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Inflammation Research

Anti-inflammatory drugs and their mechanism of action

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Abstract. Nonsteroidal anti-inflammatory drugs (NSAIDs) produce their therapeutic activities through inhibition of cyclooxygenase (COX), the enzyme that makes prostaglandins (PGs). They share, to a greater or lesser degree, the same side effects, including gastric and renal toxicity. Recent research has shown that there are at least two COX isoenzymes. COX-1 is constitutive and makes PGs that protect the stomach and kidney from damage. COX-2 is induced by inflammatory stimuli, such as cytokines, and produces PGs that contribute to the pain and swelling of inflammation. Thus, selective COX-2 inhibitors should be anti-inflammatory without side effects on the kidney and stomach. Of course, selective COX-2 inhibitors may have other side effects and perhaps other therapeutic potential. For instance, COX-2 (and not COX-1) is thought to be involved in ovulation and in labor. In addition, the well-known protective action of aspirin on colon cancer may be through an action on COX-2, which is expressed in this disease. Moreover, NSAIDs delay the progress of Alzheimer's disease. Thus, selective COX-2 inhibitors may demonstrate new important therapeutic benefits as anticancer agents, as well as in preventing premature labor and perhaps even retarding the progression of Alzheimer's disease.

Key words: Anti-inflammatory drugs – Mechanism – Cyclooxygenase-1 (COX-1) – Cyclooxygenase-2 (COX-2) – Nonsteroidal anti-inflammatory drugs (NSAIDs) – Therapeutic use

Introduction

Early history

About 3,500 years ago in ancient Egypt, the Ebers papyrus recommended the application of a decoction of the dried leaves of myrtle to the abdomen and back to expel rheumatic pains from the womb. A thousand years later, Hippocrates recommended the juices of the poplar tree for treating eye diseases, and those of willow bark to relieve the pain of childbirth and to reduce fever. All of these medicinal remedies contain salicylates.

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In A.D. 30, Celsus described the 4 classic signs of inflammation (rubor, calor, dolor, and tumor, or redness, heat, pain, and swelling), and used extracts of willow leaves to relieve them. Throughout the Roman times of Pliny the Elder, Dioscorides, and Galen, the use of salicylate-containing plants was further developed, and willow bark was recommended for mild to moderate pain. In China and other parts of Asia also, salicylate-containing plants were being applied therapeutically. The curative effects of *Salix* and *Spirea* species were even known to the early inhabitants of North America and South Africa.

Through the Middle Ages, further uses for salicylates were found, such as plasters to treat wounds and various other external and internal applications, including the treatment of menstrual pain and the discomfort of dysentery. However, willows were needed for basket making, so the women herbalists of those days turned to other related plants. For instance, they grew meadowsweet (*Spirea ulmaria*) in their herb gardens, and made decoctions from the flowers.

Willow bark

The first 'clinical trial' of willow bark to be published in England was made by a country parson, the Reverend Edward Stone of Chipping Norton in Oxfordshire [1]. On June 2, 1763, Edward Stone read a report to the Royal Society on the use of willow bark in fever. He had accidentally tasted it, and was surprised by its extraordinary bitterness, which reminded him of the taste of cinchona bark (containing quinine), then being used to treat malaria. He believed in the 'doctrine of signatures', which dictated that the cures for diseases would be found in the same locations where the malady occurs. Since the 'willow delights in a moist and wet soil, where agues chiefly abound', he gathered a pound of willow bark, dried it over a baker's oven for 3 months, then ground it to a powder. His greatest success was with doses of 1 dram (1.8 g), which he reported using in about 50 patients, with safety and success.

He concluded his paper by saying, 'I have no other motives for publishing this valuable specific, than that it may have a fair and full trial in all its variety of circumstances and situations, and that the world may reap the benefits accruing from it'. His wishes have certainly been realized. World

production of aspirin has been estimated at 45 thousand tons a year, with an average consumption of about 80 tablets per person per year. Without the discovery in recent years of a great many replacements for aspirin and its variants, consumption would have surely been very much higher.

Synthetic salicylates

Salicylic acid was synthesized chemically in Germany in 1859, and its ready supply led to even more extended usage as an external antiseptic, as an antipyretic, and in the treatment of rheumatism. The father of Felix Hoffman, a young chemist working for Bayer, urged his son to make a more palatable form of salicylate to treat his severe rheumatism. Felix made acetylsalicylate or aspirin, and asked his father to try it. Bayer's Research Director, Dr. Heinrich Dreser, recognized that he had an important new drug on his hands and introduced it in 1899, at the same time writing a paper, suggesting that aspirin was a convenient way of supplying the body with the active substance, salicylate [2]. This point is still debated, but most of the evidence now shows that aspirin works in its own right.

By the early 1900s, the main therapeutic actions of aspirin (and sodium salicylate itself) were recognized as its antipyretic, anti-inflammatory, and analgesic effects. With the passing of time, several other drugs were discovered that shared some or all of these actions. These drugs include antipyrine, phenacetin, acetaminophen (paracetamol), phenylbutazone, and, more recently, the fenamates, indomethacin and naproxen. Because of the similarity of their therapeutic actions, these drugs tended to be regarded as a group, and were generally known as the aspirin-like drugs. Because they were clearly distinct from the glucocorticoids (the other major group of agents used in the treatment of inflammation), these drugs were also called the 'nonsteroidal, anti-inflammatory drugs' (NSAIDs) [3].

Despite the diversity of their chemical structures, these drugs all share the same therapeutic properties. They alleviate the swelling, redness, and pain of inflammation, reduce a general fever, and cure a headache. More than that, they also share, to a greater or lesser extent, a number of similar side effects. Depending on dose, they can cause gastric upset, in high doses, they can delay the birth process, and in overdose, may damage the kidney. A particularly interesting 'side effect', now known as a therapeutic action, is the antithrombotic effect. When a chemically diverse group of drugs all share not only the same therapeutic qualities (which in themselves have not much connection with one another), but also the same side effects, it is fairly certain that the actions of those drugs are based on a single, biochemical intervention. For many years, pharmacologists and biochemists had searched for such a common mode of action, without finding a generally acceptable scientific explanation.

Salicylates and the prostaglandin system

In the late 1960s, Priscilla Piper, who had been working with Harry Collier at the Parke Davis Laboratories in Hounslow, Middlesex, came to Vane's laboratory at the Royal College of Surgeons as a graduate student. Piper and Vane employed the technique of continuous bioassay, using the cascade bioassay system [4], developed earlier by Vane for use with blood or an artificial salt solution. The method involved perfusing isolated guinea pig lungs with Krebs' solution, and using the effluent to successively superfuse strips of vascular or gastrointestinal tissues, selected for their sensitivity to different substances.

Piper and Vane found, as expected, the release of histamine and slow-reacting substance of anaphylaxis (SRS-A) during anaphylaxis. However, they also found some previously unreported substances, namely, prostaglandins (mainly prostaglandin E_2, but also with some prostaglandin $F_{2\alpha}$) [5], and another, very ephemeral, substance that, from the assay tissue that picked it up, was called 'rabbit aorta-contracting substance' (RCS). In the lung perfusate, RCS had a half-life of about 2 minutes, and was identified in 1975 as thromboxane A_2 by Samuelsson's group [6].

It was RCS that provided the first clue to the relationship between aspirin and the prostaglandins, when Piper and Vane presented experimental evidence that the release of RCS from the isolated guinea pig lungs during anaphylaxis was blocked by aspirin [7]. Indeed, almost any type of chemical or mechanical stimulus released prostaglandins, in addition to RCS. The result of these experiments was to move Vane's attention from RCS to prostaglandins, and he postulated that the various stimuli which released prostaglandins were in fact 'turning on' the synthesis of these compounds. A logical corollary was that aspirin might well be blocking their synthesis.

He tested this idea immediately, using the supernatant of a broken cell homogenate from guinea pig lung as a source of prostaglandin synthase. There was a dose-dependent inhibition of prostaglandin formation by aspirin, salicylate, and indomethacin, but not by morphine [8] (Fig. 1). Two other reports from the same laboratory, in the same issue of Nature, lent support to, and extended his finding. Smith and Willis found that aspirin prevented the release of prostaglandins from aggregating human platelets [9], and Vane,

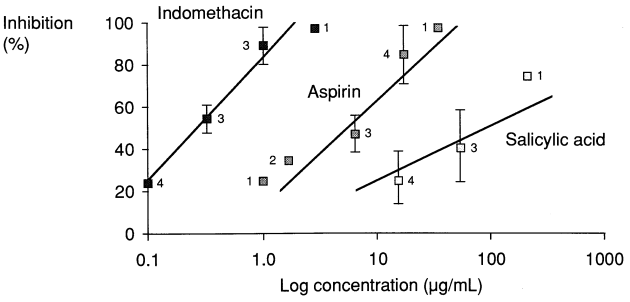

Fig. 1. Inhibition of prostaglandin biosynthesis by aspirin-like drugs in crude enzyme from guinea pig lungs. Logarithms of the concentrations of indomethacin, aspirin, and salicylate are plotted against the percentage inhibition of prostaglandin synthesis, assayed as prostaglandin $F_{2\alpha}$ on rat isolated colon. Lines are those calculated for best fit. Numbers by points indicate number of experiments. When 3 or more estimates were averaged, standard error of the mean is shown. Reprinted with permission from: Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol 1971;231:232–5, © 1998 Macmillan Magazines Ltd.

Ferreira, and Moncada demonstrated that aspirin-like drugs blocked prostaglandin release from the perfused, isolated spleen of the dog [10]. The discovery that each and every chemically diverse member of this large group of drugs all act by inhibiting the enzyme that leads to the generation of prostaglandins, which we now call 'cyclooxygenase' [8], provided a unifying explanation of their therapeutic actions, and firmly established certain prostaglandins as important mediators of inflammatory disease (for reviews, see [11–13]).

The two isoforms of COX

A homogeneous, enzymatically active cyclooxygenase (COX), or prostaglandin endoperoxide synthase (PGHS) was isolated in 1976 [14] and cloned in 1988 [15–17]. This membrane-bound haemo- and glycoprotein has a molecular weight of 71 kd, and is found in the greatest amounts in the endoplasmic reticulum of prostanoid-forming cells [18]. It exhibits cyclooxygenase activity, which both cyclizes arachidonic acid, and adds the 15-hydroperoxy group to form prostaglandin G_2. The hydroperoxy group of prostaglandin G_2 is reduced to the hydroxy group of prostaglandin H_2 by a peroxidase that uses a wide variety of compounds to provide the requisite pair of electrons. Both cyclooxygenase and hydroperoxidase activities are contained in the same dimeric protein molecule.

We now know that cyclooxygenase exists in at least 2 isoforms, COX-1 and COX-2. Over the last 2 decades, several new NSAIDs have reached the market, based on enzyme screens that turned out to be against COX-1. Garavito and his colleagues [19] have determined the 3-dimensional structure of COX-1, providing a new understanding for the actions of COX inhibitors. This bifunctional enzyme is composed of 3 independent folding units, an epidermal growth factor-like domain, a membrane-binding motive, and an enzymatic domain. The sites for peroxidase and cyclooxygenase activity are adjacent but spatially distinct. The conformation of the membrane-binding motive strongly suggests that the enzyme integrates into only a single leaflet of the lipid bilayer, and is thus a monotopic membrane protein. Three of the helices of the structure form the entrance to the cyclooxygenase channel, and their insertion into the membrane could allow arachidonic acid to gain access to the active site from the interior of the bilayer.

The cyclooxygenase active site is a long, hydrophobic channel, and Garavito and others [19] present arguments that some of the aspirin-like drugs, such as flurbiprofen, inhibit COX-1 by excluding arachidonate from the upper portion of the channel. Tyrosine 385 and serine 530 are at the apex of the long active site. Aspirin irreversibly inhibits COX-1 by acetylation of the serine 530, thereby excluding access for arachidonic acid [20]. The *S*(-) stereoisomer of flurbiprofen interacts, via its carboxylate, with arginine 120, thereby placing the second phenyl ring within Van der Waal's contact of tyrosine 385. There may be a number of other subsites for drug binding in the narrow channel.

The roentgenogram crystal structure of COX-2 closely resembles that of COX-1, and the binding sites for arachidonic acid on these enzymes are also very similar [21]. Selectivity for inhibitors may be conferred by alternative conformations at the NSAID's binding site in the COX channel. The active site of COX-2 is slightly larger, and can accommodate bigger structures than those which are able to reach the active site of COX-1. A secondary, internal pocket of COX-2 contributes significantly to the larger volume of the active site in this enzyme, although the central channel is also bigger by 17%. Selectivity for COX-2 inhibitors can be conferred by replacing the His^{513} and Ile^{523} of COX-1 with Arg and Val, respectively. This replacement removes the constriction at the mouth of the secondary side channel, and allows access for the more bulky selective COX-2 inhibitors [22].

Physiology of COX-1 and COX-2

The constitutive isoform of COX, COX-1, has clear physiological functions. Its activation leads, for instance, to the production of prostacyclin, which when released by the endothelium, is antithrombogenic [23], and when released by the gastric mucosa, is cytoprotective [24]. The inducible isoform, COX-2, was discovered some 7 years ago, and is induced in a number of cells by proinflammatory stimuli [25]. Its existence was first suspected when Needleman and his group found that bacterial lipopolysaccharide increased the synthesis of prostaglandins in human monocytes in vitro [26], and in mouse peritoneal macrophages in vivo [27]. This increase was inhibited by dexamethasone, and associated with de novo synthesis of new COX protein.

A year or so later, an inducible COX was identified as a distinct isoform of cyclooxygenase, encoded by a different gene from COX-1 [28–31]. The amino acid sequence of its cDNA shows a 60% homology with the sequence of the noninducible enzyme, with the size of the mRNA for the inducible enzyme approximating 4.5 kb, and that of the constitutive enzyme being 2.8 kb. Both enzymes have a molecular weight of 71 kd, and slightly different active sites for the natural substrate and for blockade by NSAIDs. The inhibition by the glucocorticoids of the expression of COX-2 is an additional aspect of the anti-inflammatory action of the corticosteroids. The levels of COX-2, normally very low in cells, are tightly controlled by a number of factors, including cytokines, intracellular messengers, and by the availability of substrate. Since COX-2 is induced by inflammatory stimuli and by cytokines in migratory and other cells, it is attractive to suggest that the anti-inflammatory actions of NSAIDs are due to the inhibition of COX-2, whereas the unwanted side effects, such as irritation of the stomach lining and toxic actions on the kidney, are due to inhibition of the constitutive enzyme, COX-1.

Over the years, the theory that inhibition of prostaglandin formation accounts for the therapeutic activity and the side effects of the aspirin-like drugs has been challenged, notably by Weissmann [32]. His arguments were partly based on comparing the actions of salicylate and aspirin, which are said to be equally effective against arthritis in the clinic [33], whereas in the original observations on COX [8], aspirin was 10 times stronger than salicylate as an inhibitor. As Weissmann's comparisons were based on COX-1, this apparent contradiction may now be explained by the existence of the 2 isoforms of COX, for salicylate and aspirin are both almost similarly weak inhibitors of COX-2, although the mechanism of action of salicylate may

be compounded by a suppression of the induction of COX [34].

Paracetamol also posed a problem for the original theory, for in therapeutic doses, it has analgesic and antipyretic actions, but little or no anti-inflammatory activity [35]. In 1972, Flower and Vane showed that COX preparations from the brain were more sensitive to paracetamol than those from the spleen, and suggested that there may be different isoforms of COX [36]. Perhaps in the light of recent discoveries, there is also a COX-3, on which paracetamol has a preferential action.

Functions of COX-1 and COX-2

Gastrointestinal tract

The so-called 'cytoprotective' action of prostaglandins in preventing gastric erosions and ulceration is mainly brought about by endogenously produced prostacyclin and PGE_2, which reduce gastric acid secretion, and exert a direct vasodilator action on the vessels of the gastric mucosa. In addition to these major actions, prostanoids stimulate the secretion of viscous mucus, which forms a protective barrier, as well as gastric fluid and duodenal bicarbonate [37]. In most species, including humans, the bulk of the protective prostaglandins are synthesized by COX-1, although small quantities of COX-2 have been found in the rat stomach [38]. This COX-2 may be expressed as a result of subliminal bacterial infections of the gastric mucosa.

Kidney

Maintenance of kidney function, both in animal models of disease states and in patients with congestive heart failure, liver cirrhosis, or renal insufficiency, is dependent on vasodilator prostaglandins. These patients are therefore at risk of renal ischemia when prostaglandin synthesis is reduced by NSAIDs. Synthesis of PGE_2 and prostacyclin is mainly by COX-1, although low levels of mRNA for COX-2 have also been reported [39]. Up-regulation of COX-2 expression has been observed in the macula densa after salt deprivation [39].

Central nervous system

COX-1 is found in neurones throughout the brain, but it is most abundant in the forebrain, where prostaglandins may be involved in complex, integrative functions, such as control of the autonomic nervous system and in sensory processing [40, 41]. COX-2 mRNA is induced in brain tissue and in cultured glial cells by pyrogenic substances, such as lipopolysaccharide (LPS) and interleukin (IL)-1 [42, 43]. However, low levels of COX-2 protein and COX-2 mRNA have been detected in neurones of the forebrain, without previous stimulation by proinflammatory substances [40, 41, 44]. These 'basal' levels of COX-2 are particularly high in neonates, and are probably induced by physiological nervous activity. Intense nerve stimulation, leading to seizures, induces COX-2 mRNA in discrete neurones of the hippocampus [45], whereas acute stress raises levels in the cerebral cortex [40]. COX-2 mRNA is also constitutively expressed in the spinal cord of normal rats, and is likely to be involved with processing of nociceptive stimuli [46]. Endogenous, fever-producing PGE_2 is thought to originate from COX-2, induced by LPS or IL-1 in endothelial cells lining the blood vessels of the hypothalamus [43]. The selective inhibitor of COX-2, NS-398, is a potent antipyretic agent in rats [47].

Lungs

Airway hyper-reactivity, a feature of allergic asthma, is associated with inflammation of the airways. Increased expression of COX-2 mRNA and of enzyme protein, with no change in COX-1 levels, has been detected in pulmonary epithelial cells, airway smooth muscle cells, pulmonary endothelial cells, and alveolar macrophages, treated with lipopolysaccharide or proinflammatory cytokines. In the carrageenin-induced pleurisy model of inflammation, levels of COX-2 protein increased maximally at 2 hours in the cell pellets of pleural exudate [48]. This could be accounted for by the induction of COX-2 found in mast cells, mononuclear leukocytes, and neutrophils present in the exudate [49]. However, lung tissue can also express COX-2 constitutively. Human lungs obtained from accident victims [50], and human pulmonary epithelial cells in culture, expressed more constitutive COX-2 than constitutive COX-1 [51, 52]. Exposure to environmental pollutants from car exhausts increases the expression of COX-2 protein and the formation of PGE_2 and $PGF_{2\alpha}$ in these cells in culture [53].

Reproductive system

Expression of COX-1 is much greater than that of COX-2 in fetal hearts, kidneys, lungs, and brains, as well as in the decidual lining of the uterus [54, 55]. Constitutive COX-1 in the amnion could also contribute prostaglandins for the maintenance of a healthy pregnancy [56]. In human amnion cells, chorionic gonadotrophin increases the mRNA for COX-1 [57], whereas glucocorticoids, epidermal growth factor (EGF), IL-1β, and IL-4 all stimulate COX-2 expression [58, 59]. Both COX-1 and COX-2 are expressed in the uterine epithelium at different times in early pregnancy, and may be important for implantation of the ovum and for the angiogenesis necessary to establish the placenta [60].

Gene knockout animals

Several papers on COX-1 and COX-2 gene-deficient mice have now been published [61–63]. At first sight, some of the results are surprising, until it is remembered that both in physiology and pathology, the body uses several parallel pathways to reinforce a common result. For instance, it might have been expected that without the ability to generate prostacyclin, the gastric mucosa of COX-1 knockout mice would show the kind of erosions produced by NSAIDs. However, COX-1 (-/-) mice have normal gastric mucosa, albeit with a decreased sensitivity to the damaging effects of indomethacin [61]. The normality of the mucosa in these mice could well be brought about by the continued release of

nitric oxide and calcitonin gene-related peptide (CGRP), both also known to contribute to the maintenance of a healthy mucosa [64]. Possibly, in the COX-1 (-/-) mice, these mechanisms have been accentuated to compensate for the lack of prostacyclin.

It is more difficult to explain the reduced ulcerogenic actions of indomethacin in these knockout mice, except that it is well known that, in higher concentrations than those needed to inhibit cyclooxygenase, indomethacin inhibits many other enzyme systems. Furthermore, several aspirin-like drugs, including indomethacin, have a local irritant effect on the mucosa, as well as inhibiting COX through a systemic action [65]. Such a local irritation might explain the erosions in the COX-1 (-/-) mice, in which case, treatment with subcutaneous indomethacin would not cause this mucosal irritation.

In both COX-1 [61] and COX-2 knockout mice [62, 63], local application of arachidonic acid still produces some ear inflammation. Here, it should be noted that arachidonic acid leads not only to COX products, but also to leukotrienes via the 5-lipoxygenase pathway. Indeed, the fact that the second pathway contributes to this inflammatory response is shown by 5-lipoxygenase knockout mice, in which inflammation of the ear, produced by arachidonic acid, is substantially reduced [66]. When stronger and more general inflammatory stimuli, such as phorbol esters, are used, many additional mediators will be called into play, such as 5-hydroxytryptamine, bradykinin, nitric oxide, and histamine. Thus, it is not surprising to a pharmacologist that cancelling out a single enzyme, such as COX-1 [61], COX-2 [62, 63], or 5-lipoxygenase [66], has little or no effect on the gross inflammation induced by painting the ears with such a strong irritant as a phorbol ester. Thus, the apparent paradox presented by studies in knockout mice may be logically explained. However, there are many relevant measurements still to be made in these gene-deficient strains of mice.

Mice that lack the gene for production of COX-1 appear to be perfectly healthy, and do not show significant signs of pathological changes in the kidney. This is in accord with the finding that inhibition of COX-1 by NSAIDs does not alter renal function under normal physiological conditions. However, in COX-2 (-/-) null mice, the kidneys failed to develop fully after birth, with the result that the animals died before they were 8 weeks old [62].

Prostaglandins synthesized by COX-1 are apparently essential for the survival of fetuses during parturition, since the majority of offspring born to homozygous COX-1 knockout mice do not survive [61]. This high mortality of the pups may be due to premature closure of the ductus arteriosus. Female COX-2 knockout mice are mostly infertile, producing very few offspring, due to a reduction in ovulation [63].

Selective inhibitors of COX-2

The importance of the discovery of the inducible COX-2 is highlighted by the differences in pharmacology of the two enzymes [67]. Aspirin, indomethacin, and ibuprofen are much less active against COX-2 than against COX-1 [68]. Indeed, the strongest inhibitors of COX-1, such as aspirin, indomethacin, and piroxicam, are the NSAIDs that cause the most damage to the stomach [69]. The spectrum of activities of some 10 standard NSAIDs against the 2 enzymes ranges from a high selectivity towards COX-1 (166-fold for aspirin), through to equal activity on both [70].

The range of activities of NSAIDs against COX-1, as compared with COX-2, nicely explains the variations in the side effects of NSAIDs at their anti-inflammatory doses. Drugs that have the highest potency on COX-2 and a better COX-2/COX-1 activity ratio will have potent anti-inflammatory activity, with fewer side effects on the stomach and kidney. Garcia Rodriguez and Jick [71] have published a comparison of epidemiologic data on the side effects of NSAIDs. Piroxicam and indomethacin in anti-inflammatory doses were found to produce high gastro-intestinal toxicity. These drugs have a much higher potency against COX-1 than against COX-2 [72]. Thus, when epidemiological results are compared with COX-2/COX-1 ratios, a parallel relationship is seen between gastrointestinal side effects and COX-2/COX-1 ratios.

Inhibitors in therapeutic use

Meloxicam, nimesulide, and etodolac (Fig. 2) were not designed specifically as COX-2 inhibitors, but were identified from pharmacological tests as potent anti-inflammatory drugs, with low ulcerogenic activity in the rat stomach. In some instances, this was shown to parallel low activity against prostaglandin synthesis in the rat stomach. After the discovery of COX-2, these 3 drugs were each found preferentially to inhibit COX-2, rather than COX-1, with a variation in their COX-2/COX-1 ratios of between 0.1 and 0.01.

Meloxicam, which has a selectivity towards COX-2 of up to 100-fold over COX-1 (depending on the test system), is marketed around the world for use in rheumatoid arthritis and osteoarthritis [73]. In double-blind trials in more than 5000 patients with osteoarthritis and rheumatoid arthritis, meloxicam, in doses of 7.5 mg or 15 mg once daily, compared in efficacy with standard NSAIDs, such as naproxen 750-1000 mg, piroxicam 20 mg, and diclofenac 100 mg. Both doses of meloxicam produced significantly fewer gastrointestinal adverse effects than the standard NSAIDs ($p < 0.05$). Discontinuation of treatment due to gastrointestinal side effects was also significantly less frequent with meloxicam. Perforations, ulcerations, and bleedings occurred in fewer meloxicam-treated patients than in patients treated with piroxicam, diclofenac, or naproxen. The frequency of adverse events with meloxicam was significantly less ($p < 0.05$), when compared to piroxicam and naproxen [74, 75].

Nimesulide is currently sold in several European countries and in South America for the relief of pain associated with inflammatory conditions. It is a preferential inhibitor of COX-2, with a 10- to 50-fold greater potency against this enzyme than against COX-1 (Table 1) [76]. In limited clinical trials for its use in acute and chronic inflammation in patients, it was more effective than a placebo, or had comparable anti-inflammatory activity to established NSAIDs [81–83]. Epidemiologic data suggests that, in long-term therapeutic use at anti-inflammatory doses (100 mg twice daily), it causes no more serious

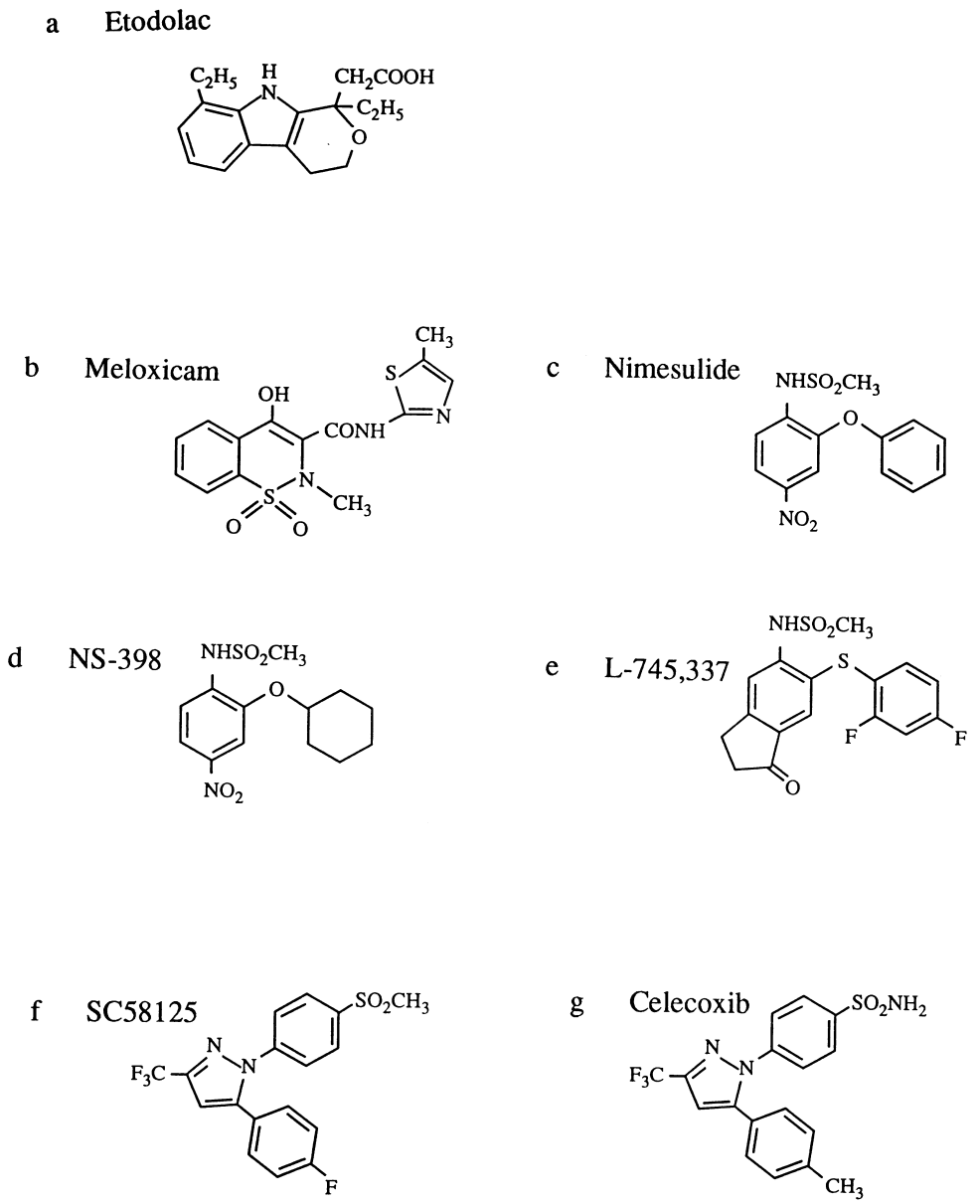

Fig. 2. Chemical structures of some selective COX-2 inhibitors.

gastrointestinal symptoms than did a placebo [84]. Moreover, nimesulide seems safe to use in aspirin-sensitive asthmatics. Several recent studies in NSAID-intolerant patients with asthma demonstrated that therapeutic doses of nimesulide did not induce asthmatic attacks, while high doses of 400 mg only precipitated mild asthma in 10% of patients [85]. A disadvantage of nimesulide may be the need for dosing more than once a day, because of its relatively short half-life.

Etodolac is marketed in Europe and North America for the treatment of osteoarthritis and rheumatoid arthritis. It has a COX-2/COX-1 ratio of 0.09, when tested on recombinant human enzymes, and a ratio of 0.1 in the human whole blood assay (Table 1) [86]. In healthy human volunteers, etodolac twice daily did not suppress gastric mucosal prostaglandin production, and caused less gastric damage than did naproxen [87]. Patients with osteoarthritis or rheumatoid arthritis obtained relief from symptoms with etodolac, equal to other commonly used NSAIDs, but with a lower incidence of serious gastrointestinal toxicity [88].

Inhibitors in clinical development

The discovery of COX-2 stimulated several laboratories to develop highly selective inhibitors of this enzyme. Needleman and his group at Monsanto/Searle have made inhibitors that are some 1000-fold more potent against COX-2 than against COX-1 [89]. One of these, SC-58635 (celecoxib, Fig. 2), is an effective analgesic for moderate-to-severe pain after tooth extraction [90]. Celecoxib, given for 7 days to human volunteers, provided no evidence of gastric damage [91]. It is currently in phase III clinical trials in patients with arthritis [89].

Table 1. COX-2/COX-1 ratios of NSAIDs in the human whole blood assay.

Drug	Year of study			
	1996 [77]	1995 [78]	1996 [79] 1997 [80]	1997[a]
Ketoprofen	1.7	–	50.0	2.0
Flurbiprofen	1.0	1.2	14.3	100.0
Indomethacin	0.5	2.5	3.3	33.0
Piroxicam	0.3	0.2	12.5	2.0
Naproxen	1.7	0.5	10.0	100.0
Ibuprofen	2.0	5.0	5.0	10.0
Diclofenac	–	0.3	0.5	0.33
Etodolac	–	0.12	0.20	0.19
Nimesulide	0.06	–	–	0.15
Meloxicam	0.09	–	0.33	0.20
Celecoxib	–	–	–	0.10
NS-398	0.006	0.006	0.10	0.24
SC58125	0.007	<0.01	<<0.08	0.004
L-745,337	0.004	≪0.09	≪0.33	<0.1

[a] Unpublished observations (Warner T., Vojnovic I., 1997).

A similar, highly selective COX-2 inhibitor from Merck-Frosst, MK-966, which resembles an earlier Merck compound L-745337 (Fig. 2), is currently undergoing phase III clinical trials [92]. In phase I studies, a single dose of 250 mg daily for 7 days (which is 10 times the anti-inflammatory dose) produced no adverse effects on the stomach mucosa, as evidenced by gastroscopy [93]. After a single dose of 1 g, there was no evidence of COX-1 inhibition in platelets, but the activity of COX-2 in lipopolysaccharide-stimulated monocytes ex vivo was reduced. For postoperative dental pain, MK-966, at 25 mg per dose, had equal analgesic activity to ibuprofen, and provided relief from symptoms in a 6-week study of osteoarthritis [94].

New uses for selective COX-2 inhibitors

Premature labor

Prostaglandins are important for inducing uterine contractions during labor. NSAIDs such as indomethacin will delay premature labor by inhibiting the production of prostaglandins, but will at the same time cause early closure of the ductus arteriosus, and reduce urine production by the foetal kidneys [95]. The delay in the birth process is most likely due to inhibition of COX-2, since the mRNA for COX-2 increases substantially in the amnion and placenta immediately before and after the start of labor [96], whereas the side effects on the fetus are due to inhibition of COX-1. One cause of preterm labor could be an intrauterine infection, resulting in the release of endogenous factors that increase PG production by up-regulating COX-2 [59]. Nimesulide reduces prostaglandin synthesis in isolated foetal membranes, and was used successfully for a prolonged period to delay premature labor, without manifesting the side effects of indomethacin on the fetus [95].

Colon cancer

Epidemiological studies have established a strong link between the ingestion of aspirin and a reduced risk of developing colon cancer [97, 98]. It has also been reported that sulindac caused reduction of prostaglandin synthesis and regression of adenomatous polyps in 11 of 15 patients with familial adenomatous polyposis (FAP), a condition in which many colorectal polyps develop spontaneously, with eventual progression to tumors [99–101]. This indication that COX activity is involved in the process leading to colon cancer is supported by the demonstration that COX-2, and not COX-1, is highly expressed in human and animal colon cancer cells, as well as in human colorectal adenocarcinomas [102, 103]. Further support for the close connection between COX-2 and colon cancer has come from studies in the mutant *Apc* mouse, which is a model of FAP in humans. The spontaneous development of intestinal polyposis in these mice was strongly reduced, either by deletion of the COX-2 gene, or by treatment with a selective COX-2 inhibitor [104–106]. Thus, it is highly likely that COX-2 inhibitors could be used prophylactically to prevent colon cancer in genetically susceptible individuals, without causing gastrointestinal damage themselves.

Alzheimer's disease

The connection between COX and Alzheimer's disease has been based entirely on epidemiological studies, largely due to the lack of an animal model of the disease. A number of studies have shown a significantly reduced odds ratio for Alzheimer's disease in those taking NSAIDs as anti-inflammatory therapy [107–109]. The Baltimore Longitudinal Study of Aging, with 1686 participants, reported in 1997 that the risk of developing Alzheimer's disease is reduced among users of NSAIDs, especially those who have taken the medications for 2 years or more [110]. No decreased risk was evident with acetaminophen or aspirin use. However, aspirin was probably taken in a dose too low to have an anti-inflammatory effect. The protective effect of NSAIDs is consistent with evidence of inflammatory activity in the pathophysiology of Alzheimer's disease [111, 112]. However, the content of COX-2 in the brain tissue of patients with Alzheimer's disease was lower than normal [113], which may reflect the large loss of neuronal tissue in the late stages of the disease. Chronic treatment with selective COX-

2 inhibitors may therefore slow the progress of Alzheimer's disease, without damaging the stomach mucosa [110].

Conclusions

All the results so far published, and many yet to be published from the drug industry, support the hypothesis that the unwanted side effects of NSAIDs are due to their ability to inhibit COX-1, whilst their anti-inflammatory (therapeutic) effects are due to inhibition of COX-2. Other roles for COX-2 will surely be found in the next few years, for prostaglandin formation is under strong control in organs such as the uterus. It is likely that the hormonal induction of COX-2 leads to, for example, the prostaglandin production associated with parturition. The identification of selective inhibitors of COX-1 and COX-2 will not only provide an opportunity to test the new hypothesis, but also lead to advances in the therapy of inflammation. New uses will also be found for selective COX-2 inhibitors.

References

[1] Stone E. An account of the success of the bark of the willow in the cure of agues. Philosophical Transactions of the Royal Society 1763;53:195–200.

[2] Dreser H. Pharmakologisches über Aspirin (Acetylsalicylsäure). Pflügers Arch 1899;76:306–18.

[3] Flower RJ. Drugs which inhibit prostaglandin biosynthesis. Pharmacol Rev 1974;26:33–67.

[4] Vane JR. The use of isolated organs for detecting active substances in the circulating blood. Br J Pharmacol Chemother 1964;23:360–73.

[5] Piper PJ, Vane JR. The release of prostaglandins during anaphylaxis in guinea-pig isolated lungs. In: Mantegazza P, Horton EW, editors. Prostaglandins, Peptides and Amines. London/New York: Academic Press, 1969:15–9.

[6] Hamberg M, Svensson J, Samuelsson B. Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci USA 1975;72:2994–8.

[7] Palmer MA, Piper PJ, Vane JR. The release of RCS from chopped lung and its antagonism by anti-inflammatory drugs. Br J Pharmacol 1970;40:581P.

[8] Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol 1971;231:232–5.

[9] Smith JH, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. Nature 1971;231:235–7.

[10] Ferreira SH, Moncada S, Vane JR. Indomethacin and aspirin abolish prostaglandin release from spleen. Nature 1971;231: 237–9.

[11] Flower RJ, Vane JR. Inhibition of prostaglandin biosynthesis. Biochem Pharmacol 1974;23:1439–50.

[12] Higgs GA, Moncada S, Vane JR. Eicosanoids in inflammation. Ann Clin Res 1984;16:287–99.

[13] Vane JR, Botting RM. The mode of action of anti-inflammatory drugs. Postgrad Med J 1990;66(Suppl 4):S2–17.

[14] Hemler M, Lands WEM, Smith WL. Purification of the cyclooxygenase that forms prostaglandins. Demonstration of the two forms of iron in the holoenzyme. J Biol Chem 1976; 251:5575–9.

[15] DeWitt DL, Smith WL. Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. Proc Natl Acad Sci USA 1988;85:1412–6.

[16] Merlie JP, Fagan D, Mudd J, Needleman P. Isolation and characterization of the complementary DNA for sheep seminal vesicle prostaglandin endoperoxide synthase (cyclooxygenase). J Biol Chem 1988;263:3550–3.

[17] Yokoyama C, Takai T, Tanabe T. Primary structure of sheep prostaglandin endoperoxide synthase deduced from cDNA sequence. FEBS Lett 1988;231:347–51.

[18] Smith WL. Prostaglandin biosynthesis and its compartmentation in vascular smooth muscle and endothelial cells. Ann Rev Physiol 1986;48:251–62.

[19] Picot D, Loll PJ, Garavito RM. The X-ray crystal structure of the membrane protein prostaglandin H_2 synthase-1. Nature 1994;367:243–9.

[20] Roth GJ, Stanford N, Majerus PW. Acetylation of prostaglandin synthetase by aspirin. Proc Natl Acad Sci USA 1975;72: 3073–6.

[21] Luong C, Miller A, Barnett J, Chow J, Ramesha C, Browner MF. Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. Nat Struct Biol 1996;3:927–33.

[22] Wong E, Bayly C, Waterman HL, Riendeau D, Mancini JA. Conversion of prostaglandin G/H synthase-1 into an enzyme sensitive to PGHS-2-selective inhibitors by a double His^{513} to Arg and Ile^{523} to Val mutation. J Biol Chem 1997;272:9280–6.

[23] Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature 1976;263:663–5.

[24] Whittle BJR, Higgs GA, Eakins KE, Moncada S, Vane JR. Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. Nature 1980;284:271–3.

[25] Xie W, Robertson DL, Simmons DL. Mitogen-inducible prostaglandin G/H synthase: A new target for nonsteroidal anti-inflammatory drugs. Drug Dev Res 1992;25:249–65.

[26] Fu J-Y, Masferrer JL, Seibert K, Raz A, Needleman P. The induction and suppression of prostaglandin H_2 synthase (cyclooxygenase) in human monocytes. J Biol Chem 1990; 265:16737–40.

[27] Masferrer JL, Zweifel BS, Seibert K, Needleman P. Selective regulation of cellular cyclooxygenase by dexamethasone and endotoxin in mice. J Clin Invest 1990;86:1375–9.

[28] Xie W, Chipman JG, Robertson DL, Erikson RL, Simmons DL. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci USA 1991;88:2692–6.

[29] O'Banion MK, Sadowski HB, Winn V, Young DA. A serum- and glucocorticoid-regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein. J Biol Chem 1991;266:23261–7.

[30] Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR. 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.

[31] Sirois J, Richards JS. Purification and characterisation of a novel, distinct isoform of prostaglandin endoperoxide synthase induced by human chorionic gonadotropin in granulosa cells of rat preovulatory follicles. J Biol Chem 1992;267:6382–8.

[32] Weissmann G. Prostaglandins as modulators rather than mediators of inflammation. J Lipid Mediat 1993;6:275–86.

[33] Weissmann G. Aspirin. Sci Am 1991;January:84–90.

[34] Wu KK, Sanduja R, Tsai AL, Ferhanoglu B, Loose-Mitchell DS. Aspirin inhibits interleukin-1-induced prostaglandin H synthase expression in cultured endothelial cells. Proc Natl Acad Sci USA 1991;88:2384–7.

[35] Clissold SP. Paracetamol and phenacetin. Drugs 1986;32(Suppl 4):46–59.

[36] Flower RJ, Vane JR. Inhibition of prostaglandin synthetase in brain explains the antipyretic activity of paracetamol (4-acetamidophenol). Nature 1972;240:410–1.

[37] Whittle BJR, Vane JR. Prostanoids as regulators of gastrointestinal function. In: Johnston LR, editor. Physiology of the Gastrointestinal Tract, Vol. 1. 2nd ed. New York: Raven Press, 1987:143–80.

[38] Kargman S, Charleson S, Cartwright M, Frank J, Riendeau D, Mancini J, et al. Characterization of prostaglandin G/H synthase 1 and 2 in rat, dog, monkey and human gastrointestinal tracts. Gastroenterology 1996;111:445–54.

[39] Harris RC, McKanna JA, Akai Y, Jacobson HR, Dubois RN, Breyer MD. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. J Clin Invest 1994;94:2504–10.

[40] Yamagata K, Andreassson KI, Kaufman WE, Barnes CA, Worley PF. Expression of a mitogen-inducible cyclooxygenase in brain neurons; regulation by synaptic activity and glucocorticoids. Neuron 1993;11:371–86.
[41] Breder CD, Dewitt D, Kraig RP. Characterization of inducible cyclooxygenase in rat brain. J Comp Neurol 1995;355:296–315.
[42] Breder CD, Saper CB. Expression of inducible cyclooxygenase mRNA in the mouse brain after systemic administration of bacterial lipopolysaccharide. Brain Res 1996;713:64–9.
[43] Cao C, Matsumura K, Yamagata K, Watanabe Y. Endothelial cells of the brain vasculature express cyclooxygenase-2 mRNA in response to systemic interleukin-1β: A possible site of prostaglandin synthesis responsible for fever. Brain Res 1996; 733:263–72.
[44] Cao C, Matsumura K, Yamagata K, Watanabe Y. Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in rat brain: Its possible role in the febrile response. Brain Res 1995;697:187–96.
[45] Marcheselli VL, Bazan NG. Sustained induction of prostaglandin endoperoxide synthase-2 by seizures in hippocampus. J Biol Chem 1996;271:24794–9.
[46] Beiche F, Scheuerer S, Brune K, Geisslinger G, Goppelt-Struebe M. Up-regulation of cyclooxygenase-2 mRNA in the rat spinal cord following peripheral inflammation. FEBS Lett 1996;390:165–9.
[47] Futaki N, Yoshikawa K, Hamasaka Y, Arai I, Higuchi S, Iizuka H, et al. NS-398, a novel non-steroidal anti-inflammatory drug with potent analgesic and antipyretic effects which causes minimal stomach lesions. Gen Pharmacol 1993;24:105–10.
[48] Tomlinson A, Appleton I, Moore AR, Gilroy DW, Willis D, Mitchell JA, Willoughby DA. Cyclo-oxygenase and nitric oxide synthase isoforms in rat carrageenin-induced pleurisy. Br J Pharmacol 1994;113:693–4.
[49] Hatanaka K, Harada Y, Kawamura M, Ogino M, Saito M, Katori M. Cell types expressing COX-2 in rat carrageenin-induced pleurisy. Jpn J Pharmacol 1996;71(Suppl I):304P.
[50] O'Neill GP, Ford-Hutchinson AW. Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. FEBS Lett 1993;330:156–60.
[51] Asano K, Lilly CM, Drazen JM. Prostaglandin G/H synthase-2 is the constitutive and dominant isoform in cultured human lung epithelial cells. Am J Physiol 1996;271:L126–31.
[52] Walenga RW, Kester M, Coroneos E, Butcher S, Dwivedi R, Statt C. Constitutive expression of prostaglandin endoperoxide G/H synthase (PGHS)-2 but not PGHS-1 in human tracheal epithelial cells in vitro. Prostaglandins 1996;52:341–59.
[53] Samet JM, Reed W, Ghio AJ, Devlin RB, Carter JD, Dailey LA, et al. Induction of prostaglandin H synthase 2 in human airway epithelial cells exposed to residual oil fly ash. Toxicol Appl Pharmacol 1996;141:159–68.
[54] Bennett P, Slater D. COX-2 expression in labor. In: Vane J, Botting J, Botting R, editors. Improved Non-steroid Anti-inflammatory Drugs. COX-2 Enzyme Inhibitors. Lancaster, London: Kluwer Academic Publishers and William Harvey Press, 1996:167–88.
[55] Gibb W, Sun M. Localization of prostaglandin H synthase type 2 protein and mRNA in term human fetal membranes and decidua. J Endocrinol 1996;150:497–503.
[56] Trautman MS, Edwin SS, Collmer D, Dudley DJ, Simmons D, Mitchell MD. Prostaglandin H synthase-2 in human gestational tissues: Regulation in amnion. Placenta 1996;17:239–45.
[57] Toth P, Li X, Lei ZM, Rao CV. Expression of human chorionic gonadotropin (hCG)/luteinizing hormone receptors and regulation of the cyclooxygenase-1 gene by exogenous hCG in human fetal membranes. J Clin Endocrinol Metab 1996;81:1283–8.
[58] Zakar T, Hirst JJ, Milovic JE, Olson DM. Glucocorticoids stimulate the expression of prostaglandin endoperoxide H synthase-2 in amnion cells. Endocrinology 1995;136:1610–9.
[59] Spaziani EP, Lantz ME, Benoit RR, O'Brien WF. The induction of cyclooxygenase-2 (COX-2) in intact human amnion tissue by interleukin-4. Prostaglandins 1996;51:215–23.
[60] Chakraborty I, Das SK, Wang J, Dey SK. Developmental expression of the cyclo-oxygenase-1 and cyclo-oxygenase-2 genes in the peri-implantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids. J Mol Endocrinol 1996; 16:107–22.
[61] Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 1995;83:483–92.
[62] Morham SG, Langenbach R, Loftin CD, Tiano HF, Vouloumano N, Jenette JC, et al. Prostaglandin synthase 2 gene disruption causes renal pathology in the mouse. Cell 1995;83: 473–82.
[63] Dinchuck JE, Car BD, Focht RJ, Johnston JJ, Jaffee BD, Covington MB, et al. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. Nature 1995; 378:406–9.
[64] Whittle BJR. Neuronal and endothelium-derived mediators in the modulation of the gastric microcirculation: integrity in the balance. Br J Pharmacol 1993;110:3–17.
[65] Rainsford KD, Willis C. Relationship of gastric mucosal damage induced in pigs by antiinflammatory drugs to their effects on prostaglandin production. Dig Dis Sci 1982;27:624–35.
[66] Chen XS, Sheller JR, Johnson EN, Funk CD. Role of leukotrienes revealed by targeted disruption of the 5-lipoxygenase gene. Nature 1994;372:179–82.
[67] 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 USA 1993;90;11693–7.
[68] Meade EA, Smith WL, DeWitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J Biol Chem 1993;268:6610–4.
[69] Lanza FL. A review of gastric ulcer and gastroduodenal injury in normal volunteers receiving aspirin and other non-steroidal anti-inflammatory drugs. Scand J Gastroenterol 1989;24(Suppl 163): 24–31.
[70] Akarasereenont P, Mitchell JA, Thiemermann C, Vane JR. Relative potency of nonsteroid anti-inflammatory drugs as inhibitors of cyclooxygenase-1 or cyclooxygenase-2. Br J Pharmacol 1994; 112(Suppl.):183P.
[71] Garcia Rodriguez LA, Jick H. Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs. Lancet 1994;343:769–72.
[72] Vane JR, Botting RM. New insights into the mode of action of anti-inflammatory drugs. Inflamm Res 1995;44:1–10.
[73] Churchill L, Graham A, Shih C-K, Pauletti D, Farina PR, Grob PM. Selective inhibition of human cyclooxygenase-2 by meloxicam. Inflammopharmacology 1996;4;125–35.
[74] Barner A. Review of clinical trials and benefit/risk ratio of meloxicam. Scand J Rheumatol 1996;25(Suppl 102):29–37.
[75] Distel M, Mueller C, Bluhmki E, Fries J. Safety of meloxicam: A global analysis of clinical trials. Br J Rheumatol 1996;35(Suppl 1):68–77.
[76] Tavares IA, Bishai PM, Bennet A. Activity of nimesulide on constitutive and inducible cyclooxygenases. Arzneimittelforschung/Drug Res 1995;45:1093–5.
[77] Partrignani P, Panara MR, Santini G, Sciulli MG, Padovano R, Cipollone F. Differential inhibition of cyclooxygenase activity of prostaglandin endoperoxide synthase isozymes in vitro and ex vivo in man. Prostaglandins Leukot Essent Fatty Acids 1996;55(Suppl 1):P115.
[78] Glaser K. Cyclooxygenase selectivity and NSAIDs: cyclooxygenase-2 selectivity of etodolac (Lodine). Inflammopharmacology 1995;3:335–45.
[79] Brideau C, Kargman S, Liu S, Dallob AL, Ehrich EW, Rodger IW, et al. A human whole blood assay for clinical evaluation of biochemical efficacy of cyclooxygenase inhibitors. Inflamm Res 1996;45:68–74.
[80] Prasit P. New highly selective COX-2 inhibitors. William Harvey Research Conference: Selective COX-2 Inhibitors. Phuket, Sept 17-19, 1997.
[81] Weissenbach R. Clinical trials with nimesulide, a new non-

steroid anti-inflammatory agent, in rheumatic pathology. J Int Med Res 1981;13:237–45.
[82] Pais JM, Rosteiro FM. Nimesulide in the short-term treatment of the inflammatory process of dental tissues: a double-blind controlled trial against oxyphenbutazone. J Int Med Res 1983; 11:149–54.
[83] Emami Nouri E. Nimesulide for treatment of acute inflammation of the upper respiratory tract. Clin Ther 1984;6:142–50.
[84] Fusetti G, Magni E, Armandola MC. Tolerability of nimesulide. Epidemiological data. Drugs 1993;46(Suppl 1):277–80.
[85] Senna GE, Passalacqua G, Andri G, Dama AR, Albano M, Fregonese L, et al. Nimesulide in the treatment of patients intolerant of aspirin and other NSAIDs. Drug Saf 1996;14:94–103.
[86] Glaser K, Sung M-L, O'Neill K, Belfast M, Hartman D, Carlson R, et al. Etodolac selectively inhibits human prostaglandin G/H synthase 2 (PGHS-2) versus human PGHS-1. Eur J Pharmacol 1995;281:107–11.
[87] Laine L, Sloane R, Ferretti M, Cominelli F. A randomized double-blind comparison of placebo, etodolac and naproxen on gastrointestinal injury and prostaglandin production. Gastrointest Endosc 1995;42:428–33.
[88] Cummings DM, Amadio P Jr. A review of selected newer nonsteroidal anti-inflammatory drugs. Am Fam Physician 1994;49:1197–202.
[89] Isakson P, Zweifel B, Masferrer J, Koboldt C, Seibert K, Hubbard R, et al. Specific COX-2 inhibitors: From bench to bedside. In: Vane J, Botting J, editors. Selective COX-2 Inhibitors. Pharmacology, Clinical Effects and Therapeutic Potential. London: Kluwer Academic Publishers and William Harvey Press, 1998:1–17.
[90] Hubbard RC, Mehlisch DR, Jasper DR, Nugent MJ, Yu S, Isakson PC. SC-58635, a highly selective inhibitor of COX-2, is an effective analgesic in an acute post-surgical pain model. J Invest Med 1996;44:293A.
[91] Lanza FL, Rack MF, Callison DA, Hubbard RC, Yu SS, Talwalker S, et al. A pilot endoscopic study of the gastroduodenal effects of SC-58635, a novel COX-2 selective inhibitor [abstract]. Gastroenterology 1997;112:A194.
[92] Ford-Hutchinson AW. New highly selective COX-2 inhibitors. In: Vane J, Botting J, editors. Selective COX-2 Inhibitors. Pharmacology, Clinical Effects and Therapeutic Potential. London: Kluwer Academic Publishers and William Harvey Press, 1998:117–25.
[93] Lanza F, Simon T, Quan H, Bolognese J, Rack MF, Hoover M, et al. Selective inhibition of cyclooxygenase-2 (COX-2) with MK-0966 (250 mg q.d.) is associated with less gastroduodenal damage than aspirin (ASA) 650 mg q.i.d. or ibuprofen (IBU) 800 mg t.i.d. [abstract]. Gastroenterology 1997;112:A194.
[94] Ehrich E, Mehlisch D, Perkins S, Brown P, Wittreich J, Lipschutz K, et al. Efficacy of MK-966, a highly selective inhibitor of COX-2, in the treatment of postoperative dental pain [abstract]. Arthritis Rheum 1996;39(Suppl 9): S81.
[95] Sawdy R, Slater D, Fisk N, Edmonds DK, Bennett P. Use of a cyclo-oxygenase type-2-selective non-steroidal anti-inflammatory agent to prevent preterm delivery. Lancet 1997;350:265–6.
[96] Gibb W, Sun M. Localization of prostaglandin H synthase type 2 protein and mRNA in term human fetal membranes and decidua. J Endocrinol 1996;150:497–503.
[97] Thun MJ, Namboodiri MM, Heath CWJ. Aspirin use and reduced risk of fatal colon cancer. N Engl J Med 1991;325: 1593–6.
[98] Luk GD. Prevention of gastrointestinal cancer – the potential role of NSAIDs in colorectal cancer. Schweiz Med Wochenschr 1996;126:801–12.
[99] Nugent KP, Spigelman AD, Phillips RKS. Tissue prostaglandin levels in familial adenomatous polyposis patients treated with sulindac. Dis Colon Rectum 1996;39:659–62.
[100] Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hyland LM, Celano P, et al. Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med 1993;328:1313–6.
[101] Matsuhashi N, Nakajima A, Fukushima Y, Yazaki Y, Oka T. Effects of sulindac on sporadic colorectal adenomatous polyps. Gut 1997;40:344–9.
[102] Kutchera W, Jones DA, Matsunami N, Groden J, McIntyre TM, Zimmerman GA, et al. Prostaglandin H synthase 2 is expressed abnormally in human colon cancer: Evidence for a transcriptional effect. Proc Natl Acad Sci USA 1996;93:4816–20.
[103] Gustafson-Svärd C, Lilja I, Hallböök O, Sjödahl R. Cyclooxygenase-1 and cyclooxygenase-2 gene expression in human colorectal adenocarcinomas and in azoxymethane induced colonic tumours in rats. Gut 1996;38:79–84.
[104] 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;104:1183–8.
[105] 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;99:2254–9.
[106] Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, et al. Suppression of intestinal polyposis in *Apc*$^{\Delta 716}$ knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 1996;87:803–9.
[107] Cochran FR, Vitek MP. Neuroinflammatory mechanisms in Alzheimer's disease: New opportunities for drug discovery. Expert Opin Invest Drugs 1996;5:449–55.
[108] Breitner JCS, The role of anti-inflammatory drugs in the prevention and treatment of Alzheimer's disease. Ann Rev Med 1996;47:401–11.
[109] McGeer PL, McGeer EG. The inflammatory response system of brain: Implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 1995;21:195–218.
[110] Stewart WF, Kawas C, Corrada M, Metter EJ. Risk of Alzheimer's disease and duration of NSAID use. Neurology 1997;48:626–32.
[111] Hampel H, Muller N. Inflammatory and immunological mechanisms in Alzheimer's disease. Drug News Perspect 1995;8:599–608.
[112] Yan SD, Zhu H, Fu J, Yan SF, Roher A, Tourtellotte WW, et al. Amyloid-β peptide-receptor for advanced glycation endproduct interaction elicits neuronal expression of macrophage-colony stimulating factor: A proinflammatory pathway in Alzheimer disease. Proc Natl Acad Sci USA 1997;94:5296–301.
[113] Chang JW, Coleman PD, O'Banion MK. Prostaglandin G/H synthase-2 (cyclooxygenase-2) mRNA expression is decreased in Alzheimer's disease. Neurobiol Aging 1996;17:801–8.

Post-Presentation Discussion

Questioner: Carlson, Abbott Laboratories, United States. I've been unhappy about clinical trials of the so-called early COX-2 selective drugs, such as meloxicam and nimesulide. Most of these compounds, when I have looked at them, have the equivalent doses, say, in my animal experiments, but what they've used in man are analgesic doses, and not anti-inflammatory, and so with these early ones, if you go high enough, you're going to get GI irritation, especially for anti-inflammatory doses, and I think we need to know that most of these compounds that have been given in the clinic, meloxicam, nabumetone, all these, etodolac... I came from Wyeth-Ayerst, I can tell you. That's an analgesic dose. So I just want your comment on that.

Sir John: I haven't got all of the results of the meloxicam clinical trial on slides here today, but its quite clear to me that in every adverse event on the GI tract, I've only used the serious ones to illustrate, but in every other adverse event, including irritation and all of the minor events, there are less adverse events with meloxicam than with either diclofenac or piroxicam. And I recently looked at etodolac fairly rigorously, and there were less adverse events for that, also.