Clinical Effects of NSAIDs and COXIBs in Colon Cancer Prevention

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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.

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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].

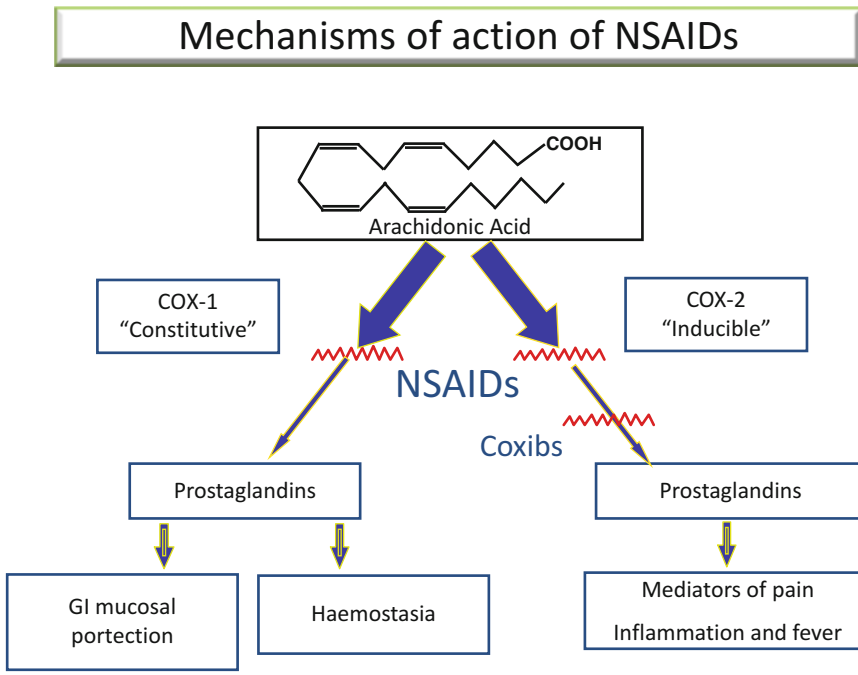

Fig. 13.1 Mechanisms of action of NSAIDs

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

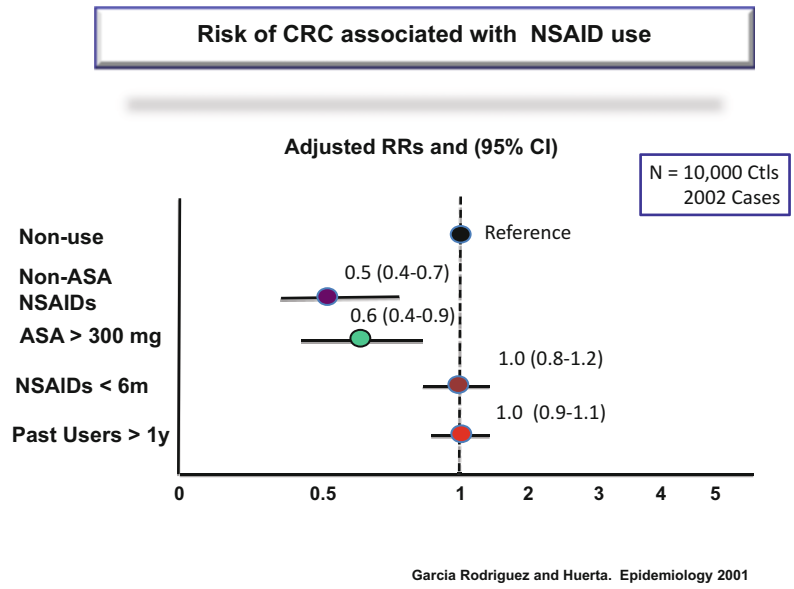

Fig. 13.2 Risk of CRC associated with NSAID use

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].

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