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Review articles

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THE FIGHT AGAINST RHEUMATISM: FROM WILLOW BARK TO COX-1 SPARING DRUGS

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Man has been fighting rheumatism for thousands of years. Early therapy began with the use around the world of decoctions or extracts of herbs or plants such as willow bark or leaves. Most or all of these turned out to contain salicylates. The first record was about 3,500 years ago in the Ebers papyrus. Hippocrates, Celsus, Pliny the Elder, Dioscorides and Galen all recommended decoctions containing salicylate for rheumatic pain. A country parson, the Reverend Edward Stone of Chipping Norton in Oxfordshire, made the first "clinical trial" of willow bark (1). He was surprised by its bitter taste, which reminded him of cinchona bark (containing quinine), then being used to treat malaria. He harvested a pound of willow bark, dried it, pulverized it and dispersed it in tea, small beer or water. He found in 50 patients that doses of 1 dram (1.8g) cured their fever. He concluded "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". Salicylic acid was chemically synthesised in 1860 by Kolbe in Germany and its ready supply led to even more extended usage as an external antiseptic, as an antipyretic and in the treatment of rheumatism.

Key words: *rheumatism, aspirin, prostaglandins, COX-1, COX-2, NSAIDS, selective COX-2 inhibitor*

INTRODUCTION

Aspirin was born just over 100 years ago. Felix Hoffman was a young chemist working for Bayer in Germany. Legend has it that his father was taking salicylate for his severe rheumatism and urged Felix to find a more palatable form. Nowadays, it is easy to disguise the taste of a medicine by putting a hard sugar coating on the tablet, but neither tablets nor coatings were available. Instead, Felix made acetylsalicylic acid, named as aspirin. Bayer's Research Director, Dr Heinrich Dreser, tested aspirin in animals and found it to be antipyretic, analgesic and anti-inflammatory. He recognized that he had an important new drug on his hands and introduced it to the market as

a powder in 1899 and as tablets soon thereafter. The wishes of the Reverend Edward Stone have certainly been realised; world production of acetylsalicylic acid is now estimated at 50 thousand tons a year, with an average consumption of about 80 tablets per person per year. Without the discovery in recent years of many other aspirin-like drugs, such as ibuprofen, the fenamates, indomethacin and naproxen, consumption would have surely been very much higher.

Despite the diversity of their chemical structures, these non-steroid anti-inflammatory drugs (NSAIDS) all share the same therapeutic properties, alleviating the swelling, redness and pain of inflammation, reducing fever and curing headaches. Importantly, they also share to a greater or lesser extent the same group of side effects, including interfering with the birth process and damaging the kidney. However, the most troublesome side effect is on the stomach. Indeed, epidemiological studies have characterised the degree of gastric damage caused by different compounds (2). An estimated 34—46% of patients on NSAID therapy will have some form of gastrointestinal adverse events (3). In the USA alone, some 100,000 patients on NSAIDs are hospitalised each year because of perforations, ulcers or bleeding in the stomach (PUBs) (4) and about 15,000 of these die in intensive care. Of course, these hospitalisations only represent the extreme of gastric irritation, which ranges from mild dyspepsia all the way through to PUBs. Even ibuprofen, recognised as one of the mildest gastric irritants, causes problems in a significant proportion of patients. Clearly, there is dramatic need for anti-inflammatory drugs that do not affect the stomach.

Discovery of the mode of action of aspirin

For many years pharmacologists and biochemists searched for a common mode of action without finding a generally acceptable scientific explanation. In the late 1960 s I was working on a newly discovered group of chemical mediators called the prostaglandins. Many types of chemical or mechanical stimuli cause their synthesis and release in different parts of the body. Interestingly, one or more of this group of lipid-derived mediators caused pain, swelling and redness. They also contracted many kinds of smooth muscle, including that of the uterus. In addition, they increased renal blood flow and reduced gastric acid secretion. These were all activities with which aspirin interfered in some way. Could it be that aspirin was blocking the biosynthesis of prostaglandins?

I tested this idea immediately in vitro, using as a source of prostaglandin synthase the supernatant of a broken cell homogenate from guinea pig lung (5). There was a dose-dependent inhibition of prostaglandin formation by aspirin, salicylate and indomethacin but not by morphine (6). Two other reports (7, 8) from my laboratory in the same issue of Nature lent support to these findings. The discovery that each and every chemically diverse member of this large

group of drugs all act by inhibiting the key enzyme in prostaglandin biosynthesis (which we now call cyclooxygenase or COX) provided a unifying explanation for their therapeutic actions and shared side effects (for reviews see refs 9—11). This theory became well accepted, although there was always the puzzle as to why the different drugs, at therapeutic concentrations, varied widely in the severity of their side effects.

Some companies found new compounds that were anti-inflammatory, but were developed because they were less damaging to the stomach, usually tested in rats. Meloxicam, nimesulide and etodolac, all now recognised as selective COX-2 inhibitors, were discovered in this way.

The Discovery of COX-2

There were various clues in the literature suggesting that there may be a second COX enzyme. As early as 1972, Smith and Lands (12) and Flower and Vane (13) speculated on the existence of isoenzymes. Maddox (14) showed the presence of two separate prostaglandin synthase complexes in sheep vesicular tissue. Others (15—17) also suggested two distinct forms of COX. Rosen *et al.*. (1989) (18) were studying the regulation of cyclooxygenase in cultures of epithelial cells from trachea and found an increase in activity of COX during prolonged cell culture. The increase in activity was not accounted for by the increase in 70 kDa cyclooxygenase protein, nor by the mRNA of 2.8 kb. They did find a second mRNA of 4.0 kb and suggested that their evidence was consistent with the 4.0 kb mRNA being derived from a distinct cyclooxygenase-related gene which encode for a protein with COX activity.

Needleman and his group (19—21) reported that bacterial lipòpolysaccharide (LPS) increased the synthesis of prostaglandins in human monocytes *in vitro* and in mouse peritoneal macrophages *in vivo*. This increase, but not the basal level of enzyme, was inhibited by dexamethasone and associated with *de novo* synthesis of new COX protein. This further gave rise to the concept of "constitutive" and "inducible" forms of COX.

The breakthrough came from molecular biologists outside the field of prostaglandins. Simmons and his colleagues in 1991 (22, 23) were studying early response genes and discovered an inducible second form of COX in chicken embryo cells. It was encoded by a 4.1 kb mRNA similar in size to that reported by Rosen *et al.* (18). They cloned the gene, deduced the protein structure and found it homologous to COX, but to no other known protein.

This work was closely followed by Herschmann and his colleagues (24), who found a similar gene in the mouse, as did Simmons *et al.* (25), O'Banion *et al.* (26) and Sirois and Richards (27), Both enzymes have a molecular weight of 71 Kd and the amino acid sequence of the cDNA for COX-2 shows a 60% homology with the sequence of the non-inducible enzyme. The mRNA for the

inducible enzyme approximates 4.5 kb and that of the constitutive enzyme, 2.8 kb. The inhibition by 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.

Thus, the constitutive isoform COX-1 has clear physiological functions. Its activation leads, for instance, to the production of prostacyclin which when released by the endothelium is anti-thrombogenic (28) and when released by the gastric mucosa is cytoprotective (29). The second isoform, COX-2, is inducible in a number of cells by pro-inflammatory stimuli (30). Since COX-2 is induced by inflammatory stimuli and by cytokines in migratory and other cells it was attractive to suggest, as I and others did in 1993 (31, 32), 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 mucosa and toxic effects on the kidney are due to inhibition of the constitutive enzyme, COX-1. This very important hypothesis is now well supported, not only by a wealth of data on COX-2 inhibitors in animal tests, but also by the better tolerability in man of selective COX-2 inhibitors (see below).

The design of selective COX-2 inhibitors

Two brilliant scientists led the field, racing with their teams to produce selective COX-2 inhibitors. Tony Ford-Hutchinson, then at Merck Frosst in Canada and Phil Needleman, then Research Director at Monsanto Searle (now Pharmacia) committed substantial resources to the projects. The results were the marketing within ten years of rofecoxib (VIOXX) by Merck and of celecoxib (Celebrex) by Searle. Favourable clinical trial results are already available for meloxicam (Mobic) (33—35): both rofecoxib and celecoxib have shown similar superiority to the NSAIDs from the safety point of view in extensive clinical trials yet to be published. Rofecoxib and celecoxib are effective analgesics in man for moderate to severe pain following tooth extraction (36, 37).

Measuring the effects of aspirin-like drugs on the two enzymes

Mitchell *et al.* (31) were the first to measure the effects of NSAIDs on both COX-1 and COX-2. Recently, several methods have been published to determine the relative actions of NSAIDs on COX-1 and COX-2. These range from isolated enzymes (now usually recombinant human enzymes) through whole cell preparations in vitro to the human whole blood assay (see Ref 38 for discussion of relative merits). The isolated enzyme assays give the highest ratios for COX-2/COX-1. For instance, meloxicam has a ratio of 100, whereas celecoxib and rofecoxib have ratios of more than 1000 in favour of COX-2 (39).

However, such assays do not take into account the avid, but variable binding of some of these drugs to plasma protein and other aspects of the kinetics of drug distribution. It is now generally accepted that the human whole blood assay, first described by Patrono and colleagues (40) best reflects activity *in vivo* in man. The activity of compounds on COX-2 is measured in the platelets of the blood sample and that on COX-2 in white cells induced over 24 hours to express COX-2 by LPS or a cytokine. A useful modification by Warner *et al.* (41) reduces the time to measure COX-2 effects down to 1.5 hours.

The advantages of these methods are that they use human cells that importantly are in a physiological environment (plasma), which automatically takes any protein binding into account. What is more, the assays give reproducible results between various laboratories. For significant differences between drugs, changes in ratio of an order of magnitude are needed. Interestingly, the ratios change substantially in this assay. In our hands (41), for example, meloxicam has a ratio in favour of COX-2 of about 5, celecoxib is hardly different at 10, whereas rofecoxib, has a ratio of >60 (see *Table 1*).

Table 1. The COX-2/COX-1 ratios for some NSAIDs and selective COX-2 inhibitors in whole blood assays in different laboratories. The data from Warner *et al.* (41) is for the modified whole blood assay (a) and for the usual whole blood assay (b).

DRUG	Warner *et al.* (41) (a)	Warner *et al.* (41) (b)	Patrignani *et al.* (40)	Brideau *et al.* 1996	Pairet *et al.* (38)	Glaser (42)
Ketoprofen	5.1	61	1.7	5.4		
Flurbiprofen	10.0	73	1.0	14.6		
Indomethacin	10.0	80	0.53	2.88	0.82	5.7
Piroxicam	0.1	3.3	0.32	11.8	1.1	
Naproxen	3.8	3.0	1.67	9.5		13.1
Ibuprofen	2.6	0.9	2.0	6.3		
6-MNA	2.6	> 5	0.67			
Diclofenac	0.3	0.5		0.36	0.39	1.5
Etodolac	0.1	0.2				0.09
Nimesulide	0.038	0.19	0.006			
Meloxicam	0.04	0.37	0.009		0.08	
Celecoxib	0.3	0.7			0.029	
NS 398	0.0061	0.051	0.006	0.09		0.00003
SC58125		< 0.01	0.007	< 0.033	0.027	< 0.001
L745,337	< 0.01	< 0.01	0.007	< 0.3		
Rofecoxib	0.0049	0.013				

Selective COX-2 inhibitors in current therapeutic use

Meloxicam, nimesulide and etodolac were identified in the 1980s as potent anti-inflammatory drugs with low ulcerogenic activity in the rat stomach. In some instances, this was also shown to parallel low activity against prostaglandin synthesis in the rat stomach. After the characterisation of the COX-2 gene, these three drugs were each found selectively to inhibit COX-2 rather than COX-1 (see *Table 1*).

Meloxicam, which has a selectivity towards COX-2 of about 5 in the human whole blood assay is marketed around the world for use in rheumatoid arthritis and osteoarthritis. In double blind trials (33—35) in many thousands of patients with osteoarthritis, 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 or 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 at $p < 0.05$ when compared to piroxicam and naproxen. These large-scale clinical trials with a selective COX-2 inhibitor add weight to the concept that the sparing of COX-1 inhibition will reduce gastric damage.

Etodolac is marketed in Europe and North America for the treatment of osteoarthritis and rheumatoid arthritis. It has about five fold selectivity for COX-2 in human whole blood (42). In healthy human volunteers, etodolac twice daily did not suppress gastric mucosal prostaglandin production and caused less gastric damage than naproxen (43). Patients with osteoarthritis or rheumatoid arthritis obtained relief from symptoms equal to other commonly used NSAIDs with etodolac, but with a lower incidence of serious gastrointestinal toxicity (44).

Nimesulide is currently sold in Europe and South America for the relief of pain associated with inflammatory conditions. It is a selective inhibitor of COX-2 with about five fold greater potency against this enzyme than against COX-1 in the human whole blood assay (*Table 1*). In limited clinical trials for its use in acute and chronic inflammation in patients it was more effective than placebo or had comparable anti-inflammatory activity to established NSAIDs. Interestingly, nimesulide seems safe to use in aspirin-sensitive asthmatics. Several recent studies in NSAID-intolerant asthmatic patients 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 (45). Perhaps aspirin-induced asthma is associated with COX-1 inhibition?

Interestingly, COX-2 is the constitutive and dominant form of the enzyme in human cultured lung epithelial cells (46).

A recent epidemiological study (47) identified 1505 patients with upper gastrointestinal tract bleeding. It showed nimesulide to have a similar relative risk to that of naproxen (4.4 times control) and more than diclofenac (2.7 times control). Clearly, other factors are also involved, such as frequency of dosage etc. As with other NSAIDs, nimesulide is used in different dosages and when these are separated, the higher doses give a much higher relative risk.

Constitutive COX-2

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 ischaemia when prostaglandin synthesis is reduced by NSAIDs. Synthesis of PGE_2 is mainly by COX-1, although there are discrete cells in the macula densa that contain constitutitive COX-2 (48, 49). Prostacyclin, made by constitutive COX-2 may drive the renin-angiotensin system (49) Schneider and Stahl (50) have reviewed this rapidly evolving field.

Fitzgerald's group (51) compared the renal effects of the non-selective COX inhibitor, indomethacin with those of the COX-2 inhibitor, rofecoxib and with placebo in healthy older adults over two weeks treatment. Both active regimes were associated with a transient but significant decline in urinary sodium excretion during the first 72 hours. The glomerular filtration rate (GFR) was decreased by indomethacin but not changed significantly by rofecoxib. Thus, acute sodium retention by NSAIDs in healthy adults is mediated by inhibition of COX-2, whereas depression of GFR is due to inhibition of COX-1.

The urinary excretion of the prostaglandin metabolite 2,3-dinor-6keto prostaglandin $F_{1\alpha}$ was decreased by both rofecoxib and indomethacin, but not by placebo. The implication of this is that the endothelial cell uses COX-2 to make prostacyclin, this enzyme is possibly induced by the shear stress in the arterial wall, rather than being present constitutitively.

COX-1 is found in neurones throughout the brain but it is most abundant in forebrain where prostaglandins may be involved in complex integrative functions such as control of the autonomic nervous system and in sensory processing. COX-2 mRNA is induced in brain tissue and in cultured glial cells by pyrogenic substances such as LPS, IL-1 or TNF (52). However, low levels of COX-2 protein and COX-2 mRNA have been detected in neurones of the forebrain without previous stimulation by pro-inflammatory stimuli. 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 (53),

whereas acute stress raises levels in the cerebral cortex. COX-2 mRNA is also constitutively expressed in the spinal cord of normal rats and may be involved with processing of nociceptive stimuli (54). 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 (52).

Li *et al.* (55) tested the effects of LPS in producing a fever in knockout mice. Wild type, and $COX\text{-}1_{(+/-)}$ and $COX\text{-}1_{(-/-)}$ mice all responded to LPS with a 1°C rise in core temperature within 1 hour: the fever gradually abated over the next 4 hours. By contrast, $COX\text{-}2_{(+/-)}$ and $COX\text{-}2_{(-/-)}$ mice displayed no temperature rise after LPS. Thus, COX-2 is necessary for the production of fever produced by LPS. A corollary of this finding is that there is unlikely to be a COX-3 through which paracetamol brings down a fever. Furthermore, Oates *et al.* (56) has recently shown that in HUVEC cells in culture, paracetamol inhibits COX-2 with an IC_{50} of 66um, well within the therapeutic range in humans. Furthermore, the selective COX-2 inhibitor, rofecoxib, is a potent antipyretic agent in man (57).

Nomenclature

Merck and Searle refer to their new COX-2 inhibitors as "specific", arguing that at therapeutic doses, there is only inhibition of COX-2 and not of COX-1. Pharmacologists use the word "specific" far more rigorously, and „selective" is a more appropriate description. Even more accurate would be „COX-1 sparing drugs".

FUTURE THERAPEUTIC USES FOR SELECTIVE COX-2 INHIBITORS

Premature labour

Prostaglandins (PGF_{2a}) induce uterine contractions during labour. NSAIDs such as indomethacin will delay premature labour 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 fetal kidneys (58). The delay in the birth process is most likely due to inhibition of COX-2 since mRNA for COX-2 increases substantially in the amnion and placenta immediately before and after the start of labour (59), whereas the side effects on the fetus are due to inhibition of COX-1. One cause of pre-term labour could be an intra-uterine infection resulting in release of endogenous factors that increase prostaglandin production by up-regulating COX-2 (ref 60). Nimesulide reduces prostaglandin synthesis in isolated fetal membranes and has been used successfully for a prolonged period to delay premature labour without manifesting the side effects of indomethacin on the fetus (58).

Colon cancer

Epidemiological studies have established a strong link between ingestion of aspirin and a reduced risk of developing colon cancer (61, 62). Sulindac also caused reduction of prostaglandin synthesis and regression of adenomatous polyps in 11 out of 15 patients with familial adenomatous polyposis (FAP), a condition in which many colorectal polyps develop spontaneously with eventual progression to tumors. 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 (63, 64). 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 highly selective COX-2 inhibitor (65—67). Nimesulide also reduced the number and size of intestinal polyps in *Min* mice (68). Furthermore, the development of azoxymethane--induced colon tumours over a year was inhibited in celecoxib-fed rats (69). A clinical trial of celecoxib in patients with FAP (70) has shown a positive reduction in polyps and this indication for the drug has been allowed by the FDA.

Alzheimer's disease

The connection between COX and Alzheimer's disease has been based mostly on epidemiology, because of 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 (71—73). The Baltimore Longitudinal Study of Ageing (74), with 1686 participants showed 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. 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. There is a strong interest in COX-2 in Alzheimer's disease, and expression of COX-2 has been shown in the frontal cortex of brain from Alzheimer's patients (75).

CONCLUSIONS

Selective inhibitors of COX-2 clearly provide important advances in the therapy of inflammation. Conventional NSAIDs are associated with gastro-intestinal side effects, which include ulceration of the stomach,

sometimes with subsequent perforation and deaths estimated at several thousand a year in the USA alone. Selective COX-2 inhibitors have substantially reduced side effects on the stomach. Already, the published extensive clinical results with meloxicam show this improved safety and tolerability. The clinical trial results with rofecoxib or celecoxib are just as dramatic. In addition to their beneficial actions in inflammatory diseases, these drugs may be useful in the future for the prevention of colon cancer, Alzheimer's disease or premature labour.

Finally, the suppression of prostacyclin release from endothelial cells by "specific" COX-2 inhibitors (34) suggests the possibility of interference with the cardio-vascular system. However, we have been using COX-2 inhibitors for many years, because this is how the NSAIDs produce their therapeutic effects. Thus, the "selective COX-2" inhibitors will do nothing different to prostacyclin production than a conventional NSAID, although the prostacyclin thromboxane balance may be changed because of their lack of effect on platelet COX-1.

New side effects of the selective COX-2 inhibitors, if any, may arise from the fact that they cross the blood-brain barrier, far more easily than do the conventional carboxy-acid NSAIDs.

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. Henry D, Lim LL-Y, Rodriguez LAG *et al.* Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. *Br. J. Med.* 1996; 312: 1563—1566.
3. Coles LS, Fries JF, Kraines RG, Roth SH. From experiment to experience: side effects of nonsteroidal anti-inflammatory drugs. *Am J Med.* 1983; 74: 820—828.
4. Fries J. Toward an understanding of NSAID-related adverse events: the contribution of longitudinal data. *Scand J Rheumatol* 1996; 25 (Suppl 102): 3—8.
5. Änggard E, Samuelsson B. Biosynthesis of prostaglandins from arachidonic acid in guinea pig lung. Prostaglandins and related factors. 38. *J Biol Chem* 1965; 240: 3518–21.
6. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biology* 1971; 231: 232—235.
7. Ferreira SH, Moncada S, Vane JR. Indomethacin and aspirin abolish prostaglandin release from spleen. *Nature* 1971; 231: 237—239.
8. Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. *Nature New Biology*. 1971; 231: 235—237
9. Flower RJ, Vane JR. Inhibition of prostaglandin biosynthesis. *Biochem Pharmacol.* 1974; 23: 1439—1450.
10. Vane JR, Botting RM. Anti-inflammatory drugs and their mechanism of action. *Inflamm Res* 1998; 47 (Suppl 2): S78-S87.
11. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol 1998; 38: 97—120.

12. Smith WL, LandsWEM. Oxygenation of polyunsaturated fatty acids during prostaglandin biosynthesis by sheep vesicular gland. *Biochemistry* 1972; 11: 3276—3285
13. Flower RJ and Vane JR. Inhibition of prostaglandin synthetase in brain explains the antipyretic activity of paracetamol (4-Acetamidophenol). *Nature* 1972; 240: 410—411
14. Maddox IS.The role of copper in prostaglandin synthesis. *Biochem Biophys Acta* 1973; 306: 74—81.
15. Lysz TW and Needleman P. Evidence for two distinct forms of fatty acid cyclooxygenase in brain. *J Neurochem* 1982; 38: 1111—1117.
16. Lysz TW, Zweig A, Keeting PE. Examination of mouse and rat tissues for evidence of dual forms of the fatty acid cyclo-oxygenase. *Biochem Pharmacol* 1988; 37: 921—927.
17. Tsai AI, Sanduja R and Wu KK. Evidence for two pools of prostaglandin H synthase in human endothelial cells. 7th International Conference on Prostaglandins and related compounds. Florence, Italy. 1990 Abstract No 41
18. Rosen GD, Birkenmeier TM, Raz A and Holtzman MJ. Identification of a cyclooxygenase-related gene and its potential role in prostaglandin formation. *Biochem Biophys Res Commun* 1989; 164: 1358—1365
19. Raz A, Wyche A and Needleman P. Temporal and pharmacological division of fibroblast cyclooxygenase expression into transcriptional and translational phases. *Proc Natl Acad Sci USA* 1989; **86**: 1657—1661.
20. 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—16740.
21. 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—1379.
22. Simmons DL, Levy DB, Yannoni Y and Erikson RL. Identification of a phorbol ester-repressible v-*src*-inducible gene. *Proc. Natl Acad Sci USA* 1989; 86: 1178—1182.
23. Xie W, Chipman JG, Robertson DL, Erikson RL and Simmons DL. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc. Natl. Acad. Sci. USA* 1991; 88: 2692—2696.
24. Kujubu DA, Fletcher BS, Varnum BC, Lim RW and 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—12872.
25. Simmons DL, Xie W, Chipman J and Evett G. Multiple cyclooxygenases: Cloning of a mitogen-inducible form. In Prostaglandin, Leukotrienes, Lipoxins and PAF, Bailey M (ed). London, Plenum Press, 1991, pp. 67—68.
26. O'Banion MK, Sadowski HB, Winn V and Young DA. A serum- and glucocorticoid-regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein. *J Biol Chem.* 1991; 266: 23261—23267.
27. 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—6388.
28. 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—665
29. 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—273.
30. Meade EA, Smith WL, De-Witt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. *J Biol Chem* 1993; 268: 6610—6614.

31. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ and Vane JR. Selectivity of nonsteroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci USA* 1993: 90; 11693—11697.
32. Vane JR. Towards a better aspirin. *Nature* 1994; 367: 215—216.
33. Hawkey C, Kahan A, Steinbruck K *et al.* Gastrointestinal tolerability of meloxicam compared to diclofenac in osteoarthritis patients. *Br J Rheumatol* 1998; 37: 937—945.
34. Dequeker J, Hawkey C, Kahan A *et al.* Improvement in gastrointestinal tolerability of the selective cyclooxygenase (COX)-2 inhibitor, meloxicam, compared with piroxicam: results of the safety and efficacy large-scale evaluation of COX-inhibiting therapies (SELECT) trial in osteoarthritis. *Br J Rheumatol* 1998; 37: 946—951.
35. Distel M, Meuller C, Bluhmki E, Fries J. Safety of meloxicam: a global analysis of clinical trials. *Br J Rheumatol* 1996; 35: (1): 68—77.
36. 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.
37. Ehrich EW, Dallob A, De Lepleire I, Van Hecken A, Riendeau D, Yuan W, Porras A, Wittreich J, Scibold JR, De Schepper P, Mehlisch DR, Gertz B. Characterization of rofecoxib as a cyclooxygenase-2 isoform inhibitor and demonstration of analgesia in the dental pain model. *Clin Pharmacol Ther* 1999; 65: 336—347.
38. Pairet M, van Ryn J, Mauz A, Schierok H, Diederen W, Turck D, Engelhardt G. Differential inhibition of COX-1 and COX-2 by NSAIDs: a summary of results obtained using various test systems. In: Selective COX-2 Inhibitors. Pharmacology, Clinical Effects and Therapeutic Potential. Vane, J., Botting, J. eds. Kluwer Academic Publishers, Lancaster; William Harvey Press, London. 1998; pp. 27—46.
39. Churchill L, Graham A, Shih C-K, Pauletti D, Farina PR, Grob PM. Selective inhibition of human cyclooxygenase-2 by meloxicam. *Inflammopharmocology* 1996; 4; 125—135.
40. Patrignani P, Panara MR, Greco A. *et al.* Biochemical and pharmacological characterization of the cyclooxygenase activity of human blood prostaglandin endoperoxide synthases. *J Pharmacol Exp Ther* 1994; 271: 1705—1710.
41. TD Warner, F Giuliano, I Vojnovic, A Bukasa, JA Mitchell and JRVane. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: A full *in vitro* analysis. *Proc Natl Acad Sci USA* 1999; 9: 7563—7568.
42. Glaser KB. Cyclooxygenase selectivity and NSAIDs: cyclooxygenase-2 selectivity of etodolac (Lodine). *Inflammopharmacology* 1995; 3: 335—345
43. Laine L, Sloane R, Ferretti M, Cominelli F. A randomised double-blind comparison of placebo, etodolac and naproxen on gastrointestinal injury and prostaglandin production. *Gastrointest Endosc* 1995; 42: 428—433.
44. Cummings DM, Amadio P Jr. A review of selected newer nonsteroidal anti-inflammatory drugs. *Am Fam Physician* 1994; 49: 1197—1202.
45. 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 Safety* 1996; 14: 94—103.
46. Garcia Rodriguez LA, Cattaruzzi C, Troncom MG, Agostinis L. Risk of hospitalization for upper gastrointestinal tract bleeding associated with ketorolac, other nonsteroidal anti-inflammatory drugs, calcium antagonists, and other antihypertensive drugs. *Arch Intern Med* 1998; 158: 33—39.
47. 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: 126—131
48. 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—2510.

49. Harris RC. The macula densa: recent developments. *J Hypertension* 1996; 14: 815—22.
50. Schneider A, Stahl RAK. Cyclooxygenase-2 (COX-2) and the kidney: current status and potential perspectives. *Nephrol Dial Transplant* 1998; 13: 10—12.
51. Catella-Lawson F, McAdam B, Morrison BW, Kapoor S, Kujubu D, Antes L, Lasseter KC, Quan H, Gertz BJ, Fitzgerald GA. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics, and vasoactive eicosanoids. *J Pharmac Exp Ther* 1999;289: 735—741
52. Cao C, Matsumura K, Yamagata K, Watanabe Y. Cyclooxygenase-2 is induced in brain blood vessels during fever evoked by peripheral or central administration of tumor necrosis factor. *Mol Brain Res* 1998; 56: 45—56
53. Marcheselli VL, Bazan NG. Sustained induction of prostaglandin endoperoxide synthase-2 by seizures in hippocampus. *J Biol Chem* 1996; 271: 24794—24799.
54. 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—169.
55. Li S, Wang Y, Matsumura K, Ballou LR, Morehan SG, Blatteis CM. The febrile response to lipopolysaccharide is blocked in cyclooxygenase-$2_{-/-}$ mice. *Brain Research* 1999;825: 86—94
56. Oates JA, Marnett LJ, Boutaud O. The antipyretic action of acetaminophen: inhibition of the cyclooxygenase activity of endothelial cells treated with IL-1. *Abstracts of the 11th International Conference on Advances in Prostaglandin and Leukotriene Research* (Florence June 4—8 2000); p. 37.
57. Schwartz J, Mukhopadhyay S, McBride K, Jones T, Adcock S Sharp P, Hedges K *et al.* (1998) Antipyretic activity of a selective cyclooxygenase (COX-2) inhibitor, MK-0966. *Clin Pharmacol Therap* 1998; 63: 167.
58. 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—266.
59. 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.
60. 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—223.
61. Thun MJ, Manboodiri MM, Heath, CWJ. Aspirin use and reduced risk of fatal colon cancer. *N Engl J Med* 1991; 325: 1593—1596.
62. Luk GD. Prevention of gastrointestinal cancer — the potential role of NSAIDs in colorectal cancer. *Schweiz Med Wochenschr* 1996 126: 801—812.
63. 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(10): 4816—4820.
64. 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.
65. 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—1188.
66. Oshima M, Dinchuk JE, Kargman SL, *et al.* Suppression of intestinal polyposis in $Apc^{\Delta 716}$ knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 1996; 87: 803—809.
67. 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—2259.
68. Nakatsugi S, Fukutake M, Takahashi M, Fukuda K, Isoi T, Taniguchi Y *et al.* Suppression of intestinal polyp development by nimesulide, a selective cyclooxygenase-2 inhibitor, in Min mice. *Jpn J Cancer Res* 1997; 88: 1117—1120.

69. Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. *Cancer Res* 1998; 58: 409—12.
70. Steinbach G, Lynch PM, Lynch, PM *et al.* The Effect of celecoxib, a cyclo-oxygenase-2 inhibitor, in familial adenomatous polyposis. *New Eng J Med* 2000; 342: 1964—52(440).
71. 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.
72. Cochran FR, Vitek MP. Neuroinflammatory mechanisms in Alzheimer's disease: new opportunities for drug discovery. *Expert Opin Invest Drugs* 1996; 5: 449—55.
73. Breitner JCS. The role of anti-inflammatory drugs in the prevention and treatment of Alzheimer's disease. *Annu Rev Med* 1996; 47: 401—11.
74. Stewart WF, Kawas C, Corrada M, Metter EJ. Risk of Alzheimer's disease and duration of NSAID use. *Neurology* 1997; 48: 626—32.
75. Pasinetti GM, Aisen PS. Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease brain. *Neuroscience* 1998; 87: 319—24.

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