirin. Most cell types constitutively express the COX-1 (PTGS1) isoform. COX-2 (PTGS2), in contrast, is constitutively expressed only in limited number of tissues, but can be rapidly induced by a variety of stimuli including tissue injury, hypoxia, growth factors, cytokines, and activated oncogenes [73]. The COX enzymes catalyze the conversion of arachidonic acid to prostaglandin (PG) H_2, which is the rate-limiting step in the generation of prostanoids such as PGE_2, PGI_2, and thromboxane (TX) A_2 [74]. The conversion of PGH_2 to prostaglandins and other biologically active mediators is achieved by tissue-specific isomerases.

COX-2 is overexpressed in around 80 % of colorectal cancers and is upregulated at an early stage in a proportion of adenomas [75]. Most hypotheses relating to the chemopreventive mechanisms of aspirin have therefore tended to focus on COX-related pathways and COX-2 in particular [76]. Among the COX-2 metabolites present in colorectal cancer tissues, the pro-inflammatory prostanoid, PGE_2, is the most abundant and appears to act in an autocrine and paracrine fashion to modulate neoplastic cellular attributes such as proliferation, resistance to apoptosis, migration, and invasion [77]. PGE_2 has also been identified as a driver of tumor-associated angiogenesis and is implicated in colorectal cancer metastasis [78–80]. Accumulating evidence also points to a critical role for PGE_2 in facilitating tumor evolution by suppressing myeloid cell activation and promoting tumor immune evasion [81].

The ability of PGE_2 to effect a pro-tumorigenic cellular phenotype is likely to

depend on a number of different molecular mechanisms including MEK-ERK and PI3K-AKT signal transduction via epidermal growth factor receptor activation [82, 83], deregulation of Wnt signaling [84, 85], and modulation of gene transcription as a result of aberrant DNA methylation [86]. In $Apc^{min/+}$ and $Apc^{\Delta 716}$ mice, animal models of human FAP, genetic or pharmacologic inactivation of COX-2 markedly reduces the number and size of intestinal polyps [87–89]. Moreover, administration of PGE_2 augments intestinal tumorigenesis in $Apc^{min/+}$ mice [90] and increases colon tumor multiplicity in a rat model of chemical-induced carcinogenesis [91]. Indirect evidence supporting the importance of COX-2 inhibition in colorectal cancer chemoprevention in humans comes from adenoma prevention trials using selective COX-2 inhibitors [41, 92, 93]. Although these drugs effectively prevent adenoma recurrence, coxibs have been reported to have pleiotropic effects involving COX-independent pathways, such as inhibition of AKT pathway signal transduction and altered sphingolipid signaling [94, 95].

Aspirin may also exert an antineoplastic effect on COX-dependent tumorigenesis through mechanisms other than blockade of PG synthesis. Aspirin appears to be capable of transcriptional repression of COX-2 [96] and has been shown to prevent COX-2-peroxidase-mediated activation of co-carcinogens [97]. Furthermore, acetylation of COX-2 by aspirin renders the enzyme capable of generating 15-epi-lipoxin-A4, or "aspirin-triggered lipoxin," which has anti-inflammatory and growth inhibitory properties [98].

COX-2 appears to be a promising target for chemoprevention. However, it remains uncertain whether aspirin's antineoplastic effects in vivo result primarily from inhibition of COX-2. Aspirin has a short plasma half-life of around 20 min, and it has been estimated that aspirin is 60–170 times more effective at acetylating COX-1 than COX-2 [99]. Orally administered aspirin is subject to first-pass metabolism in the gut and liver, resulting in negligible systemic bioavailability following low-dose administration [100]. Inhibition of COX-1 in the pre-systemic (i.e., portal) circulation therefore may be an important contributor to the antiplatelet effect of low-dose aspirin [101]. Although inhibition of COX in anucleate platelets is irrecoverable, nucleated cells can overcome COX inhibition within 2–4 h by regenerating COX enzymes. Thus, although once-daily low-dose aspirin appears sufficient to influence colorectal cancer risk [35], it seems doubtful that this is achieved by sustained inhibition of systemic COX-2 alone.

Several COX-independent mechanisms have been proposed to account for the antitumor effect of aspirin; these include activation of NFkB [102], direct interference with Wnt or MEK signaling [103–105], interaction with cell cycle regulators [106, 107], disruption of mitochondrial and proteasome function [108, 109], acetylation of non-COX proteins [110], enhanced catabolism of polyamines [111, 112], and attenuation of MMR deficiency [113]. Data supporting these alternative mechanisms derive almost entirely from in vitro experiments, which generally require high concentrations of aspirin, several orders of magnitude greater than peak plasma levels achieved after ingestion of standard therapeutic doses.

Could inhibition of COX-1 therefore be relevant to aspirin's chemopreventive effect? It is interesting that genetic inactivation of COX-1 is as effective as COX-2 knockout in reducing tumor burden in $Apc^{min/+}$ mice [88]. In humans, daily administration of low-dose (81 mg) aspirin, which is considered inadequate to inhibit peripheral COX-2, can reduce PGE_2 levels in colonic mucosal biopsies taken over 3 days after the last dose of aspirin [114]. While this might suggest the involvement of COX-1 inhibition, one would expect once-daily aspirin dosing to only transiently inactivate COX-1 in nucleated colonic epithelial cells. Furthermore, over the course of several days, much of the colonic epithelium itself will have been regenerated. The only cell type susceptible to durable inhibition of COX-1 over this time frame is platelets, and it has thus been hypothesized that the antineoplastic and cardiovascular effects of aspirin might share a common mechanism [115].

The role of platelets in cancer metastasis has been appreciated for several decades [116, 117];

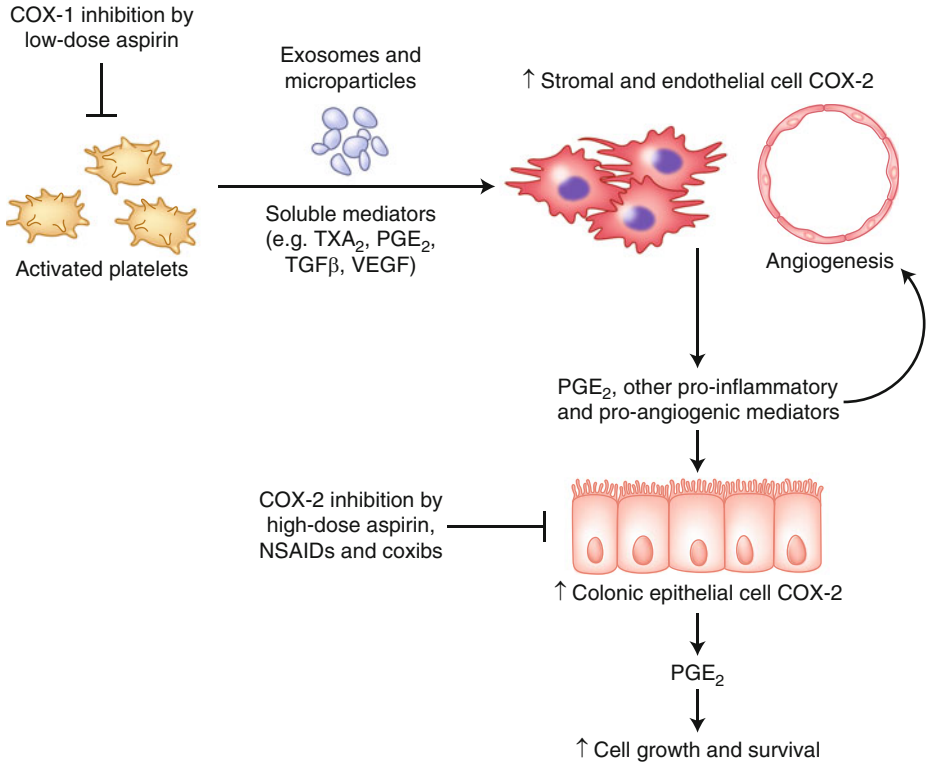


Fig. 14.1 Proposed mechanism through which inhibition of platelet COX-1 by low-dose aspirin can influence colorectal tumorigenesis [119, 120]. Platelet activation generates soluble mediators, microparticles, and exosomes, which alter the behavior of adjacent nucleated cells. Induction of COX-2 expression in stromal and endothelial cells in the colorectal mucosal tissue microenvironment leads to the release of prostanoids and other pro-inflammatory mediators that, in turn, induce COX-2 expression in epithelial cells. Epithelial COX-2 expression generates PGE_2, which, along with mediators from stromal and endothelial cells, promotes cell growth, resistance to apoptosis, and angiogenesis. Abbreviations: *COX* cyclooxygenase, *NSAID* nonsteroidal anti-inflammatory drug, *PGE_2* prostaglandin E_2, *TGF-β* transforming growth factor beta, *TXA_2* thromboxane A_2, *VEGF* vascular endothelial growth factor

however, it has been suggested more recently that platelets could influence earlier phases of tumorigenesis by contributing to chronic inflammation [118]. A model has been proposed whereby activated platelets act as a source of inflammatory mediators that induce COX-2 expression in non-epithelial cells in the mucosal tissue microenvironment (Fig. 14.1) [119, 120]. Platelet-induced COX-2 expression by stromal and endothelial cells then acts as a source of PGE_2 that promotes epithelial cell transformation and growth [119, 120]. This model is attractive since it accommodates both platelet COX-1 and tumoral COX-2 and PGE_2-dependent mechanisms. Activated platelets have the potential to influence the behavior of other cells through direct contact, via the release of soluble pro-inflammatory, growth promoting, and pro-angiogenic molecules, including PGE_2, transforming growth factor beta (TGF-β), and vascular endothelial growth factor [121], or by shedding microparticles and exosomes [121, 122]. Platelets have been shown to be capable of inducing COX-2 expression in the colorectal cancer cell line, HT29, during co-culture [123], and evidence from a mouse model of metastasis suggests that platelet-derived TGF-β can induce epithelial-mesenchymal transition and promote tumor cell metastasis [124]. These data derive from experimental conditions where there is direct contact between platelets and neoplastic cells, such as

might occur during vascular transit. Data supporting an influence of platelets on earlier stages of tumor evolution are currently limited [118].

Clinical Considerations in Adopting Aspirin for CRC Chemoprevention

Aspirin Toxicity

Toxicity associated with regular aspirin use represents the major factor that has limited the recommendation of aspirin for cancer chemoprevention [125]. Among the adverse events associated with regular aspirin use, serious bleeding-related complications, comprising gastrointestinal and intracranial hemorrhage, are the most clinically important. For regular use of aspirin at standard doses (> 325 mg/day), the risk of upper GI bleeding appears to increase in a dose-dependent manner [126, 127]. Evidence for a dose-response relationship for low-dose aspirin (75–325 mg/day) is conflicting [128–133]. Nonetheless, low-dose aspirin has consistently been found to increase GI bleeding risk. In a meta-analysis of 35 randomized controlled trials, compared to control agents, daily low-dose aspirin increased the risk of major GI bleeding by 55 % (HR, 1.55; 95 % CI, 1.27–1.90), equivalent to an additional one to two significant GI bleeds per 1000 person-years [134]. Data from epidemiologic studies and randomized trials suggest that the elevated GI bleeding risk associated with aspirin use diminishes with increasing time since the initiation of therapy [135, 136]. In a meta-analysis of six primary prevention trials of daily low-dose aspirin, no excess major extracranial bleeding was observed in the treatment group when the follow-up period was restricted to ≥3 years [136]. Furthermore, the case fatality rate for major extracranial bleeding across these trials was lower for individuals on aspirin compared to controls, suggesting a protective effect of aspirin on death from major extracranial bleeding (OR, 0.32; 95 % CI, 0.12–0.93) [136]. The GI bleeding risk associated with long-term low-dose aspirin may be partly mitigated by *H. pylori* eradication [137, 138], which could potentially reduce upper GI complications of aspirin therapy in the general population by up to 30 % [139]. The UK-based Helicobacter Eradication Aspirin Trial (HEAT) aims to recruit in excess of 6000 *H. pylori*-positive individuals (≥60 years of age) who are taking low-dose aspirin [140]. The study will address whether eradication therapy, compared to placebo, is effective in preventing ulcer bleeding complications. Concomitant administration of proton pump inhibitors (PPIs) has been estimated to reduce upper GI complications of aspirin therapy by 66 % in a meta-analysis of three randomized trials [134]; however, the overall benefit and cost effectiveness of this approach in the general population remain uncertain [141].

Although intracerebral and subarachnoid hemorrhage attributable to aspirin use is relatively rare, it is considered the most serious complication of aspirin therapy on account of the associated risk of death or long-term disability [142]. A meta-analysis by the Antithrombotic Trialists' (ATT) Collaboration, which included individual participant data from six primary prevention trials and 16 secondary prevention trials of low-dose aspirin, found that aspirin increased the relative risk of intracranial bleeding by 39 % (RR, 1.39; 95 % CI, 1.08–1.78), which translates to an absolute risk of one or two excess bleeds per 10,000 patient-years [143]. Hypertension is a major risk factor for intracranial bleeding, and it has been proposed that adequate blood pressure control may reduce the risk associated with aspirin use [142]. In the Hypertension Optimal Treatment (HOT) study, which enrolled hypertensive subjects to targeted blood pressure reduction, there was no difference in the rate of intracranial bleeding between the aspirin and control groups after achieving blood pressure control [144].

Based on data from randomized trials, the absolute risk of major bleeding associated with low-dose aspirin use appears modest; however, data from a population-based cohort study suggest that the "real-world" hemorrhagic risks associated with aspirin may have been underestimated [145]. In the analysis, which

utilized administrative data from 12 regional health authorities in Puglia, Italy, over 186,000 individuals being prescribed low-dose aspirin were propensity sore-matched 1:1 to controls who did not take prescribed aspirin [145]. Among aspirin users, the incidence of major bleeding was around fivefold higher than estimates obtained from meta-analyses of randomized trials (incidence rate ratio, 1.55; 95 % CI, 1.48–1.63) [145]. The rate of bleeding in controls in this population was also considerably higher than that observed in clinical trials, which may reflect differences in the prevalence of other bleeding risk factors such as hypertension and the use of non-aspirin NSAIDs [145]. It has therefore been suggested that results from the ATT Collaboration's meta-analysis [143] remain the most robust risk estimates for general populations in Europe and North America [146].

Dose and Duration of Treatment

Since the adverse effects of aspirin appear to be largely dose related, at least for standard doses (>325 mg/day), it is important to establish the smallest dose of aspirin capable of effectively preventing colorectal neoplasia. In the meta-analysis of long-term trial follow-up data by Rothwell and colleagues [24], there appeared to be no difference in the effectiveness of lower doses of aspirin (75 mg–300 mg/day) compared to higher doses (500–1200 mg/day). Data on cancer outcomes from trials including head-to-head comparisons of aspirin doses are limited [28, 147]; however, follow-up of participants in the Dutch TIA study [147] showed an increased risk of fatal colorectal cancer in the group who received a very low-dose of aspirin, 30 mg/day, compared to those who were assigned to 283 mg/day [24]. These findings suggest that long-term daily aspirin doses of at least 75 mg are required to prevent colorectal cancer incidence and mortality. For alternate-day dosing, analysis of extended follow-up in the WHS demonstrated a reduction in the risk of incident CRC with 100 mg of aspirin [40], although men assigned 325 mg on alternate days did not experience a reduction in CRC risk over 12 years of follow-up in the PHS [39].

Observational data tend to suggest that higher aspirin doses, ≥300 mg/day, might be necessary to prevent incident colorectal cancer [148], although many of these studies captured limited information on aspirin dose and duration of use. In the NHS and HPFS prospective cohorts, where data on aspirin use frequency is available over a prolonged period, the greatest reduction in CRC risk was associated with the maximum use category of 14 or more standard aspirin tablets per week [19, 20].

In meta-analysis of adenoma prevention trials, which employed aspirin doses between 81 mg and 325 mg/day [42], lower doses (≤160 mg/day) resulted in a reduction in the risk of recurrent adenoma that was of comparable magnitude to that observed for any aspirin dose [42]. Comparison of higher aspirin doses (≥300 mg/day) to placebo also demonstrated a statistically significant reduction in absolute risk for any adenoma. However, in a pooled analysis of the two studies that directly compared lower-dose to higher-dose aspirin, significantly greater risk reduction was found with low-dose aspirin [42]. This atypical dose-response relationship precludes firm conclusions about the relative effectiveness of lower vs. higher doses of aspirin for adenoma prevention.

The results of two ongoing multinational, placebo-controlled, primary prevention trials of low-dose aspirin (100 mg/day), the Aspirin in Reducing Events in the Elderly (ASPREE) [149] and the Aspirin to Reduce Risk of Initial Vascular Events (ARRIVE) [150], may provide additional evidence for low-dose aspirin in colorectal cancer chemoprevention, although the scheduled follow-up duration in both trials is only five years. In addition, the outcome of the CaPP3 trial may be extrapolated to help inform choice of aspirin dose for sporadic colorectal cancer prevention [63].

Randomized trials and observational studies have consistently shown that there is a duration-risk relationship between aspirin use and CRC [148]. In analyses of data from the UK-TIA and BDAT studies, reduction in CRC incidence was

observed after a latency period of around 10 years following assignment to aspirin for 5 or more years ($P_{interaction} = 0.004$ for aspirin and follow-up time in the UK-TIA) [148]. In the UK-TIA study [28], no unblinding was performed at the end of the 5-year treatment period, and, since participants would have been unaware of their study assignment, one would not expect there would be significant differences in self-selected use of aspirin post-trial. Thus, the reduction in CRC incidence and mortality observed beyond 10 years post-randomization is likely be attributable to aspirin taken for 5 years during the study period [148]. These findings are generally consistent with data from additional randomized trials and observational studies [24, 148].

Tumor Location

A number of observational studies have suggested that aspirin, or non-aspirin NSAIDs, may have differential associations with CRC risk depending on tumor location, with some studies reporting stronger associations for proximal colon cancer risk and weaker or nonexistent associations for distal colon and rectal cancer risk [22, 151, 152]. Site-specific associations are inconsistent across the literature, and in a meta-analysis of 19 case-control studies and 11 cohort studies, no convincing differential associations were observed for aspirin and CRC risk according to tumor location, age, sex, race, or family history [148]. In the meta-analysis of long-term individual data from four randomized trials by Rothwell and colleagues, assignment to daily aspirin for an average of 5.8 years reduced the 20-year risk of colon cancer (HR, 0.76; 95 % CI, 0.60–0.96), but not rectal cancer (HR, 0.90; 95 % CI, 0.63–1.30) [24]. Where data on colonic subsite were available, aspirin reduced the risk of proximal (HR, 0.45; 95 % CI, 0.28–0.74), but not distal (HR, 1.10; 95 % CI, 0.73–1.64; $P_{difference} = 0.04$) colon cancer [24]. When analyses were restricted to participants with a treatment duration of at least 5 years, a 70 % reduction in the risk of proximal colon cancer was observed in addition to a statistically significant reduction in rectal cancer risk (HR, 0.58; 95 % CI, 0.36–0.92); however, no effect on the incidence of distal colon cancer was observed [24]. Thus, the magnitude of benefit from aspirin may differ according to tumor anatomic location as well as duration of use.

Overall Risk-Benefit of Aspirin and Strategies to Personalize Chemoprevention

Any decision to recommend regular aspirin for primary disease prevention must take into account the balance of absolute risks and benefits, including effects on total cancer incidence and mortality. In a recent analysis of prophylactic aspirin use, modeled using data from the UK population, 10 years of aspirin use starting at age 50, 55, 60, or 65 years was associated with a consistently favorable benefit-harm profile over a 15- to 20-year period [146]. The net absolute mortality reduction associated with commencing aspirin at age 55 years was 1.43 % for men and 0.7 % for women, with almost all of this benefit (89 %–96 %) resulting from prevention of deaths from cancer [146]. It has been suggested that the estimates for aspirin-associated harms used in this analysis were excessively high and failed to take into account the diminution in the risk of extracranial bleeding that occurs over time [153]. Since the study authors aimed to use conservative estimates of harm, it is possible that the actual net benefits of aspirin use in the general population may be greater than their predictions. Even in the absence of increased cardiovascular risk, the beneficial effect of aspirin on cancer mortality makes it possible that aspirin prophylaxis would be cost saving or cost-effective, at least in men [154].

In 2007, the US Preventive Services Task Force recommended against the routine use of aspirin or NSAIDs for colorectal cancer prevention, citing a lack of evidence and concerns over toxicity [125]. Having recently reevaluated the available evidence, the USPSTF has put forward

a draft proposal that recommends 10 years of low-dose aspirin use for combined cardiovascular and cancer prevention among individuals aged 50–59 years who have a 10-year cardiovascular risk of >10 % and are not at increased risk for bleeding complications [155]. This is a significant step forward from the previous USPSTF position statement and will no doubt lead to an increase in aspirin use in the general population. There will, however, be a proportion of the population who stand to benefit from the cancer preventive effects of aspirin, but who do not fulfill the cardiovascular risk criteria. It would therefore be desirable to be able to personalize chemoprevention by stratifying individuals according to their predicted benefit from aspirin.

Several metabolic and genetic markers have recently emerged that may facilitate personalized CRC chemoprevention with aspirin. 15-Hydroxyprostaglandin dehydrogenase (15-PGDH) acts as a metabolic "brake" on PGE_2 synthesis [156]. 15-PGDH null mice are resistant to the antineoplastic effects of celecoxib, and, among 16 participants in the Adenoma Prevention with Celecoxib (APC) trial, higher pretreatment mucosal 15-PGDH predicted response to celecoxib [156]. In an analysis of a subset of participants from the NHS and HPFS cohorts, higher normal mucosal 15-PGDH expression was associated with a >50 % reduction in CRC risk with regular aspirin use, but there was no association between aspirin and CRC risk for those with lower levels of 15-PGHD expression [157]. Prostaglandin E metabolite (PGE-M), the major urinary metabolite of PGE_2, has also been proposed as a metabolic biomarker of colorectal neoplasia. In a nested case-control study within the NHS, aspirin use was associated with a reduced risk of colorectal adenoma only among participants with urinary PGE-M concentrations in the highest three quartiles [158]. In contrast, no association between urinary PGE-M and adenoma recurrence according to aspirin assignment was observed in the Aspirin/Folate Polyp Prevention Study (AFPPS) [159]. Thus, additional large prospective analyses are required to further evaluate PGE-M as a potential predictive marker of aspirin responsiveness.

In a recent genetic association study, exploiting the databases of the Colon Cancer Family Registry and Genetics and Epidemiology of Colorectal Cancer Consortium, two common genetic polymorphisms demonstrated interaction with aspirin use status in their associations with CRC risk [160]. Compared to nonusers, a reduced risk of CRC was associated with aspirin or NSAID use among those with the most common, AA, genotype of rs2965667, at 12p12.3, whereas increased CRC risk was observed with aspirin use in the minority of individuals with AT or TT genotypes ($P_{\text{interaction}} = 4.6 \times 10^{-9}$) [160]. Similarly, the commonest, AA, genotype of rs16973225, at 15q25.2, was associated with reduced CRC risk among aspirin or NSAID users; however, the minor, AC and CC, genotypes were not associated with differential CRC risk according to aspirin or NSAID use [160]. While the functional significance of these variants remains unknown, a possible mechanism for rs2965667 might relate to its proximity to the microsomal glutathione S-transferase 1 gene (*MGST1*), a xenobiotic metabolizing enzyme that has high sequence homology with PGE_2 synthase and whose activity confers cellular resistance to oxidative stress [161]. It is also notable that rs16973225 polymorphism is located downstream of the gene encoding the pro-inflammatory cytokine, interleukin 6, which has been implicated in the pathogenesis of colorectal cancer [162]. It is possible that these two genetic variants could help identify a minority of individuals for whom aspirin use is ineffective or harmful; however, validation in additional populations is required.

Conclusions

The balance of evidence is shifting in favor of aspirin as an agent for the chemoprevention of colorectal neoplasia. This trend toward the acceptance of aspirin for broader indications, beyond cardiovascular prophylaxis, is reflected in the recent USPSTF draft recommendation. Nonetheless, many areas of uncertainty remain that are fundamental to the adoption of aspirin

for population-based CRC chemoprevention. Most important among these are the optimal dose and duration of aspirin therapy, which have yet to be defined. It is not clear what the durability or persistence of aspirin's protective effect is and at what age aspirin should be discontinued for maximal net benefit. Although ongoing clinical studies of aspirin, combined with accumulating data from post-trial follow-up of completed randomized trials, may help shed light on some of these areas of uncertainty, the prospect of a large randomized trial of aspirin for CRC primary prevention seems highly unlikely given the long follow-up that would be required, the already high prevalence of aspirin use with the potential for "drop-in" off protocol use of aspirin, and the attendant logistical and cost issues. Elucidating molecular mechanisms that participate in the chemopreventive effect of aspirin at physiologically relevant doses remains a crucial research goal. Defining these mechanisms may help inform the optimal dosing for cancer prevention and could yield targets for synergistic chemopreventive approaches in combination with other agents.

A further area of uncertainty is whether there are subgroups of the population who do not stand to benefit from aspirin use. A recent analysis conducted using data from the WHS suggests that alternate-day low-dose aspirin may be ineffective or harmful for the majority of women aged $\geq$45 years of age [163]. Furthermore, the impact of aspirin use on CRC incidence and mortality in individuals who are already participating in colorectal screening is not known. The identification of predictive biomarkers of benefit and harm from aspirin therapy should also, therefore, be a research priority as we strive to develop precision colorectal cancer chemoprevention strategies.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):5–29.
2. Pritchard CC, Grady WM. Colorectal cancer molecular biology moves into clinical practice. Gut. 2011;60(1):116–29.
3. O'Brien MJ, Zhao Q, Yang S. Colorectal serrated pathway cancers and precursors. Histopathology. 2015;66(1):49–65.
4. Atkin WS, Edwards R, Kralj-Hans I, Wooldrage K, Hart AR, Northover JM, et al. Once-only flexible sigmoidoscopy screening in prevention of colorectal cancer: a multicentre randomised controlled trial. Lancet. 2010;375(9726):1624–33.
5. Nishihara R, Wu K, Lochhead P, Morikawa T, Liao X, Qian ZR, et al. Long-term colorectal-cancer incidence and mortality after lower endoscopy. N Engl J Med. 2013;369(12):1095–105.
6. Loberg M, Kalager M, Holme O, Hoff G, Adami HO, Bretthauer M. Long-term colorectal-cancer mortality after adenoma removal. N Engl J Med. 2014;371(9):799–807.
7. Early DS, Gray 2nd DM. Patient attitudes and issues in colon cancer screening. J Natl Compr Canc Netw. 2014;12(5):673–8.
8. Rutter CM, Johnson E, Miglioretti DL, Mandelson MT, Inadomi J, Buist DS. Adverse events after screening and follow-up colonoscopy. Cancer Causes Control. 2012;23(2):289–96.
9. Chan AT, Arber N, Burn J, Chia WK, Elwood P, Hull MA, et al. Aspirin in the chemoprevention of colorectal neoplasia: an overview. Cancer Prev Res (Phila). 2012;5(2):164–78.
10. Cuzick J, Otto F, Baron JA, Brown PH, Burn J, Greenwald P, et al. Aspirin and non-steroidal anti-inflammatory drugs for cancer prevention: an international consensus statement. Lancet Oncol. 2009;10(5):501–7.
11. Mackowiak PA. Brief history of antipyretic therapy. Clin Infect Dis. 2000;31 Suppl 5:S154–6.
12. Fuster V, Sweeny JM. Aspirin: a historical and contemporary therapeutic overview. Circulation. 2011;123(7):768–78.
13. Drazen JM. COX-2 inhibitors—a lesson in unexpected problems. N Engl J Med. 2005;352(11):1131–2.
14. Waddell WR, Loughry RW. Sulindac for polyposis of the colon. J Surg Oncol. 1983;24(1):83–7.
15. Kune GA, Kune S, Watson LF. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Res. 1988;48(15):4399–404.
16. Paganini-Hill A, Chao A, Ross RK, Henderson BE. Aspirin use and chronic diseases: a cohort study of the elderly. BMJ. 1989;299(6710):1247–50.
17. Thun MJ, Namboodiri MM, Heath Jr CW. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med. 1991;325(23):1593–6.
18. Jacobs EJ, Thun MJ, Bain EB, Rodriguez C, Henley SJ, Calle EE. A large cohort study of long-term daily use of adult-strength aspirin and cancer incidence. J Natl Cancer Inst. 2007;99(8):608–15.
19. Chan AT, Giovannucci EL, Meyerhardt JA, Schernhammer ES, Wu K, Fuchs CS. Aspirin dose and duration of use and risk of colorectal cancer in men. Gastroenterology. 2008;134(1):21–8.

20. Chan AT, Giovannucci EL, Meyerhardt JA, Schernhammer ES, Curhan GC, Fuchs CS. Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. JAMA. 2005;294(8):914–23.
21. Chan AT, Manson JE, Feskanich D, Stampfer MJ, Colditz GA, Fuchs CS. Long-term aspirin use and mortality in women. Arch Intern Med. 2007;167 (6):562–72.
22. Ruder EH, Laiyemo AO, Graubard BI, Hollenbeck AR, Schatzkin A, Cross AJ. Non-steroidal anti-inflammatory drugs and colorectal cancer risk in a large, prospective cohort. Am J Gastroenterol. 2011;106(7):1340–50.
23. Algra AM, Rothwell PM. Effects of regular aspirin on long-term cancer incidence and metastasis: a systematic comparison of evidence from observational studies versus randomised trials. Lancet Oncol. 2012;13(5):518–27.
24. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet. 2010;376(9754):1741–50.
25. Peto R, Gray R, Collins R, Wheatley K, Hennekens C, Jamrozik K, et al. Randomised trial of prophylactic daily aspirin in British male doctors. Br Med J (Clin Res Ed). 1988;296 (6618):313–6.
26. The Medical Research Council's General Practice Research Framework. Thrombosis prevention trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in the primary prevention of ischaemic heart disease in men at increased risk. Lancet. 1998;351 (9098):233–41.
27. Swedish Aspirin Low-Dose Trial (SALT) of 75 mg aspirin as secondary prophylaxis after cerebrovascular ischaemic events. The SALT Collaborative Group. Lancet. 1991;338(8779):1345-9.
28. Farrell B, Godwin J, Richards S, Warlow C. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. J Neurol Neurosurg Psychiatry. 1991;54(12):1044–54.
29. ETDRS Investigators. Aspirin effects on mortality and morbidity in patients with diabetes mellitus. Early Treatment Diabetic Retinopathy Study report 14. JAMA. 1992;268(10):1292–300.
30. Juul-Moller S, Edvardsson N, Jahnmatz B, Rosen A, Sorensen S, Omblus R. Double-blind trial of aspirin in primary prevention of myocardial infarction in patients with stable chronic angina pectoris. The Swedish Angina Pectoris Aspirin Trial (SAPAT) Group. Lancet. 1992;340(8833):1421–5.
31. Ogawa H, Nakayama M, Morimoto T, Uemura S, Kanauchi M, Doi N, et al. Low-dose aspirin for primary prevention of atherosclerotic events in patients with type 2 diabetes: a randomized controlled trial. JAMA. 2008;300(18):2134–41.
32. Belch J, MacCuish A, Campbell I, Cobbe S, Taylor R, Prescott R, et al. The prevention of progression of arterial disease and diabetes (POPADAD) trial: factorial randomised placebo controlled trial of aspirin and antioxidants in patients with diabetes and asymptomatic peripheral arterial disease. BMJ. 2008;337:a1840.
33. Fowkes FG, Price JF, Stewart MC, Butcher I, Leng GC, Pell AC, et al. Aspirin for prevention of cardiovascular events in a general population screened for a low ankle brachial index: a randomized controlled trial. JAMA. 2010;303(9):841–8.
34. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW. Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet. 2011;377(9759):31–41.
35. Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. Lancet. 2012;379 (9826):1591–601.
36. Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. N Engl J Med. 1989;321(3):129–35.
37. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, et al. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. JAMA. 2005;294(1):47–55.
38. Gann PH, Manson JE, Glynn RJ, Buring JE, Hennekens CH. Low-dose aspirin and incidence of colorectal tumors in a randomized trial. J Natl Cancer Inst. 1993;85(15):1220–4.
39. Sturmer T, Glynn RJ, Lee IM, Manson JE, Buring JE, Hennekens CH. Aspirin use and colorectal cancer: post-trial follow-up data from the Physicians' Health Study. Ann Intern Med. 1998;128(9):713–20.
40. Cook NR, Lee IM, Zhang SM, Moorthy MV, Buring JE. Alternate-day, low-dose aspirin and cancer risk: long-term observational follow-up of a randomized trial. Ann Intern Med. 2013;159(2):77–85.
41. Baron JA. Intermediate effect markers for colorectal cancer. IARC Sci Publ. 2001;154:113–29.
42. Cole BF, Logan RF, Halabi S, Benamouzig R, Sandler RS, Grainge MJ, et al. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. J Natl Cancer Inst. 2009;101(4):256–66.
43. Benamouzig R, Uzzan B, Deyra J, Martin A, Girard B, Little J, et al. Prevention by daily soluble aspirin of colorectal adenoma recurrence: 4-year results of the APACC randomised trial. Gut. 2012;61(2):255–61.
44. Logan RF, Grainge MJ, Shepherd VC, Armitage NC, Muir KR. ukCAP Trial Group. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. Gastroenterology. 2008;134(1):29–38.

45. Sandler RS, Halabi S, Baron JA, Budinger S, Paskett E, Keresztes R, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med. 2003;348(10):883–90.
46. Baron JA, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, et al. A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med. 2003;348(10):891–9.
47. Benamouzig R, Deyra J, Martin A, Girard B, Jullian E, Piednoir B, et al. Daily soluble aspirin and prevention of colorectal adenoma recurrence: one-year results of the APACC trial. Gastroenterology. 2003;125(2):328–36.
48. Ishikawa H, Mutoh M, Suzuki S, Tokudome S, Saida Y, Abe T, et al. The preventive effects of low-dose enteric-coated aspirin tablets on the development of colorectal tumours in Asian patients: a randomised trial. Gut. 2014;63(11):1755–9.
49. Pommergaard HC, Burcharth J, Rosenberg J, Raskov H. Aspirin, Calcitriol, and Calcium do not Prevent Adenoma Recurrence in a Randomized Controlled Trial. Gastroenterology. 2016;150 (1):114–22.
50. Mirkhel A, Peyster E, Sundeen J, Greene L, Michelson AD, Hasan A, et al. Frequency of aspirin resistance in a community hospital. Am J Cardiol. 2006;98(5):577–9.
51. Jasperson KW, Tuohy TM, Neklason DW, Burt RW. Hereditary and familial colon cancer. Gastroenterology. 2010;138(6):2044–58.
52. Schneikert J, Behrens J. The canonical Wnt signalling pathway and its APC partner in colon cancer development. Gut. 2007;56(3):417–25.
53. Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hylind LM, Celano P, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med. 1993;328(18):1313–6.
54. Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med. 2000;342(26):1946–52.
55. Higuchi T, Iwama T, Yoshinaga K, Toyooka M, Taketo MM, Sugihara K. A randomized, double-blind, placebo-controlled trial of the effects of rofecoxib, a selective cyclooxygenase-2 inhibitor, on rectal polyps in familial adenomatous polyposis patients. Clin Cancer Res. 2003;9(13):4756–60.
56. Burn J, Chapman PD, Mathers J, Bertario L, Bishop DT, Bulow S, et al. The protocol for a European double-blind trial of aspirin and resistant starch in familial adenomatous polyposis: the CAPP study. Concerted Action Polyposis Prevention. Eur J Cancer. 1995;31A(7-8):1385–6.
57. Burn J, Bishop DT, Chapman PD, Elliott F, Bertario L, Dunlop MG, et al. A randomized placebo-controlled prevention trial of aspirin and/or resistant starch in young people with familial adenomatous polyposis. Cancer Prev Res (Phila). 2011;4(5):655–65.
58. Ishikawa H, Wakabayashi K, Suzuki S, Mutoh M, Hirata K, Nakamura T, et al. Preventive effects of low-dose aspirin on colorectal adenoma growth in patients with familial adenomatous polyposis: double-blind, randomized clinical trial. Cancer Med. 2013;2(1):50–6.
59. Pande M, Wei C, Chen J, Amos CI, Lynch PM, Lu KH, et al. Cancer spectrum in DNA mismatch repair gene mutation carriers: results from a hospital based Lynch syndrome registry. Fam Cancer. 2012;11 (3):441–7.
60. Cunningham JM, Christensen ER, Tester DJ, Kim CY, Roche PC, Burgart LJ, et al. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res. 1998;58 (15):3455–60.
61. Burn J, Bishop DT, Mecklin JP, Macrae F, Moslein G, Olschwang S, et al. Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome. N Engl J Med. 2008;359(24):2567–78.
62. Burn J, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet. 2011;378 (9809):2081–7.
63. Burn J, Mathers JC, Bishop DT. Chemoprevention in Lynch syndrome. Fam Cancer. 2013;12(4):707–18.
64. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA. 2009;302(6):649–58.
65. Liao X, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, et al. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. N Engl J Med. 2012;367(17):1596–606.
66. Domingo E, Church DN, Sieber O, Ramamoorthy R, Yanagisawa Y, Johnstone E, et al. Evaluation of PIK3CA mutation as a predictor of benefit from nonsteroidal anti-inflammatory drug therapy in colorectal cancer. J Clin Oncol. 2013;31(34):4297–305.
67. Reimers MS, Bastiaannet E, Langley RE, van Eijk R, van Vlierberghe RL, Lemmens VE, et al. Expression of HLA class I antigen, aspirin use, and survival after a diagnosis of colon cancer. JAMA Intern Med. 2014;174(5):732–9.
68. Kothari N, Kim R, Jorissen RN, Desai J, Tie J, Wong HL, et al. Impact of regular aspirin use on overall and cancer-specific survival in patients with colorectal cancer harboring a PIK3CA mutation. Acta Oncol. 2015;54(4):487–92.
69. Li P, Wu H, Zhang H, Shi Y, Xu J, Ye Y, et al. Aspirin use after diagnosis but not prediagnosis improves established colorectal cancer survival: a meta-analysis. Gut. 2015;64(9):1419–25.
70. Paleari L, Puntoni M, Clavarezza M, Caviglia S, Corradengo D, Campazzi E, et al. PIK3CA mutation, aspirin use after diagnosis, and colorectal cancer

survival: A systematic review and meta-analysis of epidemiological studies. J Clin Oncol. 2015;33.
71. Clinical Trials Unit MRC. Add-Aspirin Trial Protocol London. 2015. [Accessed: 2015 October 15] Available from: www.ctu.mrc.ac.uk/13391/13399/18269/addaspirin_protocol.
72. Ali R, Toh HC, Chia WK, ASCOLT Trial Investigators. The utility of Aspirin in Dukes C and High Risk Dukes B Colorectal cancer–the ASCOLT study: study protocol for a randomized controlled trial. Trials. 2011;12:261.
73. Williams CS, Mann M, DuBois RN. The role of cyclooxygenases in inflammation, cancer, and development. Oncogene. 1999;18(55):7908–16.
74. Patrignani P, Patrono C. Cyclooxygenase inhibitors: From pharmacology to clinical read-outs. Biochim Biophys Acta. 2015;1851(4):422–32.
75. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, DuBois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994;107(4):1183–8.
76. Doherty GA, Murray FE. Cyclooxygenase as a target for chemoprevention in colorectal cancer: lost cause or a concept coming of age? Expert Opin Ther Targets. 2009;13(2):209–18.
77. Wang D, Dubois RN. Eicosanoids and cancer. Nat Rev Cancer. 2010;10(3):181–93.
78. Sonoshita M, Takaku K, Sasaki N, Sugimoto Y, Ushikubi F, Narumiya S, et al. Acceleration of intestinal polyposis through prostaglandin receptor EP2 in Apc(Delta 716) knockout mice. Nat Med. 2001;7(9):1048–51.
79. Buchanan FG, Gorden DL, Matta P, Shi Q, Matrisian LM, DuBois RN. Role of beta-arrestin 1 in the metastatic progression of colorectal cancer. Proc Natl Acad Sci U S A. 2006;103(5):1492–7.
80. Kalinski P. Regulation of immune responses by prostaglandin E2. J Immunol. 2012;188(1):21–8.
81. Zelenay S, van der Veen AG, Bottcher JP, Snelgrove KJ, Rogers N, Acton SE, et al. Cyclooxygenase-dependent tumor growth through evasion of immunity. Cell. 2015;162(6):1257–70.
82. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med. 2002;8(3):289–93.
83. Buchanan FG, Wang D, Bargiacchi F, DuBois RN. Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. J Biol Chem. 2003;278(37):35451–7.
84. Castellone MD, Teramoto H, Williams BO, Druey KM, Gutkind JS. Prostaglandin E2 promotes colon cancer cell growth through a Gs-axin-beta-catenin signaling axis. Science. 2005;310(5753):1504–10.
85. Gala MK, Chan AT. Molecular pathways: aspirin and Wnt signaling-a molecularly targeted approach to cancer prevention and treatment. Clin cancer Res. 2015;21:1543–8.
86. Xia D, Wang D, Kim SH, Katoh H, DuBois RN. Prostaglandin E2 promotes intestinal tumor growth via DNA methylation. Nat Med. 2012;18(2):224–6.
87. Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, et al. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell. 1996;87(5):803–9.
88. Chulada PC, Thompson MB, Mahler JF, Doyle CM, Gaul BW, Lee C, et al. Genetic disruption of Ptgs-1, as well as Ptgs-2, reduces intestinal tumorigenesis in Min mice. Cancer Res. 2000;60(17):4705–8.
89. Jacoby RF, Seibert K, Cole CE, Kelloff G, Lubet RA. The cyclooxygenase-2 inhibitor celecoxib is a potent preventive and therapeutic agent in the min mouse model of adenomatous polyposis. Cancer Res. 2000;60(18):5040–4.
90. Wang D, Buchanan FG, Wang H, Dey SK, DuBois RN. Prostaglandin E2 enhances intestinal adenoma growth via activation of the Ras-mitogen-activated protein kinase cascade. Cancer Res. 2005;65(5):1822–9.
91. Kawamori T, Uchiya N, Sugimura T, Wakabayashi K. Enhancement of colon carcinogenesis by prostaglandin E2 administration. Carcinogenesis. 2003;24(5):985–90.
92. Arber N, Eagle CJ, Spicak J, Racz I, Dite P, Hajer J, et al. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med. 2006;355(9):885–95.
93. Bertagnolli MM, Eagle CJ, Zauber AG, Redston M, Solomon SD, Kim K, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med. 2006;355(9):873–84.
94. Arico S, Pattingre S, Bauvy C, Gane P, Barbat A, Codogno P, et al. Celecoxib induces apoptosis by inhibiting 3-phosphoinositide-dependent protein kinase-1 activity in the human colon cancer HT-29 cell line. J Biol Chem. 2002;277(31):27613–21.
95. Schiffmann S, Sandner J, Schmidt R, Birod K, Wobst I, Schmidt H, et al. The selective COX-2 inhibitor celecoxib modulates sphingolipid synthesis. J Lipid Res. 2009;50(1):32–40.
96. Xu XM, Sansores-Garcia L, Chen XM, Matijevic-Aleksic N, Du M, Wu KK. Suppression of inducible cyclooxygenase 2 gene transcription by aspirin and sodium salicylate. Proc Natl Acad Sci U S A. 1999;96(9):5292–7.
97. Craven PA, DeRubertis FR. Effects of aspirin on 1,2-dimethylhydrazine-induced colonic carcinogenesis. Carcinogenesis. 1992;13(4):541–6.
98. Romano M, Cianci E, Simiele F, Recchiuti A. Lipoxins and aspirin-triggered lipoxins in resolution of inflammation. Eur J Pharmacol. 2015;760:49–63.
99. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of nonsteroidal

antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci U S A. 1993;90(24):11693–7.
100. Charman WN, Charman SA, Monkhouse DC, Frisbee SE, Lockhart EA, Weisman S, et al. Biopharmaceutical characterisation of a low-dose (75 mg) controlled-release aspirin formulation. Br J Clin Pharmacol. 1993;36(5):470–3.
101. Pedersen AK, FitzGerald GA. Dose-related kinetics of aspirin. Presystemic acetylation of platelet cyclooxygenase. N Engl J Med. 1984;311(19):1206–11.
102. Stark LA, Reid K, Sansom OJ, Din FV, Guichard S, Mayer I, et al. Aspirin activates the NF-kappaB signalling pathway and induces apoptosis in intestinal neoplasia in two in vivo models of human colorectal cancer. Carcinogenesis. 2007;28(5):968–76.
103. Bos CL, Kodach LL, van den Brink GR, Diks SH, van Santen MM, Richel DJ, et al. Effect of aspirin on the Wnt/beta-catenin pathway is mediated via protein phosphatase 2A. Oncogene. 2006;25 (49):6447–56.
104. Pillinger MH, Capodici C, Rosenthal P, Kheterpal N, Hanft S, Philips MR, et al. Modes of action of aspirin-like drugs: salicylates inhibit erk activation and integrin-dependent neutrophil adhesion. Proc Natl Acad Sci U S A. 1998;95(24):14540–5.
105. Pan MR, Chang HC, Hung WC. Non-steroidal anti-inflammatory drugs suppress the ERK signaling pathway via block of Ras/c-Raf interaction and activation of MAP kinase phosphatases. Cell Signal. 2008;20(6):1134–41.
106. Luciani MG, Campregher C, Gasche C. Aspirin blocks proliferation in colon cells by inducing a G1 arrest and apoptosis through activation of the checkpoint kinase ATM. Carcinogenesis. 2007;28 (10):2207–17.
107. Law BK, Waltner-Law ME, Entingh AJ, Chytil A, Aakre ME, Norgaard P, et al. Salicylate-induced growth arrest is associated with inhibition of p70s6k and down-regulation of c-myc, cyclin D1, cyclin A, and proliferating cell nuclear antigen. J Biol Chem. 2000;275(49):38261–7.
108. Zimmermann KC, Waterhouse NJ, Goldstein JC, Schuler M, Green DR. Aspirin induces apoptosis through release of cytochrome c from mitochondria. Neoplasia. 2000;2(6):505–13.
109. Dikshit P, Chatterjee M, Goswami A, Mishra A, Jana NR. Aspirin induces apoptosis through the inhibition of proteasome function. J Biol Chem. 2006;281 (39):29228–35.
110. Alfonso LF, Srivenugopal KS, Bhat GJ. Does aspirin acetylate multiple cellular proteins? (Review). Mol Med Rep. 2009;2(4):533–7.
111. Babbar N, Gerner EW, Casero Jr RA. Induction of spermidine/spermine N1-acetyltransferase (SSAT) by aspirin in Caco-2 colon cancer cells. Biochem J. 2006;394(Pt 1):317–24.
112. Martinez ME, O'Brien TG, Fultz KE, Babbar N, Yerushalmi H, Qu N, et al. Pronounced reduction in adenoma recurrence associated with aspirin use and a polymorphism in the ornithine decarboxylase gene. Proc Natl Acad Sci U S A. 2003;100 (13):7859–64.
113. Ruschoff J, Wallinger S, Dietmaier W, Bocker T, Brockhoff G, Hofstadter F, et al. Aspirin suppresses the mutator phenotype associated with hereditary nonpolyposis colorectal cancer by genetic selection. Proc Natl Acad Sci U S A. 1998;95(19):11301–6.
114. Ruffin MT, Krishnan K, Rock CL, Normolle D, Vaerten MA, Peters-Golden M, et al. Suppression of human colorectal mucosal prostaglandins: determining the lowest effective aspirin dose. J Natl Cancer Inst. 1997;89(15):1152–60.
115. Dovizio M, Bruno A, Tacconelli S, Patrignani P. Mode of action of aspirin as a chemopreventive agent. Recent Results Cancer Res. 2013;191:39–65.
116. Gasic GJ, Gasic TB, Stewart CC. Antimetastatic effects associated with platelet reduction. Proc Natl Acad Sci U S A. 1968;61(1):46–52.
117. Gay LJ, Felding-Habermann B. Contribution of platelets to tumour metastasis. Nat Rev Cancer. 2011;11(2):123–34.
118. Di Francesco L, Lopez Contreras LA, Sacco A, Patrignani P. New insights into the mechanism of action of aspirin in the prevention of colorectal neoplasia. Curr Pharm Des. 2015;21:5116–26.
119. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nat Rev Clin Oncol. 2012;9 (5):259–67.
120. Bruno A, Dovizio M, Tacconelli S, Patrignani P. Mechanisms of the antitumoural effects of aspirin in the gastrointestinal tract. Best Pract Res Clin Gastroenterol. 2012;26(4):e1–13.
121. Menter DG, Tucker SC, Kopetz S, Sood AK, Crissman JD, Honn KV. Platelets and cancer: a casual or causal relationship: revisited. Cancer Metastasis Rev. 2014;33(1):231–69.
122. Ge R, Tan E, Sharghi-Namini S, Asada HH. Exosomes in cancer microenvironment and beyond: have we overlooked these extracellular messengers? Cancer Microen. 2012;5(3):323–32.
123. Dovizio M, Maier TJ, Alberti S, Di Francesco L, Marcantoni E, Munch G, et al. Pharmacological inhibition of platelet-tumor cell cross-talk prevents platelet-induced overexpression of cyclooxygenase-2 in HT29 human colon carcinoma cells. Mol Pharmacol. 2013;84(1):25–40.
124. Labelle M, Begum S, Hynes RO. Direct signaling between platelets and cancer cells induces an epithelial-mesenchymal-like transition and promotes metastasis. Cancer Cell. 2011;20(5):576–90.
125. US Preventive Services Task Force. Routine aspirin or nonsteroidal anti-inflammatory drugs for the primary prevention of colorectal cancer: U.S. Preventive Services Task Force recommendation statement. Ann Intern Med. 2007;146(5):361–4.
126. Huang ES, Strate LL, Ho WW, Lee SS, Chan AT. - Long-term use of aspirin and the risk of gastrointestinal bleeding. Am J Med. 2011;124(5):426–33.
127. Roderick PJ, Wilkes HC, Meade TW. The gastrointestinal toxicity of aspirin: an overview of

randomised controlled trials. Br J Clin Pharmacol. 1993;35(3):219–26.
128. Derry S, Loke YK. Risk of gastrointestinal haemorrhage with long term use of aspirin: meta-analysis. BMJ. 2000;321(7270):1183–7.
129. Peters RJ, Mehta SR, Fox KA, Zhao F, Lewis BS, Kopecky SL, et al. Effects of aspirin dose when used alone or in combination with clopidogrel in patients with acute coronary syndromes: observations from the Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) study. Circulation. 2003;108 (14):1682–7.
130. Topol EJ, Easton D, Harrington RA, Amarenco P, Califf RM, Graffagnino C, et al. Randomized, double-blind, placebo-controlled, international trial of the oral IIb/IIIa antagonist lotrafiban in coronary and cerebrovascular disease. Circulation. 2003;108 (4):399–406.
131. Serebruany VL, Steinhubl SR, Berger PB, Malinin AI, Baggish JS, Bhatt DL, et al. Analysis of risk of bleeding complications after different doses of aspirin in 192,036 patients enrolled in 31 randomized controlled trials. Am J Cardiol. 2005;95 (10):1218–22.
132. McQuaid KR, Laine L. Systematic review and meta-analysis of adverse events of low-dose aspirin and clopidogrel in randomized controlled trials. Am J Med. 2006;119(8):624–38.
133. Steinhubl SR, Bhatt DL, Brennan DM, Montalescot G, Hankey GJ, Eikelboom JW, et al. Aspirin to prevent cardiovascular disease: the association of aspirin dose and clopidogrel with thrombosis and bleeding. Ann Intern Med. 2009;150(6):379–86.
134. Lanas A, Wu P, Medin J, Mills EJ. Low doses of acetylsalicylic acid increase risk of gastrointestinal bleeding in a meta-analysis. Clin Gastroenterol Hepatol. 2011;9(9):762–8. e6.
135. Garcia Rodriguez LA, Hernandez-Diaz S, de Abajo FJ. Association between aspirin and upper gastrointestinal complications: systematic review of epidemiologic studies. Br J Clin Pharmacol. 2001;52 (5):563–71.
136. Rothwell PM, Price JF, Fowkes FG, Zanchetti A, Roncaglioni MC, Tognoni G, et al. Short-term effects of daily aspirin on cancer incidence, mortality, and non-vascular death: analysis of the time course of risks and benefits in 51 randomised controlled trials. Lancet. 2012;379(9826):1602–12.
137. Chan FK, Chung SC, Suen BY, Lee YT, Leung WK, Leung VK, et al. Preventing recurrent upper gastrointestinal bleeding in patients with Helicobacter pylori infection who are taking low-dose aspirin or naproxen. N Engl J Med. 2001;344(13):967–73.
138. Chan FK, Sung JJ, Chung SC, To KF, Yung MY, Leung VK, et al. Randomised trial of eradication of Helicobacter pylori before non-steroidal anti-inflammatory drug therapy to prevent peptic ulcers. Lancet. 1997;350(9083):975–9.
139. Thorat MA, Cuzick J. Prophylactic use of aspirin: systematic review of harms and approaches to mitigation in the general population. Eur J Epidemiol. 2015;30(1):5–18.
140. Helicobacter Eradication Aspirin Trial (HEAT): ClinicalTrials.gov, [Accessed: 2015 October 20]. Available from: https://clinicaltrials.gov/ct2/show/NCT01506986.
141. Tran-Duy A, Vanmolkot FH, Joore MA, Hoes AW, Stehouwer CD. Should patients prescribed long-term low-dose aspirin receive proton pump inhibitors? A systematic review and meta-analysis. Int J Clin Pract. 2015;69(10):1088–111.
142. Gorelick PB, Weisman SM. Risk of hemorrhagic stroke with aspirin use: an update. Stroke. 2005;36 (8):1801–7.
143. Antithrombotic Trialists' Collaboration, Baigent C, Blackwell L, Collins R, Emberson J, Godwin J, et al. Aspirin in the primary and secondary prevention of vascular disease: collaborative meta-analysis of individual participant data from randomised trials. Lancet. 2009;373(9678):1849–60.
144. Hansson L, Zanchetti A, Carruthers SG, Dahlof B, Elmfeldt D, Julius S, et al. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. HOT Study Group. Lancet. 1998;351(9118):1755–62.
145. De Berardis G, Lucisano G, D'Ettorre A, Pellegrini F, Lepore V, Tognoni G, et al. Association of aspirin use with major bleeding in patients with and without diabetes. JAMA. 2012;307(21):2286–94.
146. Cuzick J, Thorat MA, Bosetti C, Brown PH, Burn J, Cook NR, et al. Estimates of benefits and harms of prophylactic use of aspirin in the general population. Ann Oncol. 2015;26(1):47–57.
147. The Dutch TIA Trial Study Group. A comparison of two doses of aspirin (30 mg vs. 283 mg a day) in patients after a transient ischemic attack or minor ischemic stroke. N Engl J Med. 1991;325(18):1261–6.
148. Flossmann E, Rothwell PM. British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet. 2007;369(9573):1603–13.
149. Aspree Investigator Group. Study design of ASPirin in Reducing Events in the Elderly (ASPREE): a randomized, controlled trial. Contemp Clin Trials. 2013;36(2):555–64.
150. Bayer HealthCare. (ARRIVE) Aspirin to Reduce Risk of Initial Vascular Events [Accessed: 2015 October 20]. http://www.arrive-study.com/EN/.
151. Mahipal A, Anderson KE, Limburg PJ, Folsom AR. Nonsteroidal anti-inflammatory drugs and subsite-specific colorectal cancer incidence in the Iowa women's health study. Cancer Epidemiol Biomarkers Prev. 2006;15(10):1785–90.

152. Smalley W, Ray WA, Daugherty J, Griffin MR. Use of nonsteroidal anti-inflammatory drugs and incidence of colorectal cancer: a population-based study. Arch Intern Med. 1999;159(2):161–6.
153. Elwood P, Morgan G. The harms of low-dose aspirin prophylaxis are overstated. Ann Oncol. 2015;26 (2):441–2.
154. Pignone M, Earnshaw S, McDade C, Pletcher MJ. Effect of including cancer mortality on the cost-effectiveness of aspirin for primary prevention in men. J Gen Intern Med. 2013;28(11):1483–91.
155. US Preventive Services Task Force. Draft Recommendation Statement: Aspirin to Prevent Cardiovascular Disease and Cancer 2015 [Accessed: 2015 October 20]. http://www.uspreventiveservicestaskforce.org/Page/Document/draft-recommendation-statement/aspirin-to-prevent-cardiovascular-disease-and-cancer#Pod4.
156. Yan M, Myung SJ, Fink SP, Lawrence E, Lutterbaugh J, Yang P, et al. 15-Hydroxyprostaglandin dehydrogenase inactivation as a mechanism of resistance to celecoxib chemoprevention of colon tumors. Proc Natl Acad Sci U S A. 2009;106(23):9409–13.
157. Fink SP, Yamauchi M, Nishihara R, Jung S, Kuchiba A, Wu K, et al. Aspirin and the risk of colorectal cancer in relation to the expression of 15-hydroxyprostaglandin dehydrogenase (HPGD). Sci Transl Med. 2014;6(233):233re2.
158. Bezawada N, Song M, Wu K, Mehta RS, Milne GL, Ogino S, et al. Urinary PGE-M levels are associated with risk of colorectal adenomas and chemopreventive response to anti-inflammatory drugs. Cancer Prev Res (Phila). 2014;7(7):758–65.
159. Fedirko V, Bradshaw PT, Figueiredo JC, Sandler RS, Barry EL, Ahnen DJ, et al. Urinary metabolites of prostanoids and risk of recurrent colorectal adenomas in the Aspirin/Folate Polyp Prevention Study (AFPPS). Cancer Prev Res (Phila). 2015;8 (11):1061–68.
160. Nan H, Hutter CM, Lin Y, Jacobs EJ, Ulrich CM, White E, et al. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. JAMA. 2015;313(11):1133–42.
161. Morgenstern R, Zhang J, Johansson K. Microsomal glutathione transferase 1: mechanism and functional roles. Drug Metab Rev. 2011;43(2):300–6.
162. Tseng-Rogenski SS, Hamaya Y, Choi DY, Carethers JM. Interleukin 6 alters localization of hMSH3, leading to DNA mismatch repair defects in colorectal cancer cells. Gastroenterology. 2015;148(3):579–89.
163. van Kruijsdijk RC, Visseren FL, Ridker PM, Dorresteijn JA, Buring JE, van der Graaf Y, et al. Individualised prediction of alternate-day aspirin treatment effects on the combined risk of cancer, cardiovascular disease and gastrointestinal bleeding in healthy women. Heart. 2015;101(5):369–76.

A Rational Approach for the Use of NSAIDs and/or Aspirin in Cancer Prevention in the Near Future: "Balancing Risk and Benefits"

15

Elizabeth Half, Ahmad Fokra, and Nadir Arber

Introduction

In 2016 colorectal cancer (CRC) is still the third most common cancer in men and women and the third leading cause of cancer-related death in men (after lung and prostate cancer) and third in women (behind lung and breast cancers) in the United States [1]. On a worldwide basis, there were more than 1,200,000 new cases in 2012, with more than 600,000 deaths [2]. Although there has been an increase in early detection and reduced number of metastatic diseases at presentation over the last few years [3], this has mainly occurred in countries with national screening programs. However, this is not sufficient, as the number of deaths due to CRC remains alarmingly high, making the prevention crucial. The development of primary prevention strategies to reduce the risk of developing colorectal neoplasia remains an important goal, particularly in view of the inherent limitations of population-based secondary prevention programs that rely on detection and removal of adenomas.

Chemoprevention is defined as the use of drugs, vitamins, or other agents to try to reduce the risk or delay the development or recurrence of cancer. The aim is to interfere with the process of carcinogenesis by targeting key molecular pathways that provides a promising approach to reduce the incidence of and mortality from cancer. Chemoprevention involves the use of a variety of natural or chemical compounds that can delay, prevent, or even reverse the adenoma to carcinoma process in the colon. Early stage of CRC fits the criteria for chemopreventive intervention as adenomatous polyps are identifiable and treatable, therefore allowing the implementation of therapeutic and preventative strategies [1].

The ideal chemopreventive agent should fulfill the following criteria: (1) the drug must be effective; (2) it should have minimal side effects or an acceptable safety profile in high-risk population; (3) it should have a convenient dosing schedule, ideally once a day; (4) it should be easily administered; and (5) it should be inexpensive. Whenever we administer any agent to a patient and in particular when we are treating healthy individuals, we must carefully assess

E. Half, M.D.
Institute of Gastroenterology, Rambam Health Care Campus, Haifa, Israel

A. Fokra, M.D.
Integrated Cancer Prevention Center, Tel Aviv Sourasky Medical Center, 6th Weizmann, St., Tel Aviv 64239, Israel

N. Arber, M.D., M.Sc., M.H.A. (✉)
Department of Gastroenterology, Integrated Cancer Prevention Center, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

Integrated Cancer Prevention Center, Tel Aviv Sourasky Medical Center, 6th Weizmann, St., Tel Aviv 64239, Israel
e-mail: nadira@tlvmc.gov.il; narber@post.tau.ac.il

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7_15

the risk/benefit ratio. We wish to emphasize that the profile of safety and efficacy for any given drug varies significantly and depends on the severity of the disease and the tolerance of the individuals receiving the specific drug.

Based on reports of chemopreventive activity in the literature and/or efficacy data from in vitro models of carcinogenesis, several agents have been studied including phytochemicals, vitamins, minerals, inhibitors of proliferation, metabolic inducers, differentiation agents, and most importantly nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin. Representative examples include folic acid, calcium, estrogen, vitamin D, oltipraz, curcumin, selenium, green tea, ursodiol, statins, and fiber, which have been encouraging, but shown modest efficacy in humans.

In this regard, the most promising drugs are aspirin and NSAIDs mainly due to their ability to inhibit cyclooxygenase, although COX-independent pathways play a major role as well. Cyclooxygenase (COX) is probably the most common therapeutic drug target in human history. Inhibitors of this enzyme have been used extensively and widely. Research in this area has been dominated by investigations of the COX-1 and COX-2 enzymes, and the therapeutic market has been revolutionized by the development of drugs that are selectively targeted against COX-2.

Supportive evidence for the role of aspirin and NSAIDs in the prevention of CRC has been derived from more than 200 well-conducted, randomized, placebo-controlled animal studies, in which the administration of various NSAIDs consistently resulted in fewer tumors per animal and fewer animals with tumors, thereby clearly showing a preventive effect on carcinogen-induced colorectal tumorigenesis in rodents [4, 5]. These findings were also supported by studies in genetically manipulated rodent models [6] that reported a reduction in number and size of colorectal neoplasms in animals treated with NSAIDs. Epidemiologic observations and population-based studies also showed that long-term use of aspirin and other NSAIDs reduced the risk of CRC [4–11]. However, treatment is associated with risks which will be discussed in details, and recommendations will be provided.

NSAIDs

The hypothesis that nonsteroidal anti-inflammatory drugs (NSAIDs) might inhibit the occurrence or growth of CRC arose in the mid-1970s. Bennett, Del Tacca [12], and Jaffe [7] reported that the concentration of prostaglandin E2 (PGE2) was higher in CRC than in the surrounding normal mucosa. The protective effect of NSAIDs has been documented in 70 of 72 epidemiologic studies that showed that the prevention of adenoma recurrence, inhibition of incidence, and even a lower mortality rate in both women and men were evident in patients that consumed NSAIDs. This protective effect depends on the dose and type of the drug but more importantly is directly related to the duration of exposure [10]. The exact mechanism by which NSAIDs exert their chemopreventive effect is not fully understood, but inhibition of the COX enzyme, also known as prostaglandin–endoperoxide synthase, has been the most extensively studied target. The COX enzyme is probably the most common therapeutic drug target in human history. Aspirin, a nonselective, COX inhibitor, has been used for almost 4000 years. Research in this area has been dominated by investigations into the COX enzymes that are central and rate-limiting enzymes in the biosynthesis of prostaglandins [13, 14]. Three COX isoforms have been identified: COX-1, COX-2, and COX-3. COX-1 and COX-2 are located on different chromosomes, and their expression is tightly regulated [14]. COX-1 is mapped to chromosome 9q32-q33.2, is encoded by the *PTGS1* gene, and is constitutively expressed in normal tissues. It serves as a "housekeeper" of mucosal integrity. COX-1 is the central enzyme in the biosynthetic pathway to prostaglandins from arachidonic acid; it produces prostacyclins, prostaglandins, and thromboxane, which protect the gastric mucosa and play a key role in platelet aggregation and renal microvasculature

dynamics. COX-2 is mapped to chromosome 1q25.2-q25.3 and is encoded by the *PTGS2* gene, an immediate early response gene that is highly inducible by either neoplastic or inflammatory stimuli. COX-2 is involved in the synthesis of prostaglandins and thromboxanes, which are regulators of processes that are relevant to cancer development. It is generally accepted that alterations in COX-2 expression and the abundance of its enzymatic product PGE_2 have key roles in influencing the development of CRC.

COX-3, a third distinct COX isozyme, is a COX-1 variant formed by intron retention, a form of alternative splicing [15]. COX-3 shares all the catalytic features of COX-1 and -2; however, its exact role is yet to be fully understood [15]. Relative to the normal mucosa, COX-2 overexpression occurs in about half of colorectal adenomas and in 85 % of human CRCs, making COX-2 an attractive therapeutic target [16, 17]. Moreover, the fact that COX-2 expression is upregulated in both premalignant and malignant colorectal tissue has also potential implications for the prevention of this type of cancer. Most NSAIDs are nonselective; however, in the last decade, selective COX-2 inhibition has been extensively studied and will be discussed at a later stage.

It is well known that not all NSAIDs that inhibit COX have similar anticancer activity and that the anticancer activity is not related directly to the degree of COX inhibition. Alternative COX-independent pathways have an important role in their chemopreventive effect. A number of COX-independent targets have been identified as being involved in the anticancer activity of NSAIDs. Cyclic guanosine monophosphate (cGMP) phosphodiesterases (PDEs) are such targets that are known to inhibit the normal apoptosis signal pathways. This inhibition permits the apoptotic signal pathway to proceed unopposed, resulting in apoptotic cell death. The potencies of certain NSAIDs for the inhibition of colon tumor cell growth correlate with their potencies for the inhibition of cGMP PDEs in vitro [5, 18–26], and certain NSAID metabolites and derivatives that lack COX inhibitory activity but maintain the anticancer activity also inhibit cGMP PDEs. The anticancer activity of NSAIDs that involves inhibition of cGMP PDEs, for example, sulindac, results in increased intracellular cGMP levels with activation of cGMP signaling [27–30].

A number of NSAIDS have called much attention over the years as chemopreventive agents among the nonselective NSAIDs sulindac and aspirin and among the selective COX-2 inhibitors celecoxib and rofecoxib have caused the most attention and will be discussed in further details.

Aspirin

Aspirin, a nonselective NSAID, was first developed in Germany by Felix Hoffman in 1897. Today it is the best-known and most widely used medication with an estimated 100 billion tablets consumed annually across the world (http://www.aspirin-foundation.com/). Over the years aspirin has been in use as a pain reliever, an anti-inflammatory drug in many chronic inflammatory diseases, as well as an antiplatelet medication in cardiovascular diseases.

The first evidence of aspirin's chemopreventive role in cancer development came from a large Australian case–control study published in 1988 exploring potential relation between numerous chronic illnesses, medication use, and CRC [31]. An inverse association was found between aspirin use and risk of CRC. Subsequently, aspirin has been investigated extensively in the chemoprevention of colorectal adenomas and CRC, based in part on their inhibition of cyclooxygenase COX-1 and COX-2 enzymes, both of which are important mediators of prostaglandin production. However COX-independent mechanisms have also been hypothesized [32]. Other molecular targets of aspirin have been extensively studied [33] but have been the focus of far fewer studies than the COX pathways.

Different pathways may play a role at diverse points during cancer development, from

inhibiting adenoma formation to staling progression [32] at later stages in carcinogenesis. Primary targets of investigation include inhibition of IkB kinase (IKK)-β, prevention of NF-kB activation, extracellular signal-regulated kinase (ERK) inhibition, mitochondrial functions, and inhibition of the Wnt/β-catenin pathway. Other reports indicate that targets of aspirin may directly or indirectly modulate the activity of transcription factors [34], cell signaling proteins, metabolic enzymes, and mitochondrial proteins [35–37].

Taken together aspirin has many mechanisms of action making the drug a potential target for the prevention and/or treatment of CRC. The Nurses' Health Study was initiated in 1976 and included >120,000 nurses aged 30–55 years [38]. Data on lifestyle, diet, and medications have been retrieved from self-administered questionnaires that had been modified over the years. Chan et al. [39] found a significantly lower rate of RC among regular aspirin users (two or more regular 325-mg tablets per week). The risk reduction was evident only after 5 years of exposure, with the protective effect being more significant after a decade. The dose of aspirin was important as well. Women consuming 2–5 regular tablets of aspirin per week experienced a modestly reduced relative risk (RR, 11 %), whereas those who consumed at least 14 doses per week experienced the highest (32 %) reduction in CRC incidence. The mortality in the Nurses' Health Study was recently evaluated after 24 years follow-up. Aspirin users had a 0.75 and 0.65 RR of cardiovascular and CRC death, respectively [40]. In the observational health professionals' study on 50,000 individuals, regular aspirin use, of more than twice a week, was associated with a 32 % reduction in mortality from CRC [41]. Jacobs et al. [42] recently examined the associations between long-term daily use of aspirin (325 mg/day) and overall cancer incidence among 69,810 men and 76,303 women participating in the Cancer Prevention Study II Nutrition Cohort. Aspirin use was reported at enrollment in 1992–1993 and updated in 1997, 1999, and 2001. Daily aspirin use for >5 years was associated with a lower incidence of CRC (RR, 0.68) among men and women combined.

On the other hand, a few large randomized trials of aspirin, in primary prevention, showed no effect on the occurrence of CRC. The Women's Health Study randomized healthy women to low-dose aspirin versus placebo. An average of 10 years of follow-up failed to show a primary preventive effect of aspirin [43]. The Physicians' Health Study was primarily designed to assess the effect of aspirin (325 mg every other day) on the risk of coronary artery disease and cancer in 22,071 male physicians in the United States [44]. After 5 years of aspirin therapy, there was no change in the incidence of CRC or polyps (nonsignificant odds ratio of 1.15 for CRC and 0.86 for adenomas) between the treatment and the placebo groups. The British Doctors Aspirin Trial ($N = 5139$, two-thirds allocated 500 mg aspirin for 5 years, one-third allocated to avoid aspirin and served as controls [45] and the United Kingdom Transient Ischemic Attack Aspirin Trial ($N = 2449$, two-thirds allocated 300 mg or 1200 mg aspirin for 1–7 years, one-third allocated to placebo control [46]) failed to show a protective effect of aspirin. The long-term effect of aspirin was analyzed in these two randomized trials with reliable posttrial follow-up for >20 years [47]. They also did a systematic review of all relevant observational studies and showed that the use of >300 mg aspirin per day for at least 5 years in the RCT was effective in primary prevention of CRC, with a latency period of about 10 years. These results were consistent with findings from their meta-analysis of the observational studies. These studies were, however, unable to circumvent confounders, such as intermittent and variable dosing and the use of other NDAIDs and risk-modifying drugs. It appears that the reduction in risk of the CRC may require prolonged follow-up (>10 years), which is biologically plausible given the prolonged time that take adenomas to progress to invasive cancer. It is, however, suggested that long-term follow-up is required to establish the effects of aspirin on the incidence and mortality from CRC.

Aspirin's effect on adenoma incidence in the general population without a history of prior adenoma or CRC has been evaluated in 27,077 healthy women, 34–77 years of age, from the Nurses' Health Study. These women underwent screening colonoscopy and adenoma occurrence was documented. Regular, short-term use of aspirin was inversely associated with the risk for colorectal adenoma. Reduced risk of adenoma incidence was associated with at least least six 325-mg aspirin tablets per week (RR, 0.68 [95 % CI, 0.55–0.84]) and with more than 14 tablets/week (RR, 0.57 [95 % CI, 0.42–0.77]) of aspirin, higher doses than those were recommended for the prevention of cardiovascular disease. The duration of follow-up was not specified, but RRs were adjusted for the duration of aspirin use [48].

In the moderate-risk setting of patients with previous adenoma or CRC, three randomized controlled trials have shown significant efficacy in preventing polyp recurrence when aspirin was given at daily doses of 81–325 mg/day. These trials were not, however, entirely in agreement as to the lowest effective aspirin dose, and they were relatively short term in duration, i.e., up to 3 years. In the first trial, aspirin (325 mg qd) or placebo was prescribed to 600 patients with a recent history of CRC. The proportion of patients who had at least one adenoma was lower in the aspirin group than in the placebo group (17 % vs. 27 %, $P = 0.004$) [49]. Aspirin also delayed the onset of recurrent adenoma. In the second trial, 81 mg or 325 mg of aspirin per day was compared to placebo in patients with a history of one or more colorectal adenomas. A reduction in the recurrence of adenomas was associated with aspirin consumption (17 % and 4 %, respectively), although it was significant only for the lower dose [50]. The 81-mg dose was associated with a 3.7 % absolute risk reduction in the incidence of advanced adenomas (RR reduction, 41 %; 95 % confidence interval, 8–62 %). Notably, protection against advanced adenomas (>1 cm, high-grade dysplasia, and villous histology) was more pronounced than the effect on the risk of recurrence of any adenoma (e.g., reduction rates of 41 % and 17 %, respectively). The third trial [51] randomized 272 patients with a history of adenoma to receive lysine acetylsalicylate (a form of aspirin) 300 mg, 150 mg, or placebo daily. Colonoscopies were done at 1 and 4 years. Both dosages were effective in reducing polyp recurrence. A lesser effect at 4 years was seen, and the lower (160 mg/day) dose was surprisingly more effective [52]. The benefit was greatest with the higher (300 mg/day) dose at 1 year [51, 52]. From the combined results of the above-cited trials, significantly fewer subjects in the low-dose aspirin group developed recurrent sporadic colorectal adenomas after 1–3 years [53]. While these trials established causality, they had relatively short duration of treatment and as such provided limited data on the necessary duration of treatment needed to prevent cancer. These trials also offered limited data on dose, and the data they did provide was somewhat conflicting.

The US Preventive Services Task Force recently [54] updated their data and reached the same conclusion that the data on aspirin in the setting of prior adenoma is limited by short-term follow-up (fewer than 5 years) and studies could not, therefore, provide sufficient information on the effect of aspirin use on the incidence in this setting. However there were some suggestions of a decreased risk of adenoma incidence in over a 3- to 4-year period [54]. No recommendations were made.

If no risk had been shown to be associated with aspirin, it would suggest that the argument in favor of its use is stronger than that of its avoidance, even with uncertainty about the reduction in the incidence of colorectal adenomas or CRC.

Secondary Prevention

In the setting of patients with history of CRC, aspirin at a dose of 325 mg for up to 3 years significantly reduced adenoma recurrence rate. In 799 patients with stage III CRC enrolled in an adjuvant chemotherapy trial, aspirin use after a median follow-up of 6.5 years was associated with improved recurrence-free survival, disease-

free survival, and reduced mortality [49–51, 55, 56]. In a prospective cohort study of 1279 men and women diagnosed with stages I–III during a median follow-up of 11.8 years, the HR (adjusted for cancer stage and location, sex, age, and body mass index) for mortality was lower in aspirin users as compared to nonusers (RR, 0.71; 95 % CI, 0.53–0.95). Regular aspirin use after the diagnosis was associated with a lower risk of CRC-specific mortality, but this was the case mainly among participants in whom primary tumors overexpressed COX-2 [57]. It is well known that CRC is a diverse disease and the response is not universal. Furthermore as discussed earlier, aspirin does carry a significant risk profile, defining biomarkers to better identify patients for response are crucial. The phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway plays an important role in CRC carcinogenesis [58], and mutations occur in 10–30 % of the cases. PI3K mutation results in an activation of the PI3K and downstream AKT pathway which enhances COX-2 activity and PGE_2 formation resulting in the inhibition of apoptosis and enhancement of cell proliferation which have been associated with the development of CRC [59–61]. In addition mutations have been associated with poor prognosis and resistance to therapy [62–64]. In this regard Domingo et al. found that regular aspirin use after CRC diagnosis was associated with a reduced rate of CRC recurrence in patients with PIK3CA-mutant cancers (HR, 0.11; $P = .027$) but not in patients lacking tumor PIK3CA mutation (HR, 0.92; $P = .71$) [65]. A recent meta-analysis pooled seven studies that received aspirin treatment CRC diagnosis. An overall survival benefit was found for treated patients with colon as well as rectal cancers. This survival benefit appeared to be confined to patients with positive COX-2 expression and with mutated PIK3CA tumors (HR = 0.58; 95 % CI, 0.37–0.90) [66]. While this evidence supports pretreatment patient selection according to tumor profile, the low number of studies prevents definitive conclusions. Trials addressing this issue are warranted to assess the efficacy of aspirin in the adjuvant setting and ultimate patient selection to maximize efficacy and reduce unnecessary toxicity.

Two cohort studies assessed aspirin use before the diagnosis. These studies found that pre-diagnosis aspirin use was associated with lower-specific mortality [50, 51]. However, a recent meta-analysis found seven publications on pre-diagnosis aspirin use in patients with CRC. They were unable to prove a positive association between pre-diagnosis aspirin use and CRC overall mortality or CRC-specific mortality [67]. Currently a large international, multicenter randomized, placebo-controlled phase 3 trial of 200 mg/day aspirin as an adjunct to adjuvant treatment for Dukes' C and high-risk Dukes' B is ongoing, in several Asian centers (ASCOLT study). This study will shed light on the effect of aspirin on disease-free and overall survival among patients with Dukes' C and high-risk Dukes' B CRC. Results are expected during the upcoming years.

What About the Use of These Agents in the Very High-Risk Population?

There is ample data regarding their efficacy in the setting of familial adenomatous polyposis (FAP), a hereditary disease with 100 % risk of developing CRC by the fifth decade. It has clearly been shown that sulindac or celecoxib is potent in preventing adenomas in FAP [66]. In this regard, aspirin has been investigated in the Colorectal Adenoma/Carcinoma Prevention Program 1 (CAPP1) study, a randomized, placebo-controlled trial of aspirin 600 mg/day and/or resistant starch 30 g/day in a two-by-two factorial design. Among 133 evaluable patients, aspirin treatment resulted in a nonsignificant reduction in polyp number (RR = 0.77; 95 % CI, 0.54–1.10) compared with nonaspirin and a significant reduction in polyp size among patients treated for more than 1 year [68]. A recent paper from Japan Familial Adenomatous Polyposis Prevention II (J-FAPP II) trial confirms that aspirin is effective in the Japanese FAP population with a lower dose of aspirin

(200 mg). No significant side effects were reported [69].

Patients with Lynch syndrome represent another high-risk group with an increased risk of 85 % for having CRC through their lifetime [70]. Evidence of a protective effect of aspirin use was provided by the CAPP2 trial. The CAPP2 trial was an RCT (aspirin 600 mg per day vs. placebo) that evaluated aspirin, for the first time, as a chemopreventive agent with cancer as the primary endpoint. At the end of two years of treatment with a daily high dose (600 mg) of aspirin, there was no evidence for a protective effect on CRC risk [71]. However, at a later follow-up, of 56 months, it was found that patients who had been randomly assigned to aspirin had about a 40 % reduction in incidence as compared to the controls that had received placebo [72]. No data for adverse events were available post-intervention. Surprisingly during the intervention, adverse events did not differ between the aspirin and the placebo group.

Cancer risk in the setting of Lynch syndrome is currently being studied in the CAPP3 study. The study intends to randomize >1000 patients with a known mutation. Patients are going to be randomized to three doses of aspirin (100, 300, 600) for 2 years followed by 3 years of open-labeled aspirin of 100 mg to all participants. Results are expected in 2020.

The association between aspirin use and CRC risk in the context of Lynch syndrome has recently been addressed in a cohort of MMR mutation carriers who are registered in a multinational family cancer registry from the United States, Australia, and Canada. 1858 MMR mutation-positive individuals were included. 714 carriers (38 %) were diagnosed with CRC at a mean age of 42.4 years. A reduced risk of CRC was associated with aspirin use (for 1 month to 4.9 years HR = 0.49, for ≥5 years; HR = 0.25, 95 % CI = 0.10–0.62, $P = .003$) compared with less than one month of use. Regarding the protective effect of aspirin in Lynch syndrome patients, CAPP3, led by John Burn, has been launched in 2015. The CAPP3 study is planned to recruit >1000 subjects with a known Lynch syndrome mutation. Patients are going to be randomized into three groups receiving 100, 300, or 600 mg of daily aspirin for 3 years to be followed with 2 years of open-labeled daily dose of 100 mg of aspirin.

Adverse Events

Aspirin treatment is not without potential harm, and it has long been recognized that treatment may be associated with serious side effects [73]. GI bleeding, ulcers, or, in the more serious scenario, intracranial bleeding and hemorrhagic stroke are among the major events. The open question is regarding the risk in average-risk population (vs. risk in patients with CVD, the population in most studies) and timing of the risk during the therapy (early or late) and whether the risk continues after treatment cessation.

Most studies reported on harms during scheduled treatment duration, the median of which was between 2.6 and 10.1 years. The Women's Health Study included events during posttrial follow-up (median of 17.5 years from randomization). Twelve RCTs (all except ETDRS and APACC) reported on gastrointestinal bleeding. Compared to controls, patients assigned to aspirin had higher risks of gastrointestinal bleeding and serious gastrointestinal bleeding (summary OR, 1.94 [95 % CI, 1.44–2.62]). Results were not consistent for fatal gastrointestinal bleeding [74].

Intracranial bleeding data were available from 12 RCTs. Patients randomized to aspirin had a higher risk of intracranial bleeding than control patients (summary OR, 1.53 [95 % CI, 1.21–1.93]). Aspirin users had a higher risk of hemorrhagic stroke than controls (summary OR, 1.47 [95 % CI, 1.16–1.88]) [74]. Regarding age-related macular degeneration, the USPSTF did not identify publications that reported on macular degeneration as part of their systematic search strategy. For all harm outcomes, very limited data were available on effect modification by

age, sex, race, comorbidities, or concomitant medication use.

Major aspirin-related complication is age dependent and has been reported to be responsible for about 2–8 % mortality rate [75]. At the same time, patients with an annual risk for coronary heart disease of 1.5 % should take aspirin to prevent cardiovascular mortality [76]. These patients will also benefit from the decrease in the incidence of colorectal neoplasia and mortality. It is also confirmed that patients with a low risk for coronary heart disease (<0.7 % per year) should not take aspirin to prevent cardiovascular events or CRC.

Dosing of Aspirin

The adverse effects of aspirin appear to be largely dose related. The minimally effective dose required for the prevention remains a critically important question. Rothwell et al. [77, 78] published a meta-analysis and reported that doses of aspirin typically used for cardiovascular disease prevention (75–325 mg daily) were as effective as high-dose (1200 mg/day) aspirin [78]. However, the short-term follow-up data from the two trials of alternate day of aspirin that were not eligible for inclusion in the meta-analysis (i.e., aspirin 325 mg in the PHS and 100 mg in the WHS) did not show a reduction in the risk of CRC [76, 77]. Although the negative findings of these studies could be attributed to their relatively short follow-up and/or alternate-day dosing, they do leave some uncertainty regarding the effects of low-dose regimens. The adenoma trials indicate that aspirin doses in the range of 81–325 mg daily reduce the risk. However, the dose–response patterns in the studies are difficult to integrate. Two trials compared higher (300–325 mg/day) and lower (81–160 mg/day) doses of aspirin; a reduction in the risk of all recurrent adenomas was found only with the lower (81–160 mg/ day) doses [79]. Nonetheless, two other trials that only studied the higher (300–325 mg/day) doses of aspirin both reported reductions in risk of all adenomas from the active treatment. Overall, the estimates for the risk reduction associated with lower (81–160 mg)- and higher (300–325 mg)-dose aspirin were similar both for all adenomas and for advanced adenomas. On the other hand, observational studies are not as conclusive. Some studies suggest that 300–325 mg/day may be required [39, 42, 47, 80, 81].

In two prospective cohort studies that could examine the use of aspirin over a long duration, greater efficacy was observed with intake as high as 14 (325 mg) tablets per week [39, 47]. An extension of the Women's Health Study which was published in 2013 included 39,876 women, aged 45 years or older, who continued to take 100 mg of aspirin on alternate-day basis or placebo. At follow-up of a median of 10 years, the CRC risk was reduced in the aspirin group (HR, 0.80 [CI, 0.67–0.97]; $P = 0.021$), primarily for proximal cancer (HR, 0.73 [CI, 0.55–0.95]; $P = 0.022$) but not distal tumors. Taking the clinical trial and observational data together, there is a very strong evidence that aspirin in doses of 325 mg per day reduces the risk. Most probably if taken for a long period of time (more than a decade), as low as 75 mg on a daily basis or even alternate day of 100 mg aspirin may be sufficient in CRC.

Screening and Aspirin Chemoprevention

An RCT of aspirin and screening colonoscopy are very much appealing but most probably will never occur. Aspirin probably cannot be considered as a substitute for screening. What about combining them? Data is lacking regarding the effect of aspirin implementation into screening programs.

However, it does make sense as approximately 30–40 % of individuals do not adhere to their screening schedule; colonoscopy is less efficient in detecting right-sided lesions with the emergence of interval cancers and aspirin that has been shown to be more effective in

preventing proximal cancer. Further data is needed before recommendations can be made.

Benefit and Risk Assessment

Currently, no health or professional organizations recommend aspirin for the primary prevention in average-risk adults. The American Cancer Society [82], the American Medical Association (AMA), and the American College of Physicians (ACP) explicitly recommend against the use of aspirin for chemoprevention, with the AMA and ACP citing the 2007 USPSTF guidelines as the basis for their recommendation [83, 84]. The American College of Gastroenterology, the National Institute for Health and Care Excellence, and the National Institutes of Health have no chemoprevention recommendations for CRC [85–87]. However, some organizations acknowledge possible roles for aspirin in both primary and secondary prevention in high-risk adults. The American Gastroenterological Association recommends that aspirin can be considered for patients with a personal history of CRC [88, 89] and advanced colorectal adenoma or a strong family history, but not for people with a history of peptic ulcer disease or hemorrhagic stroke. National Comprehensive Cancer Network [90] limits their recommendation for aspirin use to primary prevention in adults with a personal history of classical FAP or attenuated FAP to reduce polyp burden as an adjunct to endoscopic surveillance. The updated USPSTF guidelines do not provide any new recommendations for the general population; however, the USPSTF provided a grade "B" recommendation (a USPSTF assessment that there is "high or moderate certainty that the net benefit is moderate to substantial") for the use of low-dose aspirin for chronic disease prophylaxis, including the prevention, for US adults between 50 and 59 years of age with more than 10 % of 10-year risk of cardiovascular events (USPSTF September 2015). For these individuals aspirin was predicted to gain life years as well as quality-adjusted life years [91, 92]. For adults between ages 60 and 69 with a 10-year risk of CVD events of >10 %, the USPSTF issued a grade "C," indicating "at least moderate certainty that the net benefit is small." As such it is advised that physicians use a CV risk score assessment scale as the Framingham risk score, which accounts for risk differences between women and men, to estimate 10-year CVD risk, and after taking into consideration the risk for bleeding events, prescribe aspirin to adults aged 50–59 without known risk factors for bleeding who have a risk of at least 10 % for cardiovascular disease. This recommendation is in no circumstance instead of CRC screening but should be in addition to standard CRC screening programs.

Sulindac

In humans, in the high-risk setting, the efficacy of sulindac as chemopreventive agents was first suggested in a small nonrandomized study in FAP patients with desmoid tumors. Sulindac dramatically decreased the number of adenomas in four patients [8]. Similar observations were reported in 1983 [8] and 1989 and later on in a number of randomized studies of sulindac in FAP patients [93–95].

Sulindac is a prodrug, derived from sulfinylindene and converted reversibly by liver enzymes to sulindac sulfide and irreversibly to sulindac sulfone. Sulindac sulfide has antineoplastic effects via inhibition of COX and prostaglandin synthesis. Sulindac sulfone (exisulind) may induce apoptosis via suppression of cyclic guanosine monophosphate (cGMP) phosphodiesterase and subsequent increase in cGMP-dependent protein kinase G with resultant programmed cell death; despite the lack of the ability to inhibit COX, it retains the ability to induce apoptosis in colon adenocarcinoma in vitro [30, 96].

Although sulindac is a less potent anti-inflammatory drug, its effect on cancer prevention has been detrimental. In FAP setting, with many young patients with little, if any, risk for catastrophic GI bleeding and a significant risk of cancer, sulindac-proven efficacy outrages its

potential toxicity. However, to the best of our knowledge, sulindac has not been studied in average-risk individuals or as secondary prevention in subjects with history of adenomas or CRC.

Selective Cyclooxygenase (COX)-2 Inhibitors

COX-2 is an inducible enzyme that is overexpressed in sites of inflammation and neoplasia. Genetic evidence supports the role of COX-2 in the development of intestinal neoplasia [6]. Furthermore, COX-2 is overexpressed in 40–50 % of adenomas and in 85 % of CRC [97]. The benefits of the chemopreventive effects of NSAIDs without the deleterious side effects could potentially be achieved with selective COX-2 inhibition. In FAP patients the colorectal adenoma burden has been shown to be reduced by 28 % in patients treated with 400 mg of celecoxib twice daily for six months as compared with a reduction of 4.5 % in the placebo group ($P = 0.003$) [55]. Furthermore, in the same study, a significant reduction in duodenal polyposis was found as well with a 31 % reduction in involved areas compared with 8 % on placebo ($P = 0.049$) [98]. In this short-term study, celecoxib 100 and 400 mg twice daily were safe and well tolerated compared with placebo.

In patients with known sporadic adenomas, three prospective, randomized, placebo-controlled, international, multicenter trials across the globe were launched in the end of the last millennium. The primary endpoint was the number of patients with recurrent adenomatous polyps after 1 and 3 years. The Adenomatous Polyp Prevention on Vioxx (APPROVe) trial recruited 2586 patients that received 25 mg of rofecoxib (Vioxx; Merck, Whitehouse Station, NJ) daily ($N = 1257$) or placebo ($N = 1299$) for 3 years [99]. A 25 % reduction in adenoma recurrence was found in the treatment group [100]. The Adenoma Prevention with Celecoxib (APC) trial included 2026 patients, with randomization to either placebo or celecoxib (200 or 400 mg twice daily). Follow-up at 3 years disclosed a significant reduction in polyp recurrence ($P = .0001$) [101].

The Prevention of Sporadic Adenomatous Polyps (PreSAP) trial recruited 1561 patients that were randomized (3:2) to receive either 400 mg celecoxib or placebo daily. The adenoma recurrence rate was 33 % in the celecoxib group versus 49.3 % in the placebo group ($P = .0001$) [14]. Of note, in all these studies, a greater effect was observed in advanced adenomas [14, 99, 101]. Although all three trials clearly showed that selective COX-2 inhibitors reduced polyp recurrence, in the APPROVe and the APC studies, this efficacy was associated with an increased risk of cardiovascular events (mainly myocardial infarction, stroke, and heart failure). In September 2004, Merck dramatically announced the early termination of the APPROVe study. Rofecoxib was subsequently withdrawn from the market due to increased cardiovascular toxicity in patients receiving the drug for more than 18 months. A total of 46 patients in the rofecoxib group had a confirmed thrombotic event compared with 26 patients in the placebo group (RR, 1.92) [14]. In December 2004, the National Cancer Institute suspended the APC trial. The study was stopped because the analysis by an independent cardiovascular adjudication committee showed a significant dose–response excess of major cardiovascular events of 2.5 (95 % CI, 1.0–6.4) and 3.5 (95 % CI, 1.4–8.5) for the celecoxib 200 and 400 mg twice daily groups compared with the placebo group [102]. At the same time, in the PreSAP trial, the RR of the celecoxib 400 mg once daily group compared with the placebo group was nonsignificant at 1.3 (95 % CI, 0.6–2.6) [14]. A secondary analysis of the APPROVe study also found that patients assigned to rofecoxib had a higher incidence of confirmed peptic ulcer bleedings than those randomized to placebo (0.88 vs. 0.18 events per 100 patient-years; relative risk, 4.9; 95 % confidence interval, 1.98–14.54) [103]. The incidence of all confirmed complicated peptic disease including ulcer perforation,

obstruction, or bleeding was low, but was numerically higher in the rofecoxib than in the placebo group (0.23 vs. .06 events per 100 patient-years; relative risk, 3.8; 95 % confidence interval, .72–37.46; $P = .14$).

Short-term use of COX-2 inhibitors appears to be safe, while long-term use necessary for achieving the goals of chemoprevention confers significant hazards. Determining specific subpopulations of individuals who may benefit from these drugs vs. populations who are at increased risk for these side effects remains an important and open question. As a result, the COX-2 inhibitors are not recommended for CRC prevention. The goal of future studies is to develop ways of blocking COX-2 activities without disrupting the cardiovascular system and protecting the gastric mucosa [104].

Recent studies have investigated polymorphisms in the COX_2 gene and their relevance to disease risk [105–109]. No non-synonymous single-nucleotide polymorphisms (SNPs) in Caucasians have been found. In contrast, V511A, a rare COX-2 polymorphism, is found in ~5 % allele frequency in African-Americans. This allele might decrease the risk of CRC adenoma and CRC cancer [110]. More common polymorphism of COX-2 (–765G>C) (dbSNP rs20417) has been under extensive research. It is associated with lower expression level and with lower serum concentrations in the serum of the C-reactive protein in patients after coronary bypass surgery [111]. In addition, it has been shown that individuals carrying the variant –765CC genotype (about 3 % of the population) have a reduced risk of developing colorectal adenoma and hyperplastic polyps [109]. Crucially, the chemopreventive effects of NSAIDs on colorectal polyps were mostly among individuals carrying the wild-type (GG) genotype. These findings indicate that NSAID might not be useful for colorectal adenoma prevention among genetically defined subgroups, who already bear reduced expression levels of COX-2. So this supports a functional role of this variant that is relevant to the pharmacogenetics of NSAIDs and coxibs [26].

Further studies investigating the genetic polymorphisms in r enzymes that are associated with eicosanoid synthesis and the pharmacokinetics of NSAIDs are underway.

COX-Independent Effect of NSAIDs on the Prevention

Numerous studies challenge the theory that COX inhibition is solely responsible for the chemopreventive action of NSAIDs by providing evidence that these effects can be exerted, at least partially, through COX-independent mechanisms. The nonselective COX inhibitor indomethacin has much lower antiproliferative activity compared with sulindac sulfide despite having a similar chemical scaffold and an approximately tenfold lower IC_{50} to inhibit both COX-1 and COX-2 in the whole blood COX assays [112]. Similarly, while selective COX-2 inhibitors celecoxib and rofecoxib inhibit COX-2 with similar IC_{50} values, celecoxib has much higher antiproliferative activity in both COX-2-positive and COX-2-negative cell lines [113]. Other studies confirm these findings through the use of genetic methods by showing that (1) tumor cells in which the expression of COX-2 has been knocked down by antisense cDNA do not display increased apoptosis but remain sensitive to COX-2 inhibitors, (2) the level of knockdown does not affect sensitivity to COX inhibitors, and (3) fibroblasts from COX-$1^{-/-}$, COX-$2^{-/-}$, or COX-$1/2^{-/-}$knockout mice retain sensitivity to various NSAIDs [114–116].

One example of a chemopreventive agent that lacks COX inhibition is exisulind.

Exisulind, a selective apoptotic antineoplastic drug, was investigated for the treatment of a variety of malignancies including colon cancer [117]. In contrast with the parental sulindac, exisulind lacks antiprostaglandin synthetase activity and as such has no influence on levels of PGE_2 [118]. Despite its lack of effect on cyclooxygenases, exisulind inhibits cellular growth in vitro and prevents chemically induced

neoplasia in vivo [119]. The antineoplastic effects of exisulind may be due to the inhibition of cyclic guanosine monophosphate phosphodiesterase, with subsequent activation of protein kinase G, resulting in the induction of apoptosis [30]. In a phase 1 clinical trial [120–122] involving 18 FAP patients, daily administration of exisulind 600 mg, over a period of six months, produced 56 % regression of exophytic polyps. Seventeen of the 18 patients were maintained on exisulind for 24 months with continued clinical response [120]. In a study published in 2006, we studied 155 individuals with FAP who received exisulind 200 or 400 mg or placebo daily for 12 months. The decrease in median polyp size was significantly greater ($P = 0.03$) in patients who received exisulind 400 mg compared with those who received placebo, and complete or partial response was significantly higher in the exisulind 400 mg group (54.6 %) compared with the placebo group (30.2 %). In another trial of FAP patients after subtotal colectomy, exisulind (600 mg/day) significantly decreased new polyp formation by 25 % over 12 months and by an additional 54 % over 24 months [123, 124]. At the same time, however, treatment with exisulind was associated with elevated risk of toxicity. The higher dosage of exisulind was less tolerated with a significant increase in abdominal pain, hepatic transaminase elevations, and biliary events which had been previously described in FAP patients and also in prostate cancer patients treated with exisulind [117, 121]. One should mention that although toxicity was more common in treated patients, transaminase elevations that typically occurred early in the therapy were mild to moderate in intensity and resolved in many cases despite continued treatment. However, a chemopreventive agent drugs must have a very low profile of side effects as treatment will continue over many years.

Conclusions

After summarizing the benefit and risks of major NSAIDs in CRC prevention, we conclude that NSAIDs are useful chemopreventive agents. They have been shown in vitro and in vivo as well as in epidemiological and case–controlled studies to inhibit polyp formation and progression in the high-risk as well as medium-risk population (prior history of sporadic adenomas). However at the same time, one should keep in mind that the vast majority of subjects with CRC (~70 %) are considered average risk. In this population, the effect of NSAIDs as chemopreventive agents is less convincing, and COX-2 inhibitors have been found to carry major adverse effects limiting their use.

The most studied agent by far has been aspirin. Randomized trials have provided compelling evidence of a causal relationship between aspirin usage and colorectal neoplasia. Nonetheless, prospective data on long-term risk of aspirin according to the dose or duration of therapy remain limited.

The data consistently show that the rates of colorectal neoplasia are significantly lower in aspirin users than in nonusers. However, the balance of risks and benefits does not make aspirin suitable for primary prevention in all average-risk populations. Rather, its use should be considered in several groups with an increased risk of CRC (e.g., patients with a personal or a family history of colorectal neoplasia) (Table 15.1). All of these subjects should be offered surveillance colonoscopy but with the increasing recognition of lesion miss rate; during colonoscopy, aspirin may be used to decrease this miss rate.

Randomized studies of aspirin as adjuvant therapy in patients with CRC are expected in the upcoming years.

Patients with an annual risk for coronary heart disease of 1.5 % are advised to take aspirin to prevent cardiovascular mortality [74]; these patients in addition to patients aged 50–59 with a general risk of over 10 % for CVD should be prescribed low-dose aspirin as these patients will also benefit from the decrease in the incidence of colorectal adenomas and CRC mortality. However at the mean time, in the average-risk population, aspirin should not be subscribed solely for the prevention as long-time risk might outweigh its benefit.

Table 15.1 Key points balancing risks and benefits for the use of aspirin or NSAIDs in colorectal cancer prevention

1. NSAIDs, especially coxibs, have been tested in the prevention of colorectal adenomas
2. Aspirin use has been shown to reduce the incidence or recurrence of adenomas and incidence and mortality of colon cancer
3. Benefits have to be balanced against risks such as GI bleeding and CV events
4. Sulindac, celecoxib, and aspirin have been shown to be effective in high-risk patients with hereditary CRC syndromes
5. Sulindac and celecoxib have been effective in FAP. Aspirin in HNPCC
6. Aspirin seems the only agent that can be used in CRC average-risk population
7. Aspirin dose should be low but at least 75 mg/day
8. Most health or professional organizations do not recommend aspirin for the primary prevention in average-risk adults
9. The American Gastroenterological Association recommends that aspirin can be considered for patients with
(a) A personal history of CRC
(b) Advanced colorectal adenoma
(c) A strong family history
(d) But not for people with a history of peptic ulcer disease or hemorrhagic stroke
10. The USPSTF suggests high or moderate certainty that the net benefit is moderate to substantial for the use of low-dose aspirin for chronic disease prophylaxis, including the prevention of CRC, for US adults between 50–59 years of age with more than 10 % of 10-year risk of cardiovascular events
11. Aspirin can be used in average- or high-risk populations together with endoscopy screening
(a) Aspirin can be of special help to reduce the risk in the right colon, where endoscopy fails more to detect lesions
12. Biomarkers are a promising tool to detect susceptible patients that may benefit patients from taking aspirin
13. Genetic testing of targeted SNPs linked to CRC risk or susceptible to aspirin or NSAIDs may also help to detect patients that may benefit with high-intensity endoscopy screening or chemoprevention therapy with ASA or NSAIDs

References

1. Jemal A, et al. Cancer statistics, 2007. CA Cancer J Clin. 2007;57(1):43–66.
2. Seeff LC, Shapiro JA, Nadel MR. Are we doing enough to screen for colorectal cancer? Findings from the 1999 behavioral risk factor surveillance system. J Fam Pract. 2002;51(9):761–6.
3. Hong WK, et al. Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. N Engl J Med. 1990;323 (12):795–801.
4. Rao CV, et al. Chemoprevention of colon carcinogenesis by sulindac, a nonsteroidal anti-inflammatory agent. Cancer Res. 1995;55 (7):1464–72.
5. Rao CV, Reddy BS. NSAIDs and chemoprevention. Curr Cancer Drug Targets. 2004;4(1):29–42.
6. Oshima M, et al. Chemoprevention of intestinal polyposis in the ApcΔ716 mouse by rofecoxib, a specific cyclooxygenase-2 inhibitor. Cancer Res. 2001;61(4):1733–40.
7. Jaffe BM. Prostaglandins and cancer: an update. Prostaglandins. 1974;6(6):453–61.
8. Waddell WR, Loughry RW. Sulindac for polyposis of the colon. J Surg Oncol. 1983;24(1):83–7.
9. Dubé C, et al. The use of aspirin for primary prevention of colorectal cancer: a systematic review prepared for the U.S. Preventive Services Task Force. Ann Intern Med. 2007;146(5):365–75.
10. Bertagnolli MM. Chemoprevention of colorectal cancer with cyclooxygenase-2 inhibitors: two steps forward, one step back. Lancet Oncol. 2007;8 (5):439–43.
11. Arber N. Do NSAIDs prevent colorectal cancer? Can J Gastroenterol. 2000;14(4):299–307.
12. Bennett A, Del Tacca M. Proceedings: prostaglandins in human colonic carcinoma. Gut. 1975;16(5):409.
13. Agarwal B, et al. Lovastatin augments sulindac-induced apoptosis in colon cancer cells and potentiates chemopreventive effects of sulindac. Gastroenterology. 1999;117(4):838–47.
14. Arber N, et al. Celecoxib for the prevention of colorectal adenomatous polyps. N Engl J Med. 2006;355 (9):885–95.
15. Arber N. Cyclooxygenase-2 inhibitors in colorectal cancer prevention: point. Cancer Epidemiol Biomarkers Prev. 2008;17(8):1852–7.
16. Arber N, Levin B. Chemoprevention of colorectal neoplasia: the potential for personalized medicine. Gastroenterology. 2008;134(4):1224–37.

17. Arber N, et al. Five-year analysis of the Prevention of Colorectal Sporadic Adenomatous Polyps Trial. Am J Gastroenterol. 2011;106(6):1135–46.
18. Macarthur M, et al. The role of cytokine gene polymorphisms in colorectal cancer and their interaction with aspirin use in the northeast of Scotland. Cancer Epidemiol Biomarkers Prev. 2005;14 (7):1613–8.
19. Mamdani M, et al. Cyclo-oxygenase-2 inhibitors versus non-selective non-steroidal anti-inflammatory drugs and congestive heart failure outcomes in elderly patients: a population-based cohort study. Lancet. 2004;363(9423):1751–6.
20. Meyskens Jr FL, et al. Difluoromethylornithine plus sulindac for the prevention of sporadic colorectal adenomas: a randomized placebo-controlled, double-blind trial. Cancer Prev Res (Phila). 2008;1 (1):32–8.
21. Pereira C, et al. COX-2 polymorphisms and colorectal cancer risk: a strategy for chemoprevention. Eur J Gastroenterol Hepatol. 2010;22(5):607–13.
22. Reddy BS, et al. Prevention of azoxymethane-induced colon cancer by combination of low doses of atorvastatin, aspirin, and celecoxib in F 344 rats. Cancer Res. 2006;66(8):4542–6.
23. Sheehan KM, et al. THe relationship between cyclooxygenase-2 expression and colorectal cancer. JAMA. 1999;282(13):1254–7.
24. Sinicrope FA. Targeting cyclooxygenase-2 for prevention and therapy of colorectal cancer. Mol Carcinog. 2006;45(6):447–54.
25. Tuynman JB, Peppelenbosch MP, Richel DJ. COX-2 inhibition as a tool to treat and prevent colorectal cancer. Crit Rev Oncol Hematol. 2004;52 (2):81–101.
26. Ulrich CM, Bigler J, Potter JD. Non-steroidal anti-inflammatory drugs for cancer prevention: promise, perils and pharmacogenetics. Nat Rev Cancer. 2006;6(2):130–40.
27. Tinsley HN, et al. Colon tumor cell growth-inhibitory activity of sulindac sulfide and other non-steroidal anti-inflammatory drugs is associated with phosphodiesterase 5 inhibition. Cancer Prev Res (Phila). 2010;3(10):1303–13.
28. Piazza GA, et al. NSAIDs: old drugs reveal new anticancer targets. Pharmaceuticals. 2010;3(5):1652.
29. Soh JW, et al. Celecoxib-induced growth inhibition in SW480 colon cancer cells is associated with activation of protein kinase G. Mol Carcinog. 2008;47 (7):519–25.
30. Thompson WJ, et al. Exisulind induction of apoptosis involves guanosine 3',5'-cyclic monophosphate phosphodiesterase inhibition, protein kinase G activation, and attenuated beta-catenin. Cancer Res. 2000;60(13):3338–42.
31. Kune GA, Kune S, Watson LF. Colorectal cancer risk, chronic illnesses, operations, and medications: case control results from the Melbourne Colorectal Cancer Study. Cancer Res. 1988;48(15):4399–404.
32. Thun MJ, Jacobs EJ, Patrono C. The role of aspirin in cancer prevention. Nature reviews. Clin Oncol. 2012;9(5):259–67.
33. Alfonso L, et al. Molecular targets of aspirin and cancer prevention. Br J Cancer. 2014;111(1):61–7.
34. Pathi S, et al. Aspirin inhibits colon cancer cell and tumor growth and downregulates specificity protein (Sp) transcription factors. PLoS One. 2012;7(10), e48208.
35. Bellosillo B, et al. Aspirin and salicylate induce apoptosis and activation of caspases in B-cell chronic lymphocytic leukemia cells. Blood. 1998; 92(4):1406–14.
36. Zimmermann KC, et al. Aspirin induces apoptosis through release of cytochrome c from mitochondria. Neoplasia. 2000;2(6):505–13.
37. Dikshit P, et al. Aspirin induces apoptosis through the inhibition of proteasome function. J Biol Chem. 2006;281(39):29228–35.
38. Willett WC, et al. Dietary fat and the risk of breast cancer. N Engl J Med. 1987;316(1):22–8.
39. Chan AT, et al. Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. JAMA. 2005;294(8):914–23.
40. Chan AT, Manson JE, Feskanich D, Stampfer MJ, Colditz GA, Fuchs CS. Long-term aspirin use and mortality in women. Arch Intern Med. 2007;167 (6):562–72.
41. Giovannucci E, et al. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann Intern Med. 1994;121(4):241–6.
42. Jacobs EJ, et al. A large cohort study of long-term daily use of adult-strength aspirin and cancer incidence. J Natl Cancer Inst. 2007;99(8):608–15.
43. Cook NR, et al. Low-dose aspirin in the primary prevention of cancer: the women's health study: a randomized controlled trial. JAMA. 2005;294 (1):47–55.
44. Gann PH, et al. Low-dose aspirin and incidence of colorectal tumors in a randomized trial. J Natl Cancer Inst. 1993;85(15):1220–4.
45. Peto R, et al. Randomised trial of prophylactic daily aspirin in British male doctors. BMJ. 1988;296 (6618):313–6.
46. Farrell B, et al. The United Kingdom transient ischaemic attack (UK-TIA) aspirin trial: final results. J Neurol Neurosurg Psychiatry. 1991;54(12): 1044–54.
47 Chan AT, et al. Aspirin dose and duration of use and risk of colorectal cancer in men. Gastroenterology. 2008;134(1):21–8.
48. Chan AT, et al. A prospective study of aspirin use and the risk for colorectal adenoma. Ann Intern Med. 2004;140(3):157–66.
49. Sandler RS, et al. A randomized trial of aspirin to prevent colorectal adenomas in patients with previous colorectal cancer. N Engl J Med. 2003;348 (10):883–90.

50. Baron JA, et al. A randomized trial of aspirin to prevent colorectal adenomas. N Engl J Med. 2003;348(10):891–9.
51. Benamouzig R, et al. Daily soluble aspirin and prevention of colorectal adenoma recurrence: one-year results of the APACC trial. Gastroenterology. 2003; 125(2):328–36.
52. Benamouzig R, Uzzan B, Deyra J, Martin A, Girard B, Little J, Chaussade S, Association pour la Prévention par l'Aspirine du Cancer Colorectal Study Group (APACC). Prevention by daily soluble aspirin of colorectal adenoma recurrence: 4-year results of the APACC randomised trial. Gut. 2012;61(2):255–61.
53. Asano TK, McLeod RS. Non steroidal anti-inflammatory drugs (NSAID) and Aspirin for preventing colorectal adenomas and carcinomas. Cochrane Database Syst Rev. 2004;2, CD004079.
54. Chubak J, Kamineni A, Buist DSM, Anderson LA, Whitlock EP. Aspirin Use for the Prevention of Colorectal Cancer: An Updated Systematic Evidence Review for the U.S. Preventive Services Task Force [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2015 Sep. (Evidence Syntheses, No. 133.) Available from: http://www.ncbi.nlm.nih.gov/books/NBK321661/.
55. Steinbach G, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med. 2000;342(26):1946–52.
56. Bertagnolli MM, et al. Celecoxib for the prevention of sporadic colorectal adenomas. N Engl J Med. 2006;355(9):873–84.
57. Chan AT, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. JAMA. 2009;302(6):649–58.
58. Samuels Y, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004;304 (5670):554.
59. Kaur J, Sanyal SN. PI3-kinase/Wnt association mediates COX-2/PGE(2) pathway to inhibit apoptosis in early stages of colon carcinogenesis: chemoprevention by diclofenac. Tumour Biol. 2010;31 (6):623–31.
60. Uddin S, et al. Cyclooxygenase-2 inhibition inhibits PI3K/AKT kinase activity in epithelial ovarian cancer. Int J Cancer. 2010;126(2):382–94.
61. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007;129(7):1261–74.
62. Liao X, et al. Prognostic role of PIK3CA mutation in colorectal cancer: cohort study and literature review. Clin Cancer Res. 2012;18(8):2257–68.
63. Mao C, et al. PIK3CA exon 20 mutations as a potential biomarker for resistance to anti-EGFR monoclonal antibodies in KRAS wild-type metastatic colorectal cancer: a systematic review and meta-analysis. Ann Oncol. 2012;23(6):1518–25.
64. Barault L, et al. Mutations in the RAS-MAPK, PI(3)K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers. Int J Cancer. 2008;122 (10):2255–9.
65. Domingo E, et al. Evaluation of PIK3CA mutation as a predictor of benefit from nonsteroidal anti-inflammatory drug therapy in colorectal cancer. J Clin Oncol Off J Am Soc Clin Oncol. 2013;31 (34):4297–305.
66. Kim B, Giardiello FM. Chemoprevention in familial adenomatous polyposis. Best practice & research. Clin Gastroenterol. 2011;25:607–22.
67. Li P, et al. Aspirin use after diagnosis but not prediagnosis improves established colorectal cancer survival: a meta-analysis. Gut. 2015;64(9):1419–25.
68. Burn J, et al. A randomized placebo-controlled prevention trial of aspirin and/or resistant starch in young people with familial adenomatous polyposis. Cancer Prev Res (Phila). 2011;4(5):655–65.
69. Ishikawa H, et al. Chemoprevention of colorectal cancer in Japan: a brief introduction to current clinical trials. J Gastroenterol. 2009;44 Suppl 19:77–81.
70. Dowty JG, et al. Cancer risks for MLH1 and MSH2 mutation carriers. Hum Mutat. 2013;34(3):490–7.
71. Burn J, et al. Effect of aspirin or resistant starch on colorectal neoplasia in the lynch syndrome. N Engl J Med. 2008;359(24):2567–78.
72. Burn J, et al. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet. 2011;378(9809):2081–7.
73. Garcia-Albeniz X, Chan AT. Aspirin for the prevention of colorectal cancer. Best practice & research. Clin Gastroenterol. 2011;25:461–72.
74. Guirguis-Blake JM, Evans CV, Senger CA, et al. Aspirin for the primary prevention of cardiovascular events: a systematic evidence review for The U.S. Preventive Services Task Force [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2015 Sep. (Evidence Syntheses, No. 131.) [Preface] http://www.ncbi.nlm.nih.gov/books/NBK321620/.
75. Leshno M, Moshkowitz M, Arber N. Point/counterpoint: aspirin is clinically effective in chemoprevention of colorectal neoplasia: point. Cancer Epidemiol Biomarkers Prev. 2008;17(7):1558–61.
76. Ridker PM, et al. A randomized trial of low-dose aspirin in the primary prevention of cardiovascular disease in women. N Engl J Med. 2005;352 (13):1293–304.
77. Final Report on the Aspirin Component of the Ongoing Physicians' Health Study. N Engl J Med, 1989;321(3):129–35.
78. Rothwell PM, et al. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. Lancet. 2010;376(9754):1741–50.
79. Cole BF, et al. Aspirin for the chemoprevention of colorectal adenomas: meta-analysis of the randomized trials. J Natl Cancer Inst. 2009;101 (4):256–66.

80. Flossmann E, Rothwell PM, British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet. 2007;369(9573):1603–13.
81. Mahipal A, et al. Nonsteroidal anti-inflammatory drugs and subsite-specific colorectal cancer incidence in the Iowa Women's Health Study. Cancer Epidemiol Biomarkers Prev. 2006;15(10):1785–90.
82. Colorectal Cancer: Can colorectal cancer be prevented? Atlanta, GA: American Cancer Society; 2013. [updated January 17, 2013; cited April 23 2013]; http://www.cancer.org/cancer/colonandrectumcancer/detailedguide/colorectal-cancer-prevention. 2013.
83. U.S. Preventive Services Task Force. USPSTF A and B Recommendations. Rockville, MD: U.S. Preventive Services Task Force; 2010. [updated August 2010; cited April 23 2013]; http://www.ama-assn.org/resources/doc/cpt/aca-uspstf-a-and-b-recs.pdf.
84. U.S. Preventive Services Task Force. Aspirin and NSAIDS for colorectal cancer prevention. American College of Physicians; 2012. [updated 2007; cited April 23 2013]; http://www.acponline.org/mobile/cyppocketguide/aspirin_nsaids_colorectal_cancer_prevention.html.
85. Chiu LC, Tong KF, Ooi VE. Cytostatic and cytotoxic effects of cyclooxygenase inhibitors and their synergy with docosahexaenoic acid on the growth of human skin melanoma A-375 cells. Biomed Pharmacother. 2005;59 Suppl 2:S293–7.
86. National Institute for Health and Care Excellence. Published clinical guidelines. London: National Institute for Health and Care Excellence; 2013. [updated April 25, 2013; cited April 25 2013]; http://guidance.nice.org.uk/CG/Published.
87. Lynch PM. Does regular use of aspirin reduce the risk of colorectal cancer? Nature clinical practice. Oncology. 2006;3(4):186–7.
88. Winawer SJ, Zauber AG, Fletcher RH, Stillman JS, O'Brien MJ, Levin B, Smith RA, Lieberman DA, Burt RW, Levin TR, Bond JH, Brooks D, Byers T, Hyman N, Kirk L, Thorson A, Simmang C, Johnson D, Rex DK, US Multi-Society Task Force on Colorectal Cancer, American Cancer Society. Guidelines for colonoscopy surveillance after polypectomy: a consensus update by the US Multi-Society Task Force on Colorectal Cancer and the American Cancer Society. Gastroenterology. 2006;130(6):1872–85.
89. Wilcox CM, et al. Consensus development conference on the use of nonsteroidal anti-inflammatory agents, including cyclooxygenase-2 enzyme inhibitors and aspirin. Clin Gastroenterol Hepatol. 2006;4(9):1082–9.
90. National Comprehensive Cancer Network. NCCN guidelines for detection, prevention & risk reduction: colorectal cancer. Fort Washington: National Comprehensive Cancer Network; 2013. [cited April 25 2013]; http://www.nccn.org/professionals/physician_gls/pdf/colorectal_screening.pdf.
91. Dehmer SP, Maciosek MV, Flottemesch TJ. Aspirin Use to Prevent Cardiovascular Disease and Colorectal Cancer: A Decision Analysis: Technical Report [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2015 Sep. (Evidence Syntheses, No. 131s.) Available from: http://www.ncbi.nlm.nih.gov/books/NBK321651/.
92. Chan AT, Ladabaum U. Where do we stand with aspirin for the prevention of colorectal cancer? The USPSTF Recommendations. Gastroenterology. 2016;150(1):14–8.
93. Giardiello FM, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med. 1993;328(18):1313–6.
94. Labayle D, et al. Sulindac causes regression of rectal polyps in familial adenomatous polyposis. Gastroenterology. 1991;101(3):635–9.
95. Spagnesi MT, et al. Rectal proliferation and polyp occurrence in patients with familial adenomatous polyposis after sulindac treatment. Gastroenterology. 1994;106(2):362–6.
96. Logan RFA, et al. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. Gastroenterology. 2008;134(1):29–38.
97. Eberhart CE, et al. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology. 1994;107(4):1183–8.
98. Groves CJ, et al. Duodenal cancer in patients with familial adenomatous polyposis (FAP): results of a 10 year prospective study. Gut. 2002;50(5):636–41.
99. Bresalier RS, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med. 2005;352(11):1092–102.
100. Bertagnolli MM, Hawk ET. Celecoxib reduces sporadic colorectal adenomas: result from the Adenoma Prevention with Celecoxib Trial. Proc Am Assoc Cancer Res. 2006;47(Abstract CP-3).
101. Arber N. Chemoprevention of colorectal adenomas with celecoxib in an international randomized, placebo-controlled doubleblind trial. Proc Am Assoc Cancer Res. 2006;47 (Abstract CP-4).
102. Solomon SD, et al. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med. 2005;352(11):1071–80.
103. Half EE, Arber N. Chemoprevention of colorectal cancer: two steps forward, one step back? Future Oncol. 2006;2(6):697–704.
104. Zhen X-E, et al. Preparation and characterization of a novel aspirin derivative with anti-thrombotic and gastric mucosal protection properties. PLoS One. 2014;9(6), e98513.
105. Cipollone F, et al. A polymorphism in the cyclooxygenase 2 gene as an inherited protective factor

against myocardial infarction and stroke. JAMA. 2004;291(18):2221–8.
106. Campa D, et al. Association of a common polymorphism in the cyclooxygenase 2 gene with risk of non-small cell lung cancer. Carcinogenesis. 2004;25(2):229–35.
107. Koh WP, et al. Interaction between cyclooxygenase-2 gene polymorphism and dietary n-6 polyunsaturated fatty acids on colon cancer risk: The Singapore Chinese Health Study. Br J Cancer. 2004;90(9):1760–4.
108. Cox DG, et al. Polymorphisms in prostaglandin synthase 2//cyclooxygenase 2 (PTGS2//COX2) and risk of colorectal cancer. Br J Cancer. 2004;91 (2):339–43.
109. Ulrich CM, et al. PTGS2 (COX-2) −765G > C promoter variant reduces risk of colorectal adenoma among nonusers of nonsteroidal anti-inflammatory drugs. Cancer Epidemiol Biomarkers Prev. 2005;14 (3):616–9.
110. Lin HJ, et al. Prostaglandin H synthase 2 variant (Val511Ala) in African Americans may reduce the risk for colorectal neoplasia. Cancer Epidemiol Biomarkers Prev. 2002;11(11):1305–15.
111. Papafili A, et al. Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. Arterioscler Thromb Vasc Biol. 2002;22 (10):1631–6.
112. Brideau C, et al. A human whole blood assay for clinical evaluation of biochemical efficacy of cyclooxygenase inhibitors. Inflamm Res. 1996;45 (2):68–74.
113. Waskewich C, et al. Celecoxib exhibits the greatest potency amongst cyclooxygenase (COX) inhibitors for growth inhibition of COX-2-negative hematopoietic and epithelial cell lines. Cancer Res. 2002;62(7):2029–33.
114. Song X, et al. Cyclooxygenase-2, player or spectator in cyclooxygenase-2 inhibitor-induced apoptosis in prostate cancer cells. J Natl Cancer Inst. 2002;94 (8):585–91.
115. Zhang X, et al. Malignant transformation and antineoplastic actions of nonsteroidal antiinflammatory drugs (nsaids) on cyclooxygenase-null embryo fibroblasts. J Exp Med. 1999;190(4):451–60.
116. Williams CS, et al. Celecoxib prevents tumor growth in vivo without toxicity to normal gut: lack of correlation between in vitro and in vivo models. Cancer Res. 2000;60(21):6045–51.
117. Goluboff ET. Exisulind, a selective apoptotic antineoplastic drug. Expert Opin Investig Drugs. 2001;10(10):1875–82.
118. Piazza GA, et al. Apoptosis primarily accounts for the growth-inhibitory properties of sulindac metabolites and involves a mechanism that is independent of cyclooxygenase inhibition, cell cycle arrest, and p53 induction. Cancer Res. 1997;57(12):2452–9.
119. Piazza GA, et al. Sulindac sulfone inhibits azoxymethane-induced colon carcinogenesis in rats without reducing prostaglandin levels. Cancer Res. 1997;57(14):2909–15.
120. Burke C. The effect of exisulind on rectal adenomas in adults with familial adenomatous polyposis. Cancer Invest. 2000;19:1.
121. van Stolk R, et al. Phase I trial of exisulind (sulindac sulfone, FGN-1) as a chemopreventive agent in patients with familial adenomatous polyposis. Clin Cancer Res. 2000;6(1):78–89.
122. Piazza G A, Xu S, Klein-Szanto et al Overexpression of cGMP phosphodiesterase (cG-PDE) in colonic neoplasias compared to normal mucosa. Gastroenterology 2000;1181590.
123. Burke C, Van Stolk R, Arber N. et al. Exisulind prevents adenoma formation in familial adenomatous polyposis (FAP). Gastroenterology 2000;118 (4, Part 1):A657
124. Phillips R, Hultcrantz R, Bjork J. et al Exisulind, a pro-apoptotic drug, prevents new adenoma formation in patients with familial adenomatous polyposis. Gut 2000;47A2–A3.A3.

Index

A. Lanas (ed.), *NSAIDs and Aspirin*, DOI 10.1007/978-3-319-33889-7