

Cyclo-Oxygenase Isoenzymes

How Recent Findings Affect Thinking About Nonsteroidal Anti-Inflammatory Drugs

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Summary

The discovery of at least 2 cyclo-oxygenase (COX) isoenzymes, referred to as COX-1 and COX-2, has updated our knowledge of nonsteroidal anti-inflammatory drugs (NSAIDs). This has lead investigators to reconsider what can be awaited from this class of drugs. The 2 COX isoenzymes share structural and enzymatic similarities, but are specifically regulated at the molecular level and may be distinguished apart in their functions, although some physiological overlap between them does occur.

The major goal in developing selective COX inhibitors is to improve NSAID tolerability. Classic NSAIDs preferentially inhibit COX-1 *in vitro*, but it appears hazardous to judge their gastrointestinal (GI) safety profile from these data. New compounds with a high selectivity for COX-2, especially those that are non-acidic, may be better tolerated in the GI tract.

While these compounds also might have a potential use in various diseases such as colorectal cancer and neurodegenerative diseases of the Alzheimer type, the possible appearance of adverse effects, perhaps renally-related, must be taken into consideration. Finally, well-designed large clinical trials are required to adequately estimate both the promising therapeutic advantages that may be offered by highly selective NSAIDs, and the potential drawbacks that may be inherent with prolonged COX-2 inhibition.

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In 1971, aspirin-like drugs were shown to inhibit prostaglandin (PG) synthesis in cell-free homogenates^[1] and PG release from platelets^[2] and spleen.^[3] This discovery, which was made simultaneously in cells and tissues from various species including humans, led to a comprehensive hypothesis of the therapeutic actions of aspirin-like drugs and also to the recognition that nonsteroidal anti-inflammatory drugs (NSAIDs) were potent inhibitors of PG synthesis.^[4] This hypothesis was unifying in nature relative to the mechanism of action of NSAIDs, and it has turned out to be largely correct because over 40 NSAIDs in clinical use have been found to inhibit PG synthesis.^[5]

Cyclo-oxygenase (COX), the enzyme that catalyses the synthesis of cyclic endoperoxides from arachidonic acid to form PGs, was considered to be unique. Nevertheless, the inhibitory properties of NSAIDs vary in different regions of the body,^[6,7] as exemplified by the capacity of paracetamol (acetaminophen) to inhibit PG synthesis specifically in the brain.^[6] Such 'tissue specificity' may have been related to a variable partitioning of NSAIDs between organs but suggested also that there might be several molecular forms of the PG generating system,^[8] either because there were separate isoenzymes or because the same enzyme had varying degrees of activity.^[9] The apparent discrepancy between the clinical efficacy of non-acetylated salicylates^[10] and their poor inhibitory potency on COX^[11] further questioned whether there might be multiple COX enzymes or alternative mechanisms of action for NSAIDs,^[11] as for example in analgesia.^[12]

The 1990s has seen a new dawn in inflammatory research, with biological studies demonstrating increased COX activity in a variety of cells after exposure to endotoxin,^[13,14] pro-inflammatory cytokines,^[15,16] growth factors,^[17] hormones,^[18] tumour promoters^[19] and in *v-src* transformed cells.^[20] This activity required new protein synthesis and was inhibitable by corticosteroids, giving rise to the concept that there might be a 'constitutive COX activity', further referred to as

COX-1, and an 'inducible' one, further referred to as COX-2.^[21]

These 2 COX isoforms are encoded by different genes, and COX-1 was hypothesised to be a 'housekeeping gene' while COX-2 was thought to be involved in inflammation, mitogenesis and/or specialised signal transduction.^[22] This hypothesis gave a new impetus to the COX theory and led to the idea that the anti-inflammatory, analgesic and antipyretic effects of NSAIDs might be related to their capacity to inhibit COX-2, while their antithrombotic and perhaps most of their adverse effects might be due to their capacity to inhibit COX-1.^[23,24]

Although the discovery of 2 COX isoenzymes has not provided an indisputable explanation for the mechanism of action of salicylates or paracetamol,^[15,23] it has given rise to a better understanding of the inhibition of COX by classic NSAIDs and offers the prospect of devising new and potentially safer drugs.

The aim of the present article is to highlight some key points relative to the relationships between NSAIDs and COX isoenzymes, to define the COX selectivity of NSAIDs, to present new compounds, and to discuss some clinical effects of selective COX-2 inhibition, especially in the stomach.

1. Cyclo-Oxygenase (COX) Isoenzymes

1.1 Physicochemical Properties

COX isoforms are bifunctional haemoproteins that catalyse both the bisoxygenation of arachidonic acid to form PGG₂ and the peroxidative reduction of PGG₂ to form PGH₂ (reviewed elsewhere^[25,26]). Both catalytic sites are separable and COX activity can be destroyed without inhibiting peroxidase activity.^[27]

These enzymes are the common primary step in prostanoid synthesis and lead to various derivatives depending on the type of cell or tissue. The overall length of the 2 isoenzymes is almost identical, varying from 599 (human) to 602 (mice) amino acids for COX-1 and 603 (mice) to 604 (hu-

man) amino acids for COX-2 (reviewed by Xie et al.^[28]).

For a given COX isoform there is approximately 90% (81 to 98%) identity between species, but there is only 59 to 62% homology between the 2 COXs at the nucleic acid and amino acid levels. The major differences are that COX-1 contains a sequence of 17 amino acid in its amino terminus that is not present in COX-2, while COX-2 has an additional insert of 18 amino acids in its carboxy terminus. However, the amino acid residues thought to be important for catalysis are conserved,^[22] and the two isoenzymes have about the same affinity (K_m) and capacity (V_{max}) to convert arachidonic acid to PGH_2 .^[29] COX-2 is also able to metabolise C18 and C20:3 fatty acids as substrates, while COX-1 is mainly restricted to arachidonic acid (C20:4), which suggests that the COX-2 site is probably larger.^[30]

1.2 Regulation

Unlike their high similarity in physicochemical properties, the 2 COX isoenzymes are distinctly regulated at both the transcriptional and post-transcriptional level (reviewed elsewhere^[26,31-33]). The introns are much larger in the COX-1 than in the COX-2 gene accounting for lengths of 22 to 23kb and 8 to 9kb, respectively.^[34,35] The 2 COX genes differ in their promoters and in their transcripts and these differences account for the striking difference in their regulation. The transcripts of COX-1 and COX-2 genes differ significantly in size and are, respectively, of 2.8 to 3.6kb and 4.0 to 4.1kb.^[28,31,32]

Very importantly, the 3'-untranslated region of COX-2 contains several copies of Shaw-Kamen's sequence which is found in many immediate-early genes and has been shown to confer enhanced mRNA degradation. Thus, the transcripts of the COX-2 gene are prone to degrade quickly, and their stabilisation by proinflammatory cytokines participates in the enhancement of COX-2 levels.^[16,36,37] In contrast, corticosteroids may be able to destabilise COX-2 mRNA and/or to inter-

fere with translation^[38,39] without affecting basal COX-1 expression.^[40]

The promoter of the COX-1 gene is consistent with both the classification of COX-1 as a 'house-keeping' gene and its poor inducibility.^[31] In contrast, the promoter of the COX-2 gene contains many transcriptional factor consensus sequences which have been shown to have functional relevance, such as the nuclear factor of interleukin-6 (NF-IL6) sequence for the induction of rat COX-2^[18] or the cyclic adenosine monophosphate (cAMP) response element (CRE) for the induction of mouse^[41] and human COX-2.^[42] As most of these transcription factors are known to be possibly up-regulated as a result of cell stimulation by pro-inflammatory cytokines or tumour promoters, it appears logical that COX-2 is expressed during inflammation.

In addition, the CRE sequence allows PGs to modulate their own synthesis by COX-2 through changes in cellular cAMP levels secondary to their membrane binding on receptors coupled to the adenylate cyclase pathway.^[43] As there is no glucocorticoid-negative responsive element within the promoter of the COX-2 gene,^[34,35,44] the blocking effect of dexamethasone is probably mediated by an indirect mechanism.^[45,46] In view of these molecular phenomena, it is clear that COX-1 is only marginally regulated, whereas COX-2 is a highly inducible system.

1.3 Localisation

The tissue distribution of COX-1 and COX-2 differs somewhat between species^[47-49] and can be heterogeneous within the same tissue.^[50,51] For example, COX-2 is the major isoform in rat^[50] and mouse^[49,52] brain, whereas similar levels of both isoforms have been detected in human brain. This discrepancy may be due to the problems of post-mortem tissue preparation in human brain. It may result from a true heterogeneity in the levels of COXs in brain.^[50,53]

Both isoforms were found in human brain, with COX-1 being the major isoform in most tissues.

rat,^[49,54] whereas COX-2 was found to be expressed to a similar extent as COX-1 in human tissue.^[48] In the rat stomach, COX-2 was found in the surface mucous cells while COX-1 was found in mucous neck cells.^[51] Similarly, heterogeneous distribution was observed in kidney,^[49,55,56] with COX-2 localised in the macula densa and possibly contributing to the regulation of salt, volume and blood pressure homeostasis.^[56]

These results and those obtained with other tissues, such as the stomach, suggest that both COXs play a physiological role.

In addition, COX isoenzymes also partition at the cellular level since COX-1 functions primarily in the endoplasmic reticulum, whereas COX-2 activity is located both in the endoplasmic reticulum and in the nuclear envelope.^[57] Furthermore, COX-2 immunoreactivity was twice as concentrated in the nuclear envelope as in the endoplasmic reticulum. This compartmentalisation suggests that COX isoenzymes may represent two temporally and spatially separated prostanoid biosynthetic systems (recently reviewed^[26]), with COX-2 producing prostanoids for intranuclear differentiative or replicative events.^[28,58,59]

Finally, the hypothesis of a functional compartmentalisation of COX isoenzymes is further supported by their ability to metabolise arachidonic acid from different pools (reviewed by Herschman^[32]). For example, mast cells stimulated with IgE show a biphasic production of PGD₂,^[60,61] possibly related to different eicosanoid synthetic pathways:

- an early COX-1-mediated pathway, using arachidonate released by soluble phospholipase A₂ (PLA₂);
- a late COX-2 mediated pathway, using arachidonate released by cytosolic PLA₂ and mimicking cytokine stimulation.

Although this hypothesis has not been confirmed in other cell types,^[62] it suggests the possibility that there is a transcellular pathway of PG biosynthesis involving circulating soluble PLA₂ as an arachidonate-generating system for cellular COX-1.

The compartmentalisation of COX isoenzymes is an attempt to explain their respective role at the cellular level when gene disruption techniques may allow an understanding of their biological relevance. In one study, mice homozygous for COX-1 deficiency ('knock-out') survived well, had no gastric pathology, and showed either less indomethacin-induced gastric ulceration and/or a smaller ulcerated area of the stomach surface than mice with wild type COX-1.^[63] In the ear oedema model, COX-1 null mice also had reduced platelet aggregation and decreased inflammatory response to arachidonic acid, but not to phorbol ester stimulation.

By comparison, COX-2 null mice had a reduced lifespan and had no innate gastrointestinal (GI) pathology, but had kidney abnormalities that caused a progressive deterioration as the animals aged.^[64,65] COX-2 null mice had a normal inflammatory response to arachidonic acid and phorbol ester stimulation, but some other phenotypic changes were distinctly observed between the 2 studies.^[64,65]

Although different phenotypes may be observed for disrupting identical genes in alternate genetic backgrounds, these results suggest the importance of COX-2 activity in the development of diverse organ systems and question the role of PG synthesis inhibition in gastric injury.

2. The Fundamental Basis of COX-NSAID Relationships

2.1 Structural Analysis

The x-ray crystal structure established by Picot and colleagues^[66] suggests that COX-1 is a dimeric enzyme in a monotopic arrangement with respect to the cell membrane, each monomer comprising 3 independent folding domains. The overall space arrangement defines a long hydrophobic channel which allows arachidonic acid to gain access directly from the membrane,^[67] and where several amino acids appear to play a critical role: Arg¹²⁰ and Glu⁵²⁴, which form a salt bridge together; Tyr³⁵⁵, which makes a narrow constriction with

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Arg¹²⁰ in the middle portion of the channel; Ser⁵³⁰, which is known to be acetylated by aspirin;^[68] and Tyr³⁸⁵, which is thought to support the oxygenase activity as peroxide-generated tyrosyl radicals.^[69]

In a stereochemically reasonable model, arachidonic acid would be in a bent conformation, with its carboxylate interacting with the guanidinium group of Arg¹²⁰ and its C13 in the vicinity of Tyr³⁸⁵. Flurbiprofen docks in a similar fashion, suggesting that NSAIDs can inhibit COX-1 activity by excluding arachidonate from the upper portion of the channel.^[66]

A crystallographic study of COX-1 inactivated by a potent aspirin analogue^[70] further demonstrated that:

- an inhibitory effect was obtained by steric blockage of the active site channel;
- aspirin was bound initially at Arg¹²⁰ and allowed subsequent transacetylation of Ser⁵³⁰;
- an acetyl-serine side-chain existed in 2 rotameric states, with a minor conformer allowing the enzyme to accommodate a fatty acid substrate, albeit in an abnormal conformation.

Such crystallographic studies did not support any significant conformational change of the COX-1 protein^[71] when complexed with an indomethacin derivative.^[72] Site-directed mutagenesis studies^[73,74] confirmed that Arg¹²⁰ was important for biotransformation of arachidonic acid and required for inhibitory activity of NSAIDs containing a carboxylic acid, whereas Tyr³⁵⁵ was involved in stereospecificity of COX-1 towards 2-phenylpropionic derivatives. New NSAIDs, such as DuP-697, which have shown a preferential inhibition of COX-2 over COX-1 (see below in sections 2.2 and 4), inhibited wild type and Arg¹²⁰-mutated COX-1 in the same range,^[73] suggesting interaction with amino acids other than Arg¹²⁰, whose spatial arrangement is not affected by the mutation. The data cannot be extrapolated to NSAIDs lacking a carboxylate group,^[75] such as pyrazolones and oxicams.

The x-ray crystal structure of human COX-2, which has been resolved very recently, shows a straight homology to ovine COX-1 and a highly

conserved amino acid sequence within the active site.^[76] This striking similarity is consistent with the close enzymatic properties of both enzymes towards arachidonate^[29] but fails to provide a definitive explanation for their differential inhibition by NSAIDs,^[30,77,78] although COX-2 may exist in multiple conformations and/or may be flexible to some extent.^[76]

A plausible explanation has been proposed on the basis of the differential effect of aspirin, which completely inhibits ovine COX-1 activity by acetylating^[68] Ser⁵³⁰ whereas it modifies COX-2 activity by acetylating Ser⁵¹⁶ such that it produces 15-*R*-hydroxy-eicosatetraenoic acid (15-*R*-HETE) instead of PGG₂.^[79,80] It is suggested that the minor acetylated conformer of COX-1^[70] should represent the major one in COX-2, and could lead to an abnormal biotransformation of arachidonate into 15-*R*-HETE.^[81]

Another plausible explanation is that differences between the 2 COX isoenzymes may have quite a subtle physical basis.^[82,83] Thus, a single amino acid which is different between the 2 COX hydrophobic channels seems critical for the ability of a new compound, SC-58125 (see below in section 4), to inhibit COX-2 selectively over COX-1.^[84]

Finally, it can be argued that either the active site may be in a less constrained conformation in COX-2 than in COX-1 or that there may be different subsites for drug binding within the 2 active sites.

CLASSIFICAZIONE NSAIDS

2.2 Mechanistic-Based Enzymatic Studies

NSAIDs can be classified into 3 groups based on their modes of inhibition of COXs:^[85-89]

- class I: simple, competitive inhibitors, including most NSAIDs;
- class II: competitive, time-dependent, reversible; also referred to as 'slow-tight binding' inhibitors, and includes flurbiprofen, indomethacin, meclofenamate, diclofenac and some new compounds such as DuP-697, NS-398 and nimesulide.^[78,85,87,90] This mode of inhibition is consistent with a 2-step process, involving an

Table 1. Biological systems proposed for assessing the selectivity of nonsteroidal anti-inflammatory drugs (NSAIDs) towards cyclo-oxygenase (COX) isoenzymes. Before *in vitro* induction of COX-2 in total blood systems, platelet COX-1 was sometimes inhibited either by oral aspirin intake [300mg, 48h before sampling (*)] or by the addition of aspirin to the culture median [10 µmol/L for 5h (*) or 300 µmol/L for 1h (*)]

Biological systems		Pre-incubation time (min)	AA conc. (µmol/L)	Incubation (min)	Assays	References
COX-1	COX-2					
Enzymes						
<i>Purified animal enzymes</i>						
Ram seminal vesicle	Sheep placenta	2	100	2	PGE ₂	92
		0	7	10	[¹⁴ C]PGs	77
<i>Homogenates of animal enzymes</i>						
BAEC	J744.2 ^a (+LPS 12h) ^b	COX-1: 30 COX-2: 30	30 30	15 15	6-keto-PGF _{1α} PGE ₂	77
<i>Recombinant murine enzymes</i>						
<i>Purified</i>						
rmCOX-1	rmCOX-2	10	10	10	PGE ₂	49
<i>Microsomes</i>						
COS1 mammalian cells	COS1 mammalian cells	0	10	0	O ₂ consumption	30
<i>Recombinant human enzymes</i>						
<i>Purified</i>						
rhCOX-1	rhCOX-2	0	100	0	O ₂ consumption	88
rhCOX-1	rhCOX-2	0-5	100	0	Oxidation of TMPD	87
rhCOX-1	rhCOX-2	?	20	0.8	[¹⁴ C]PGs	93
<i>Microsomes</i>						
COS7 mammalian cells	COS7 mammalian cells	5	2	40 (20°C)	PGE ₂	94
Sf9 insect cells	Sf9 insect cells	30	30	0.6	PGF _{2α}	95
COS1 mammalian cells	COS1 mammalian cells	0	10	0	O ₂ consumption	78
<i>Homogenates</i>						
Sf21 insect cells	Sf21 insect cells	10	10	10	PGE ₂	96
Whole cell systems						
<i>Animal cells</i>						
BAEC	J744.2(+LPS 12h) ^b	30	30	15	6-keto-PGF _{1α}	77
GP macrophages	GP macrophages (+LPS 6h) ^b	30	33	20	PGE ₂	97
<i>Human cell lines</i>						
U937	Osteosarcoma 143	15	10	10	PGE ₂	98
<i>Mixed cellular systems</i>						
Human platelets	Rat mesangial cells (+IL-1 24h) ^b	15	2	15	TxB ₂ 6-keto-PGF _{1α}	99
<i>Transfected COS1 cells</i>						
rhCOX-1	rhCOX-2	30	30	30	PGE ₂	100
<i>Human blood cells</i>						
Total blood clotting time (1h)	Total blood (+LPS 24h) ^b	COX-1: 0 COX-2: 15	0 0	60 24h	TxB ₂ PGE ₂	101
	(+LPS 4h or 24h) ^b	COX-1: 0 COX-2: 0	0 0	60 4h or 24h	TxB ₂ PGE ₂	102*
	Monocytes (+LPS 4/24h) ^b	COX-1: 0 COX-2: 0	0 0	60 4h or 24h	TxB ₂ TxB ₂	103*
Total blood (+A-23187° 30 min)	Total blood (+LPS 5h) ^b	COX-1: 4.5h COX-2: 15	0 0	30 5h	TxB ₂ TxB ₂	95
		COX-1: 4.5h COX-2: 0	0 0	30 5h	TxB ₂ TxB ₂	104 [†]
Human platelets	Monocytes (+LPS 4h) ^b	COX-1: 60 COX-2: 0	10 1	10 10	TxB ₂ TxB ₂	105 [‡]

a Murine macrophage.

b Stimulation with bacterial endotoxin (LPS) or interleukin-1 (IL-1) for indicated time.

c A calcium ionophore.

Abbreviations and symbols: AA = arachidonic acid; BAEC = endothelial cells from bovine aorta; GP = guinea-pig; IL-1 = interleukin-1; PG = prostaglandin; rh = recombinant human; rm = recombinant murine; TMPD = tetramethyl-*p*-phenylenediamine; TxB₂ = thromboxane B₂; U937 = human myeloid leukaemia cells.

cyclo-oxygenase
inhibited by oral aspirin
for 1h (†)

References

initial rapid and reversible equilibrium binding of enzyme and inhibitor, followed by the formation of a tightly bound enzyme-inhibitor complex which dissociates slowly as a result of a structural transition.^[87-89]

- class III: competitive, time-dependent, irreversible inhibitors, including salicylate derivatives such as aspirin, which produce a covalent modification of the enzyme.^[79,80,91]

For a given inhibitor, the type of inhibition may differ in the 2 COX isoenzymes – for example, class I inhibition for COX-1 and class II inhibition for COX-2. This would partially account for the selectivity of nimesulide,^[90] DuP-697 and NS-398^[87,88] for COX-2 over COX-1.

3. Determination of NSAID Selectivity for COX Isoenzymes

Biological systems (most of which are summarised in table I) have been extensively developed to study the selectivity of NSAIDs for COX isoenzymes. The many systems have given rise to a multitude of IC₅₀ (concentration at which 50% of activity is inhibited) values (mainly compiled in Battistini et al.^[24]) and to sometimes confusing comparisons.^[106] In fact, the indices of selectivity, usually expressed as the ratios of IC₅₀ COX-2 : IC₅₀ COX-1, are not directly comparable when obtained from different sources and under different experimental conditions.^[107]

As shown in table I, the biological systems may differ in:

- the source of enzymes;
- the purity of the enzymatic activity;
- the partition of NSAID within the system;
- the time needed for drug binding equilibrium (pre-incubation);
- the source of arachidonate as substrate and, if arachidonate is added, its concentration;
- the incubation time;
- the nature of the metabolite to be studied in similar systems;
- the drug protein binding in the medium;
- the biotransformation of the drug in the system.

As a consequence, many and sometimes controversial COX inhibitory potencies have been published for NSAIDs, such as those for fenamates.^[93,104] Given these considerations, it can be concluded that IC₅₀ data are useful in drug development and classification, but that for discussing COX selectivity of NSAIDs, it is essential to obtain an overall picture using values from several systems.^[108,109]

Thus, cellular measurement of potency and selectivity, which considers the ability of drug to concentrate within cell membranes and sometimes its biotransformation,^[102,103,110] are required to confirm the results of isolated enzyme assays, which are not physiologically relevant. Moreover, as the pharmacokinetics of the drug can influence its concentration within specialised tissues, it seems important to compare *in vitro* data with whole animal measurements,^[109] such as the ratio of inhibitory potency within inflammatory sites (thought to be mainly COX-2 dependent) to inhibitory potency within the stomach (thought to be mainly COX-1 dependent).^[111,112]

Finally, it must be remembered that the relative inhibition of COX-1 and COX-2 is not known within tissues, as it is for example in blood, when NSAIDs are administered therapeutically in humans.^[113,114] In such clinical situations, COX isoenzymes may be exposed to drug concentrations exceeding largely the IC₅₀ determined *in vitro*, so that the use of selectivity indices as an indicator of the likelihood of adverse effects is somewhat questionable.

4. COX-2 Selectivity of NSAIDs

For the first time, classic NSAIDs have been tested in various systems and have been found generally to be more active against COX-1 than COX-2,^[24] with some exception possibly attributable to changes in experimental conditions. Very interestingly, some newer NSAIDs such as nabumetone (specifically its active metabolite 6-methoxy-2-naphthylacetic acid [6-MNA]) and

= interleukin-1;
from boxane B₂;

Table II. Comparison of new nonsteroidal anti-inflammatory drugs (NSAIDs) and indomethacin for their overall selectivity towards cyclo-oxygenase (COX) isoenzymes

System	IC ₅₀ (mol/L)	NS-398	flosulide (CGP-28238)	L-745337	nimesulide	T-614	SC-58125	DuP-697	meloxicam
Isolated enzymes									
<i>Purified animal</i>									
COX-1 (seminal vesicle)	0.2-0.6 × 10 ⁻⁶ [97,130,131]				>100-985 × 10 ⁻⁶ [30,131]				4 × 10 ⁻⁶ [97]
COX-2 (sheep placenta)	4.1-15 × 10 ⁻⁶ [97,130,131]				7.1-90.3 × 10 ⁻⁶ [30,131]				6 × 10 ⁻⁶ [97]
<i>Recombinant murine</i>									
COX-1 (homogenate)	10-200 × 10 ⁻⁹ [49,112]	>100 × 10 ⁻⁶ [112]					>10 × 10 ⁻⁶ [49]		
COX-2 (homogenate)	50-300 × 10 ⁻⁹ [49,112]	30 × 10 ⁻⁹ [112]					50 × 10 ⁻⁹ [49]		
<i>Recombinant human</i>									
COX-1 S19 (purified)	0.1-1.7 × 10 ⁻⁶ [93,100]	75 × 10 ⁻⁶ [93]			70 × 10 ⁻⁶ [93]				37 × 10 ⁻⁶ [100]
COX-2 S19 (purified)	0.4-25 × 10 ⁻⁶ [93,100]	1.8 × 10 ⁻⁶ [93]			1.3 × 10 ⁻⁶ [93]				0.5 × 10 ⁻⁶ [100]
COX-1 S121 (homogenate)	0.1 × 10 ⁻⁶ [96]	>100 × 10 ⁻⁶ [96]					0.8 × 10 ⁻⁶ [96]		
COX-2 S121 (homogenate)	0.9 × 10 ⁻⁶ [96]	0.1 × 10 ⁻⁶ [96]					10 × 10 ⁻⁶ [96]		
Whole cells									
<i>Animal origin</i>									
Guinea-pig macro- phages (COX-1)	0.2 × 10 ⁻⁹ [97]								5.8 × 10 ⁻⁹ [97]
Guinea-pig macro- phages +LPS ^a (COX-2)	6.4 × 10 ⁻⁹ [97]								1.9 × 10 ⁻⁹ [97]
BAEC (COX-1)	0.03 × 10 ⁻⁶ [122,132]				27 × 10 ⁻⁶ [132]				0.21 × 10 ⁻⁶ [122]
J774.2 +LPS ^a (COX-2)	01.7 × 10 ⁻⁶ [122,132]				0.32 × 10 ⁻⁶ [132]				0.17 × 10 ⁻⁶ [122]
<i>Human origin</i>									
U937 (COX-1)	5-10 × 10 ⁻⁹ [98,123]	>10 × 10 ⁻⁶ [123]	>50 × 10 ⁻⁶ [123]	>10 × 10 ⁻⁶ [98]	>10 × 10 ⁻⁶ [123]		1.0 × 10 ⁻⁶ [123]		
Osteosarcoma 143 (COX-2)	10-50 × 10 ⁻⁹ [98,123]	1.0 × 10 ⁻⁹ [123]	50 × 10 ⁻⁹ [123]	23 × 10 ⁻⁹ [98]	10 × 10 ⁻⁹ [123]		10 × 10 ⁻⁹ [123]		
COS cells (rhCOX-1)	20 × 10 ⁻⁹ [100]				1.6 × 10 ⁻⁶ [100]				2.2 × 10 ⁻⁶ [100]
COS cells (rhCOX-2)	30 × 10 ⁻⁹ [100]				0.4 × 10 ⁻⁶ [100]				0.2 × 10 ⁻⁶ [100]
Platelets (COX-1)	3.2 × 10 ⁻⁶ [105]	1.2 × 10 ⁻⁴ [105]	2.0 × 10 ⁻⁴ [105]		3.2 × 10 ⁻⁴ [105]		1.0 × 10 ⁻⁶ [105]		
Monocytes +LPS ^a (COX-2)	1.6 × 10 ⁻⁹ [105]	3.2 × 10 ⁻⁷ [105]	1.6 × 10 ⁻⁷ [105]		1.0 × 10 ⁻⁷ [105]		3.2 × 10 ⁻¹⁰ [105]		
Whole blood (COX-1)	0.2 × 10 ⁻⁶ [101]	4.8 × 10 ⁻⁶ [101]	32.3 × 10 ⁻⁶ [101]	>30 × 10 ⁻⁶ [101]			>30 × 10 ⁻⁶ [101]		
Whole blood (COX-2)	0.5 × 10 ⁻⁶ [101]	0.5 × 10 ⁻⁶ [101]	0.8 × 10 ⁻⁶ [101]	9.7 × 10 ⁻⁶ [101]			2.3 × 10 ⁻⁶ [101]		
In vivo systems									
Safety index in the rat (drug/indomethacin)		346/0.6[134]	>50/<1.3[118]	>1.3/3.3[98]	12/2[111]	>160/2[124]	>100/1[49]	>2200/<30[120]	20/3.5[133]

^a Stimulation with bacterial endotoxin.

Abbreviations: BAEC = endothelial cells from bovine aorta; IC₅₀ = concentration at which 50% of activity is inhibited; rh = recombinant human.

etodolac were found to have a well balanced inhibitory capacity for both isoenzymes.^[78,93,95,102]

In this initial phase of the search for selective COX-2 inhibitors, the structures that turned out to be the most selective were in the two least numerous classes of NSAIDs which have been known for 20 years or more^[115,116]: the sulphonamides, including NS-398^[117] and flosulide (CGP-28238),^[118] which are close analogues of the parent drug nimesulide;^[119] and the nonacidic tricyclics, including DuP-697,^[120] which is closely related to the older anti-inflammatory drugs indoxole and bimetopryrol. At the same time, other structures such as di-ter-butylphenols, including the dual 5-lipoxygenase/COX inhibitor BF-389^[121] and the oxicam derivative meloxicam,^[97] have shown some selectivity for inhibition of COX-2.^[97,100,122]

Since then, extensive research into structure-activity relations has been carried out to develop highly selective compounds, both in the arylsulphonamide group,^[123] leading to several patented products such as T-614^[124] and L-745337,^[98] and in the 1,2-diarylheterocycle group,^[125] leading to many more patented products, such as SC-57666,^[126] SC-58125^[49] and celecoxib (SC-58635).^[127] Among these compounds, T-614 was also found to inhibit COX-2 induction in cultured fibroblasts,^[128] whereas SC-58125 displayed a similar effect in adjuvant-induced arthritis in rats.^[129]

Inhibitory properties of these molecules towards COX isoenzymes are summarised in table II, by using indomethacin as a reference (unfortunately COX-1 selective) drug. As can be seen, all the molecules inhibit COX-2 preferentially, although the following points need to be underlined: (i) the selectivity for COX-2 over COX-1 is supported by a poor inhibitory effect on COX-1 and not by an improved inhibitory effect on COX-2; (ii) the order of potency for COX selectivity generally decreases from *in vitro* assays to *in vivo* assays.

For a given drug, the therapeutic index in the rat, also referred to as the 'safety index', is based

on its ulcerogenicity relative to its effect in an experimental model.^[117,133] Clearly, the safety index varies depending on the inflammatory model used (acute *versus* chronic) and/or whether it refers to analgesia or fever,^[117] so that drugs cannot be compared directly. However, for a given drug, the comparison of the safety index with the *in vitro* COX-2 inhibitory potency highlights that the COX-selectivity is strongly influenced by the pharmacokinetics of the drug – for example, its biotransformation,^[135] cellular concentration^[136] or particular tissue distribution.

These preferential COX-2 inhibitors displayed anti-inflammatory, analgesic, and antipyretic properties in classic experimental models in the rat (see table III), confirming that COX-2 plays a pathological role in inflammation, pain and fever.^[49,112]

In humans, several compounds, such as L-745337, T-614 and celecoxib, are in preclinical development or in early clinical trials,^[109,127,139] while others, such as NS-398 and flosulide, have been discontinued.^[109,139,140] Consequently, data on the therapeutic efficacy of this class of compound in human diseases are available mainly for the 2 products marketed to date: nimesulide and meloxicam. Both drugs have been studied in well controlled clinical trials and have been found to be at least as effective as the classic NSAIDs, both in painful diseases and chronic inflammatory joint diseases.^[135,141] Nevertheless, classic nonselective NSAIDs are also effective,^[142] so that the main appeal of these new NSAIDs lies in an improved safety profile.^[113,114]

5. Clinical Relevance of Selective COX-2 Inhibition in the Stomach

5.1 NSAID-Induced Gastropathy

It is now well established that there is a relationship between NSAID intake and gastroduodenal injury, in both healthy volunteers and patients with various arthritic diseases.^[143-145] Around 15 to 20% of patients taking NSAIDs develop peptic ulcer and about 3% of this group go on to experience haemorrhage or perforation.^[143] As there are 70 to

Table III. Comparison of new nonsteroidal anti-inflammatory drugs (NSAIDs) and indomethacin for their overall effects in the rat (dose in mg/kg)

	Indomethacin	NS-398	Flosulide (CGP-28238)	L-745337	SC-58125	DuP-697	T-614	Nimesulide	Meloxicam
Pharmacological effects in experimental models									
ID ₅₀ PG inflammatory exudate	0.2-0.9 ^[49,98,111,112,118,124]	0.3 ^[112]	<2.0 ^[118]	<3.0 ^[98]	0.1 ^[49]		0.6 ^[124]	1.3 ^[111]	
ED ₃₀₋₅₀ hyperalgesia	1-3.1 ^[49,98,117,120,133,137]	0.2-1.6 ^[117]		0.4 ^[98]	10 ^[49]	3.5 ^[120]	3.8-4.4 ^[137]	2.4-3.5 ^[137]	5.6 ^[133]
ED ₃₀₋₅₀ oedema	1.5-3.4 ^[49,98,117,118,133,137]	1.15 ^[117]	2.0 ^[118]	2.0 ^[98]	10.0 ^[49]		3.6 ^[137]	2.4-3 ^[137]	3.3 ^[133]
ED ₄₀₋₅₀ adjuvant arthritis	0.3-0.8/day ^[118,120,133,137,138]		0.05/day ^[118]			0.18/day ^[120]	5.2/day ^[137]	0.6-1.6/day ^[137,138]	0.12/day ^[133]
ED ₅₀ fever	1.0-8.0 ^[98,117,118,120,137,138]	1.8 ^[117]	2.0 ^[118]	3.8 ^[98]		0.05 ^[120]	5.6 ^[137]	0.5-0.8 ^[137,138]	
Gastrointestinal tolerance									
Gastrointestinal lesions (threshold dose)	4-5 ^[10,98,112,118,138]	>30 ^[112]	30 ^[118]	>30 ^[98]				100 ^[138]	
TD ₅₀ gastric lesions	2.4-5.8 ^[49,117,120,133,137]	>1000 ^[117]			>600 ^[49]	>400 ^[120]	>1000 ^[137]	106 ^[137]	2.5 ^[133]
TD ₅₀ intestinal lesions	<8-10 ^[49,120,137]				>200 ^[49]	>400 ^[120]	>1000 ^[137]	<30 ^[137]	
ID ₅₀ PG stomach mucosa	<2.5 ^[49,98,111,112,118,124]	>10 ^[112]	>100 ^[118]	>3 ^[98]	>10 ^[49]		>100 ^[124]	15.7 ^[111]	

Abbreviations: ED₅₀ = dose at which 50% of pharmacological effect is inhibited; ID₅₀ = dose at which 50% of mediator release is inhibited; PG = prostaglandin; TD₅₀ = median dose calculated by the moving-average method of Thompson (Bacteriol Rev 1947; 11: 115).

100 million prescriptions for NSAIDs per year in the US and an estimated 7-fold higher consumption of over-the-counter drugs,^[145] gastropathy from NSAIDs is recognised as the most frequent serious complication from medication therapy.^[146]

The basis of gastrototoxic effects is multifactorial^[145] and may include both the NSAID-mediated topical damage and the deleterious effect of PG inhibition.^[147-148] In addition, several factors are associated with an increased risk for developing NSAID gastropathy^[145,149,150] including aging, history of previous GI tract disease or concurrent use of anticoagulants, suggesting that haemostasis and regenerative repair capacity may be also implicated.

Most of the local deleterious effects of NSAIDs are attributable to their acidic character which participates in (i) decreasing surface hydrophobicity of the mucus gel layer with subsequent loss of barrier properties,^[151,152] (ii) uncoupling of oxidative phosphorylation with a subsequent increase in mucosal permeability,^[153,154] and 'back-diffusion' of acidic juice, and (iii) ion trapping into the mucosal epithelium.^[147,148] The systemic injury caused by NSAIDs is thought to be mainly PG-dependent,^[144,147,155,156] although underlying mechanisms between decreased PG levels and mucosal injury are unclear, as are cellular sources of PGs in terms of COX-generating systems. The reduction of blood flow within mucosa may play a pivotal role, since transient hypoxia is associated with oxyradical production,^[155] increased adherence of leucocytes to endothelium,^[157,158] and possibly tumour necrosis factor- α (TNF- α) release.^[159,160]

In addition, nitric oxide (NO) is complementary to PGs in maintaining mucosal blood flow^[161-163] and when considering that pro-inflammatory cytokines are effective inducers of both COX-2 and inducible NO synthase,^[164,165] whereas NO is a potent inducer of COX-2 in some cell types,^[166,167] it can be supposed that an NSAID-induced PG decrease disturbs complex regulatory loops.

Furthermore, inhibition of COX could theoretically enhance the conversion of arachidonate

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through the 5-lipoxygenase pathway,^[155,156] thus potentiating the production of mediators such as peptidoleukotrienes or oxyradicals, that could worsen the ischaemic state accompanying the NSAID-induced fall in PGs within mucosa.^[155]

The role of leukotrienes in the pathogenesis of NSAID-induced mucosal injury has been questioned,^[168,169] although inhibition of the 5-lipoxygenase pathway has shown some experimental preventive effect.^[170,171] Thus, it would be interesting to compare dual COX/5-lipoxygenase inhibitors^[172,173] and selective COX inhibitors in terms of gastrointestinal tolerability.

Judging from molecular^[48,49] and immunohistochemical^[51,174] studies, the cellular source of PGs within the digestive tract may not be restricted to one COX isoenzyme. Nevertheless, the expression of COX-2 within mucous epithelial cells^[51,174] and/or submucosal and muscular layers^[174] needs to be confirmed by using antibodies from different sources, the more so that analysis of stomach samples by RNase protection^[49] or reverse transcriptase-polymerase chain reaction^[48] may not distinguish between resident and infiltrating cells. As COX-1 is thought to be the most intensively expressed isoenzyme in gastric mucosa,^[54] a preferential inhibition of COX-2 would therefore provoke a less dramatic decrease in PG levels than would nonselective inhibition. However, in view of the recent study in mice showing that COX-1 gene disruption is neither associated with spontaneous gastric pathology nor increased susceptibility to NSAID,^[63] it remains unclear as to why NSAIDs cause ulcers.

5.2 Comparison of NSAID Gastrotoxicity

All NSAIDs can initiate serious adverse gastrointestinal events, but their rank order of toxicity may depend on the methodology used to design the clinical study since, for example, NSAIDs producing little or no injury in healthy volunteers cannot be relied upon to be innocuous in elderly patients or those with other risk factors.^[143]

Randomised comparative clinical studies of NSAIDs have concluded with comments on relative toxicities, but most of these studies have been too small to derive meaningful information.^[175] Consequently, most data about the relative risks for upper GI toxicity with various NSAIDs are derived from case-control^[176-181] or cohort studies^[182-184] and from national registries.^[185]

Recent case-control studies based upon UK populations and comparing the relative gastrotoxicity of NSAIDs^[180,181] showed a broadly similar rank order which was in general agreement with that shown in the yellow card data previously presented by the Committee on Safety of Medicines.^[186] Vane^[107] claimed that when the rank order of gastrotoxicity was compared with the overall rank order of COX-2 selectivity obtained in several *in vitro* systems, a good relationship between the 2 rankings was found, suggesting that COX-2 selectivity may account for an improved profile of GI adverse effects.

In broader comparisons of published case-control studies,^[176-179] ibuprofen appears to be the least toxic, while the high ratios of IC₅₀ COX-2 : IC₅₀ COX-1 of drugs such as piroxicam and indomethacin relate to a poor safety profile.^[108] However, when such results are interpreted, the following should be borne in mind.

First, a major limitation of most epidemiological studies is the assumed dose equivalency between NSAIDs, whereas few studies have shown an increased risk for high NSAID dosage.^[180,181] This suggests that the favourable rank of ibuprofen is partly attributable to the low doses used both in the clinic and as an over-the-counter drug.^[187] Secondly, apart from ibuprofen, piroxicam, indomethacin and ketoprofen, NSAIDs showed a great variability in their rank order of gastrotoxicity since studies applied different endpoints as their criteria for considering a GI incident as a case.^[188] Thirdly, the rating of drugs into various risk categories is weakened by the extensive overlap of confidence intervals. Fourthly, none of the drugs listed in these epidemiological studies has demonstrated a firm

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COX-2 selectivity in various experimental systems, so that their comparison refers only to their relative inhibitory potency on COX-1. Finally, when studied,^[180,181,185] azapropazone was the least well tolerated NSAID whereas it is only a weak inhibitor of COX activity.^[189]

Consequently, these epidemiological studies give little weight to the hypothesis that selective inhibition of COX-2 may have a sparing effect on the GI tract.

The newer NSAIDs nabumetone and etodolac have shown an improved GI safety profile in comparison with classic NSAIDs, both in short term endoscopic^[190-192] and in large postmarketing studies.^[193-196] However, it is not known whether this effect relies upon selective inhibition of PG synthesis, since neither drug (in the case of nabumetone, the active metabolite 6-MNA) showed reproducible selectivity for COX-2 in different systems.^[30,78,95,102,104,110] Furthermore, the nonacidic character of the prodrug nabumetone is thought to account in part for its reduced ulcerogenic effect,^[197] as does the inability of 6-MNA to recirculate in the bile.^[198]

Nimesulide and meloxicam, the 2 marketed NSAIDs having shown selectivity for COX-2 over COX-1 in several *in vitro* systems (see section 4), have displayed a slightly to significantly improved GI tolerability in healthy volunteers^[199,200] and patients with joint disorders.^[201-206] A possible dose-dependence effect in the GI tolerance of meloxicam was suggested in relation to endoscopic findings.^[200] For meloxicam, the favourable safety profile was proposed to be likely due to its preferential inhibition of COX isoenzymes.^[107,207-209] Nevertheless, both drugs have been reported in association with perforations, ulcerations, and bleeding in long term use,^[135,207,209] although at very low frequencies. The later data suggest again that safety, which depends on the drug concentration achieved in gastric mucosa when effective concentrations are obtained within inflammatory sites,^[210] is greatly influenced by the acidity of the drug, its bioavailability, and possible enterohepatic recircu-

lation, and is incompletely estimated by systems used for assessing COX-2 selectivity.

At present, nimesulide and meloxicam may represent an advance in the quest for safer NSAIDs, but other drugs under clinical development may be more promising in the future, since they possess a higher selectivity for COX-2 together with additional qualities, such as lack of acidity and an inability to act as proton translocators,^[106] that are effective in preventing local gastric damage. Very few clinical data are yet available, and the GI safety of these highly selective inhibitors needs to be demonstrated in long term comparative studies. It can be supposed that these clinical studies will provide a crucial test for the hypothesis that selective COX-2 inhibition has a GI-sparing effect.

6. Conclusion and Perspectives

The discovery of at least 2 COX isoenzymes, although probably with some physiological overlap, has given a new impetus to the search for anti-inflammatory drugs.^[122]

The development of selective COX-2 inhibitors seems promising and the clinical insights they might provide can occur as a result of a dual benefit: (i) an improved safety profile of NSAIDs, which relies upon a COX-1 sparing effect, as in stomach (see above in section 5,1); and (ii) a potential use in clinical situations in which COX-2 is overexpressed. For example, in patients with colorectal cancer, selective COX-2 inhibitors might have preventive and/or therapeutic effects because COX-2 overexpression^[211-213] may be associated with resistance to apoptosis^[214] and generation of PGs and related compounds that support carcinogenesis.^[215,216] The efficacy of classic NSAIDs in decreasing both the risk of death from colon cancer^[217,218] and the incidence of colon cancer^[219-221] and in inducing apoptosis in colon cells^[222,223] does not exclude any effect on COX-2.^[215,224]

Another promising area for selective COX-2 inhibitors might be neurodegenerative diseases, because COX-2, which is constitutively expressed in discrete populations of neurones,^[50,52,225] is over-

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expressed by synaptic activity^[50,226] as in isch-
aemia-reperfusion,^[225,227] and may be linked to
apoptosis-induced cell death.^[228,229] Such experi-
mental data suggest that selective inhibitors which
pass through the blood-brain barrier could have
therapeutic potency in stroke.

In Alzheimer's disease (AD), there is evidence
for inflammatory cascades involving local produc-
tion of pro-inflammatory cytokines,^[230,231] which
are potent inducers of COX-2. It is therefore con-
ceivable that COX-2 might contribute to the main-
tenance of inflammation and that its inhibition may
account partially for the beneficial effects of
NSAIDs therapy in Alzheimer's disease.^[230-232]

Finally, some of the selective COX-2 inhibitors
that have in common the ability to inhibit both the
induction and the activity of COX-2^[128,129] may
have cytokine-inhibiting properties,^[109] suggest-
ing that their mechanism of action will not be re-
stricted to the inhibition of PG synthesis.

The development of selective COX-2 inhibitors
also deserves to take care of the appearance of new
adverse effects since the respective role of both
isoenzymes is not firmly established in normal
physiological processes, such as ovulation and
pregnancy or the control of vascular tone.^[113] This
is best exemplified by the theoretical renal toxicity
of these inhibitors.^[140] PGs are well known to sup-
port renal blood flow in patients with either de-
creased perfusion pressure or ineffective plasma
volume,^[233] and their inhibition accounts for the
NSAID-induced disturbance of renal haemody-
namics, leading to acute renal failure in susceptible
patients.^[234,235]

In addition, PGs favour natriuresis and modu-
late antidiuretic hormone release,^[233] and their in-
hibition by NSAIDs may account for salt retention,
oedema, reduction of the antihypertensive effect of
diuretics and water conservation.^[234,235] Such ad-
verse effects can be observed virtually with all
NSAIDs^[175] and, as these are PG-dependent, the
question has arisen as whether they are related to
the inhibition of COX-1, COX-2 or both.

Immunohistochemical studies have shown that
COX-1 is localised mainly to arteries and arteri-
oles, glomeruli and collecting ducts,^[55,236] sug-
gesting a major role in the control of the vascular
tone. However, several lines of evidence suggest
that COX-2 may also play a role in the kidney: (i)
it has been detected in the papilla where it can
participate to the production of medullary PGs;^[56]
(ii) it is the sole COX isoenzyme which has been
detected in the macula densa and its expression
is triggered by salt restriction;^[56] (iii) it may
have a developmental role since COX-2 'knock-
out' mice develop a progressive nephropathy
when aging;^[64,65] and (iv) it becomes expressed in
mesangial cells after stimulation with NO,^[166]
interleukin-1^[237] or endothelin.^[238]

As a result, as yet it is not possible to ascertain
whether COX-2 selective inhibitors will be totally
safe for the kidney, although DuP-697 did not alter
renal blood flow in volume-depleted rats.^[120] The
full effect of COX-2 inhibition will probably be
better appreciated from accurate postmarketing
surveillance studies.^[113]

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References

1. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol* 1971; 231: 232-5
2. Smith WL, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. *Nature New Biol* 1971; 231: 235-7
3. Ferreira SH, Moncada S, Vane JR. Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature New Biol* 1971; 231: 237-9
4. Flower RJ. Drugs which inhibit prostaglandin biosynthesis. *Pharmacol Rev* 1974; 26: 33-67
5. Cronstein BN, Weissmann G. Target for anti-inflammatory drugs. *Ann Rev Pharmacol Toxicol* 1995; 35: 449-62
6. Flower RJ, Vane JR. Inhibition of prostaglandin synthase in brain explains the antipyretic activity of paracetamol (4-acetamidophenol). *Nature New Biol* 1972; 240: 410-1

7. Whittle BJR, Higgs GA, Eakins KE, et al. Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa. *Nature New Biol* 1980; 284: 271-3
8. Vane JR. Prostaglandins and the aspirin-like drugs. *Hosp Pract* 1972; 7: 61-71
9. Lysz TW, Needleman P. Evidence for two distinct forms of fatty acid cyclooxygenase in brain. *J Neurochem* 1982; 38: 1111-7
10. Preston SJ, Arnold MH, Beller EM, et al. Comparative analgesic and anti-inflammatory properties of sodium salicylate and acetylsalicylic acid (aspirin) in rheumatoid arthritis. *Br J Pharmacol* 1989; 27: 607-11
11. Abramson SB, Weissmann G. The mechanism of action of non-steroidal anti-inflammatory drugs. *Arthritis Rheum* 1989; 32: 1-9
12. McCormack K. Non steroidal anti-inflammatory drugs and spinal nociceptive processing. *Pain* 1994; 59: 9-43
13. Fu JY, Masferrer JL, Seibert K, et al. The induction and suppression of prostaglandin H2 synthase (cyclooxygenase) in human monocytes. *J Biol Chem* 1990; 265: 16737-40
14. Masferrer JL, Zweifel BS, Seibert K, et al. Selective regulation of cellular cyclooxygenase by dexamethasone and endotoxin in mice. *J Clin Invest* 1990; 86: 1375-9
15. Raz A, Wyche A, Needleman P. Temporal and pharmacological division of fibroblast cyclooxygenase expression into transcriptional and translational phase. *Proc Natl Acad Sci USA* 1989; 86: 1657-61
16. Ristimäki A, Garfinkel S, Wessendorf J, et al. Induction of cyclooxygenase-2 by interleukin-1 α . *J Biol Chem* 1994; 269: 11769-75
17. DuBois RN, Tsujii M, Bishop P, et al. Cloning and characterization of growth factor-inducible cyclooxygenase gene from rat intestinal epithelial cells. *Am J Physiol* 1994; 266: G822-7
18. Sirois J, Richards JS. Transcriptional regulation of the rat prostaglandin endoperoxide synthase 2 gene in granulosa cells. *J Biol Chem* 1993; 268: 21931-8
19. Kujubu DA, Reddy ST, Fletcher BS, et al. Expression of the protein product of prostaglandin synthase-2/TIS10 gene in mitogen-stimulated swiss 3T3 cells. *J Biol Chem* 1991; 268: 5425-30
20. Xie W, Chipman JG, Robertson DL, et al. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci USA* 1991; 88: 2692-6
21. O'Bannon MK, Sadowski HB, Winn V, et al. A serum- and glucocorticoid-regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein. *J Biol Chem* 1991; 266: 23261-7
22. DeWitt DL, Meade E, Smith WL. PGH synthase isoenzyme selectivity: the potential for safer nonsteroidal anti-inflammatory drugs. *Am J Med* 1993; 95: 40S-4S
23. Vane JR. Towards a better aspirin. *Nature New Biol* 1994; 367: 215-6
24. Battistini B, Botting R, Bakhle YS. COX-1 and COX-2: Toward the development of more selective NSAIDs. *Drug News Perspect* 1994; 7: 501-11
25. Smith WL, Marnett LJ. Prostaglandin endoperoxide synthase: structure and catalysis. *Biochim Biophys Acta* 1991; 1083: 1-17
26. Smith WL, DeWitt DL. Prostaglandin endoperoxide H synthases-1 and -2. *Adv Immunol* 1996; 62: 167-215
27. Kulmacz R J, Pendleton RB, Lands WEM. Interaction between peroxidase and cyclooxygenase activities in prostaglandin-endoperoxide synthase. *Biochemistry* 1994; 33: 5428-39
28. 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
29. Percival MD, Ouellet M, Vincent CJ, et al. Purification and characterization of recombinant human cyclooxygenase-2. *Arch Biochem Biophys* 1994; 315: 111-8
30. Meade EA, Smith WL, DeWitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isoenzyme by aspirin and other non-steroidal anti-inflammatory drugs. *J Biol Chem* 1993; 268: 6610-4
31. Goppelt-Strube M. Regulation of prostaglandin endoperoxide synthase (cyclooxygenase) isozyme expression. *Prostaglandins Leukot Essent Fatty Acids* 1995; 52: 213-22
32. Herschman HR. Prostaglandin synthase 2. *Biochim Biophys Acta* 1996; 1299: 125-40
33. Williams CS, DuBois RN. Prostaglandin endoperoxide synthase: why two isoforms? *Am J Physiol* 1996; 270: G393-400
34. Appleby SB, Ristimäki A, Neilson K, et al. Structure of the human cyclo-oxygenase-2 gene. *Biochem J* 1994; 302: 723-7
35. Kosaka T, Miyata A, Ihara H, et al. Characterization of the human gene (PTGS2) encoding prostaglandin-endoperoxide synthase 2. *Eur J Biochem* 1994; 221: 889-97
36. Lyons-Giordano B, Pratta MA, Galbraith W, et al. Interleukin-1 differentially modulates chondrocyte expression of cyclooxygenase-2 and phospholipase A2. *Exp Cell Res* 1993; 206: 58-62
37. Srivastava KS, Tetsuka T, Daphna-Iken D, et al. IL-1 β stabilizes COX II mRNA in renal mesangial cells: role of 3'-untranslated region. *Am J Physiol* 1994; 267: F504-8
38. DeWitt DL, Meade E. Serum and glucocorticoid regulation of gene transcription and expression of the prostaglandin H synthase-1 and prostaglandin H synthase-2 isozymes. *Arch Biochem Biophys* 1993; 306: 94-102
39. Ristimäki A, Narko K, Hla T. Down-regulation of cytokine-induced cyclo-oxygenase-2 transcript isoforms by dexamethasone: evidence for post-transcriptional regulation. *Biochem J* 1996; 318: 325-31
40. Hoff T, DeWitt DL, Kaever V, et al. Differentiation-associated expression of prostaglandin G/H synthase in monocytic cells. *FEBS Lett* 1993; 320: 38-42
41. Xie W, Fletcher BS, Andersen RD, et al. v-src induction of the TIS 10/PGS2 prostaglandin synthase gene is mediated by an ATF/CRE transcription response element. *Mol Cell Biol* 1994; 14: 6531-9
42. Inoue H, Nanayama T, Hara S, et al. The cyclic AMP response element plays an essential role in the expression of the human prostaglandin-endoperoxide synthase-2 gene in differentiated U937 monocytic cells. *FEBS Lett* 1994; 350: 51-4
43. Coleman RA, Smith WL, Narumiya S. Classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* 1994; 46: 205-29
44. Xie W, Merrill JR, Bradshaw WS, et al. Structural determination and promoter analysis of the chicken mitogen-inducible prostaglandin G/H synthase gene and genetic mapping of the murine homologue. *Arch Biochem Biophys* 1993; 300: 247-52
45. Scheinman RI, Cogswell PC, Lofquist AK, et al. Role of transcriptional activation of I κ B α in mediation of immunosuppression by glucocorticoids. *Science* 1995; 270: 283-6

46. Auphan N, DiDonato JA, Rosette C, et al. Immunosuppression by glucocorticoids: inhibition of NF- κ B activity through induction of I κ B synthesis. *Science* 1995; 270: 286-9
47. Feng L, Sun W, Xia Y, et al. Cloning two isoforms of rat cyclooxygenase: differential regulation of their expression. *Arch Biochem Biophys* 1993; 307: 169-77
48. O'Neill GP, Ford-Hutchinson AW. Expression of mRNA for cyclooxygenase-1 and cyclooxygenase-2 in human tissues. *FEBS Lett* 1993; 330: 156-60
49. Seibert K, Zhang Y, Leahy K, et al. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. *Proc Natl Acad Sci USA* 1994; 91: 12013-7
50. Yamagata K, Anderasson KI, Kaufmann WE, et al. Expression of a mitogen-inducible cyclooxygenase in brain neuron: regulation by synaptic activity and glucocorticoids. *Neuron* 1993; 11: 371-86
51. Iseki S. Immunocytochemical localization of cyclooxygenase-1 and -2 in the rat stomach. *Histochem J* 1995; 27: 323-8
52. 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
53. Breder CD, DeWitt DL, Kraig RP. Characterization of inducible cyclooxygenase in the rat brain. *J Comp Neurol* 1995; 355: 296-315
54. Seibert K, Masferrer JL, Zhang Y, et al. Mediation of inflammation by cyclooxygenase-2. *Agents Actions* 1995; 46 Suppl.: 41-50
55. Smith WL, Wilkin GP. Immunocytochemistry of prostaglandin endoperoxide forming cyclooxygenases: the detection of cyclooxygenases in rat, rabbit, and guinea pig kidneys by immunofluorescence. *Prostaglandins* 1977; 13: 873-92
56. Harris RC, McKanna JA, Akai Y, et al. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. *J Clin Invest* 1994; 94: 2504-10
57. Morita I, Schinder M, Regier MK, et al. Different intracellular locations for prostaglandin endoperoxide H synthase-1 and -2. *J Biol Chem* 1995; 270: 10902-8
58. Yu K, Bayowa Y, Kallen CB, et al. Differential activation of peroxisome proliferator-activated receptors by eicosanoids. *J Biol Chem* 1995; 270: 23975-83
59. Kliewer SA, Lenhard JM, Willson TM, et al. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation. *Cell* 1995; 83: 813-9
60. Murakami M, Matsumoto R, Austen KF, et al. Prostaglandin endoperoxide synthase-1 and -2 couple to different transmembrane stimuli to generate prostaglandin D2 in mouse bone marrow-derived mast cells. *J Biol Chem* 1994; 269: 22269-75
61. Kawata R, Reddy ST, Wolner B, et al. Prostaglandin synthase 1 and prostaglandin synthase 2 both participate in activation-induced prostaglandin D2 production in mast cell. *J Immunol* 1995; 155: 818-25
62. Karim S, Habib A, Levy-Toledano S, et al. Cyclooxygenases-1 and -2 of endothelial cells utilize exogenous or endogenous arachidonic acid for transcellular production of thromboxane. *J Biol Chem* 1996; 271: 12042-8
63. Langenbach R, Morham SG, Tian HF, et al. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 1995; 83: 483-92
64. Morham SG, Langenbach R, Loftin CD, et al. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell* 1995; 83: 473-82
65. Dinchuk JE, Car BD, Focht RJ, et al. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature* 1995; 378: 406-9
66. Picot D, Loll PJ, Garavito M. The x-ray crystal structure of the membrane protein prostaglandin H2 synthase-1. *Nature* 1994; 367: 243-9
67. Blake C. Prostaglandin synthase: at the heart of the matter. *Curr Biol* 1994; 4: 452-4
68. DeWitt DL, El-Harith EA, Kraemer SA, et al. The aspirin and heme-binding sites of ovine and human prostaglandin endoperoxide synthases. *J Biol Chem* 1990; 265: 5192-8
69. Tsai AL, Kulmacz RJ, Palmer G. Spectroscopic evidence for reaction of prostaglandin H synthase-1 tyrosyl radical with arachidonic acid. *J Biol Chem* 1995; 270: 10503-8
70. Loll PJ, Picot D, Garavito M. The structural basis of aspirin activity inferred from the crystal structure of inactivated prostaglandin H2 synthase. *Nature Struct Biol* 1995; 2: 637-43
71. Kulmacz RJ. Topography of prostaglandin H synthase. *J Biol Chem* 1989; 264: 14136-44
72. Loll PJ, Picot D, Ekabo O, et al. Synthesis and use of iodinated nonsteroidal anti-inflammatory drug analogs as crystallographic probes of the prostaglandin H2 synthase cyclooxygenase active site. *Biochemistry* 1996; 35: 7330-40
73. Mancini JA, Riendeau D, Falgout JP, et al. Arginine 120 of prostaglandin G/H synthase-1 is required for the inhibition by nonsteroidal anti-inflammatory drugs containing a carboxylic acid moiety. *J Biol Chem* 1995; 270: 29372-7
74. Bhattacharyya DK, Lecomte M, Ricke CJ, et al. Involvement of Arginine 120, Glutamate 524, and Tyrosine 355 in the binding of arachidonate and 2-phenylpropionic acid inhibitors to the cyclooxygenase active site of ovine prostaglandin endoperoxide H synthase-1. *J Biol Chem* 1996; 271: 2179-84
75. Brooks PM, Day RO. Nonsteroidal anti-inflammatory drugs - differences and similarities. *N Engl J Med* 1991; 324: 1716-25
76. Luong C, Miller A, Barnett J, et al. Flexibility of the NSAID binding site in the structure of human cyclo-oxygenase-2. *Nature Struct Biol* 1996; 3: 927-33
77. Mitchell JA, Akarasereenont P, Thiemermann C, et al. Selectivity of non steroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proc Natl Acad Sci USA* 1993; 90: 11693-7
78. Laneuville O, Breuer DK, DeWitt DL, et al. Differential inhibition of human prostaglandin endoperoxide H synthase-1 and -2 by nonsteroidal anti-inflammatory drugs. *J Pharmacol Exp Ther* 1994; 271: 927-34
79. Lecomte M, Laneuville O, Chuan J, et al. Acetylation of human prostaglandin endoperoxide synthase-2 (cyclooxygenase-2) by aspirin. *J Biol Chem* 1994; 269: 13207-15
80. Mancini JA, O'Neill GP, Bayly C, et al. Mutation of serine 516 in human prostaglandin G/H synthase-2 to methionine or aspirin acetylation of this residue stimulates 15-R-HETE synthesis. *FEBS Lett* 1994; 342: 33-7
81. Allen KN. Aspirin - now we can see it. *Nature Med* 1995; 1: 882-3
82. Wennogle LP, Liang H, Quintavalla JC, et al. Comparison of recombinant cyclooxygenase-2 to native isoforms: aspirin labeling of the active site. *FEBS Lett* 1995; 371: 315-20

83. Garavito RM, Picot D, Loll PL. The x-ray structures of complexes of cyclooxygenase-1 and inhibitors. *Med Chem Res* 1995; 5: 375-83
84. Gierse JK, McDonald JJ, Hauser SD, et al. A single amino acid difference between cyclooxygenase-1 (COX-1) and -2 (COX-2) reverses the selectivity of COX-2 specific inhibitors. *J Biol Chem* 1996; 271: 15810-4
85. Rome LH, Lands WEM. Structural requirements for time-dependent inhibition of prostaglandin biosynthesis by anti-inflammatory drugs. *Proc Natl Acad Sci USA* 1975; 72: 4863-5
86. Kulmacz RJ, Lands WEM. Stoichiometry and kinetics of the interaction of prostaglandin H synthase with anti-inflammatory agents. *J Biol Chem* 1985; 260: 12572-8
87. Copeland RA, Williams JM, Giannaras J, et al. Mechanism of selective inhibition of the inducible isoform of prostaglandin G/H synthase. *Proc Natl Acad Sci USA* 1994; 91: 11202-6
88. Ouellet M, Percival MD. Effect of inhibitor time-dependency on selectivity towards cyclooxygenase isoforms. *Biochem J* 1995; 306: 247-51
89. DeWitt DL, Bhattacharyya D, Lecomte M, et al. The differential susceptibility of prostaglandin endoperoxide H synthase-1 and -2 to nonsteroidal anti-inflammatory drugs: aspirin derivatives as selective inhibitors. *Med Chem Res* 1995; 5: 325-43
90. Vago T, Bevilacqua M, Norbiato G. Effect of nimesulide action time dependence on selectivity towards prostaglandin G/H synthase / cyclooxygenase activity. *Arzneimittel Forschung* 1995; 45: 1096-8
91. Bhattacharyya DK, Lecomte M, Dunn J, et al. Selective inhibition of prostaglandin endoperoxide synthase-1 (cyclooxygenase-1) by valeryl salicylic acid. *Arch Biochem Biophys* 1995; 317: 19-24
92. Futaki N, Takahashi S, Yokoyama M, et al. NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase / cyclooxygenase (COX-2) activity *in vitro*. *Prostaglandins* 1994; 47: 55-9
93. Barnett J, Chow J, Ives D, et al. Purification, characterization and selective inhibition of human prostaglandin G/H Synthase 1 and 2 expressed in the baculovirus system. *Biochim Biophys Acta* 1994; 1209: 130-9
94. O'Neill GP, Mancini JA, Kargman S, et al. Overexpression of human prostaglandin G/H synthase-1 and -2 by recombinant vaccinia virus. Inhibition by nonsteroidal anti-inflammatory drugs and biosynthesis of 15-hydroxyicosatetraenoic acid. *Mol Pharmacol* 1994; 45: 245-54
95. Glaser K, Sung M-L, O'Neill K, et al. Etodolac selectively inhibits human prostaglandin G/H synthase 2 (PGHS-2) versus human PGHS-1. *Eur J Pharmacol* 1995; 281: 107-11
96. Gierse JK, Hauser SD, Creely DP, et al. Expression and selective inhibition of the constitutive and inducible of human cyclooxygenase. *Biochem J* 1995; 305: 479-84
97. Engelhardt G, Bögel R, Schnitzler C, et al. Meloxicam: influence on arachidonic acid metabolism: part 1. *In vitro* findings. *Biochem Pharmacol* 1996; 51: 21-8
98. Chan CC, Boyce S, Bribeau C, et al. Pharmacology of selective cyclooxygenase-2 inhibitor, L-745,337: a novel nonsteroidal anti-inflammatory agent with an ulcerogenic sparing effect in rat and non human primate stomach. *J Pharmacol Exp Ther* 1995; 274: 1531-7
99. Klein T, Nusing RM, Pfeilschifter J, et al. Selective inhibition of cyclooxygenase 2. *Biochem Pharmacol* 1994; 48: 1605-10
100. Churchill L, Graham AG, Shih CK, et al. Selective inhibition of human cyclooxygenase-2 by meloxicam. *Inflammopharmacology* 1996; 4: 125-35
101. Brideau C, Kargman S, Liu S, et al. A human whole blood assay for clinical evaluation of biological efficacy of cyclooxygenase inhibitors. *Inflamm Res* 1996; 45: 68-74
102. 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: 1705-12
103. Panara MR, Greco A, Santini G, et al. Effects of the novel anti-inflammatory compounds, N-[2-(cyclohexyloxy)-4-nitrophenyl] methanesulphonamide (NS-398) and 5-methanesulphonamido - 6 - (2,4 - difluorothiophenyl) - 1 - indanone (L-745,337), on the cyclo-oxygenase activity of human blood prostaglandin endoperoxide synthase. *Br J Pharmacol* 1995; 116: 2429-34
104. Young JM, Panah S, Satchawatcharaphong C, et al. Human whole blood assays for inhibition of prostaglandin G/H synthase-1 and -2 using A23187 and lipopolysaccharide stimulation of thromboxane B₂ production. *Inflamm Res* 1996; 45: 246-53
105. Grossman CJ, Wiseman J, Lucas LS, et al. Inhibition of constitutive and inducible cyclooxygenase activity in human platelets and mononuclear cells by NSAIDs and COX 2 inhibitors. *Inflamm Res* 1995; 44: 253-57
106. Hayllar J, Bjarnason I. NSAIDs, COX-2 inhibitors, and the gut. *Lancet* 1995; 346: 521-2
107. Vane JR. NSAIDs, COX-2 inhibitors, and the gut [letter]. *Lancet* 1995; 346: 521-2
108. Vane JR, Botting RM. Mechanisms of action of anti-inflammatory drugs. *Scand J Rheumatol* 1996; 25 Suppl 102: 9-21
109. Griswold DE, Adams JL. Constitutive cyclooxygenase (COX-1) and inducible cyclooxygenase (COX-2): rationals for selective inhibition and progress to date. *Med Res Rev* 1996; 16: 181-206
110. Cipollone F, Ganci A, Panara, et al. Effects of nabumetone on prostanoic acid biosynthesis in humans. *Clin Pharmacol Ther* 1995; 58: 335-41
111. Tofanetti O, Casciarri I, Cipolla PV, et al. Effects of nimesulide on cyclo-oxygenase activity in rat gastric mucosa and inflammatory exudate. *Med Sci Res* 1989; 17: 745-6
112. Masferrer JL, Zweifel BS, Manning PT, et al. Selective inhibition of inducible cyclooxygenase 2 *in vivo* is anti-inflammatory and nonulcerogenic. *Proc Natl Acad Sci USA* 1994; 91: 3228-32
113. Richardson C, Emery P. The clinical implications of inhibition of the inducible form of cyclo-oxygenase. *Drug Saf* 1996; 15: 249-60
114. Simon LS. Non steroidal antiinflammatory drugs and their effects. The importance of COX 'selectivity'. *J Clin Rheumatol* 1996; 2: 135-40
115. Aslanian R, Carrathers NI, Kaminski JJ. Cyclooxygenase-2: a novel target for therapeutic intervention. *Expert Opin Invest Drugs* 3: 1323-5, 1994
116. Jensen NP. NSAIDs revisited cyclooxygenase 2 (COX-2) inhibitors. *Med Chem Res* 1995; 5: 319-24
117. Futaki N, Yoshikawa K, Hamasaka Y, et al. NS-398, a novel nonsteroidal anti-inflammatory drug with potent analgesic and antipyretic effects, which causes minimal stomach lesions. *Gen Pharmacol* 1993; 24: 105-10

118. Wiesenberger-Böttcher I, Schweizer A, Green JR, et al. The pharmacological profile of CGP-28238, a highly potent anti-inflammatory compound. *Agents Actions* 1989; 26: 240-2
119. Swingle KF, Moore GI, Grant TJ. 4-nitro-2-phenoxy-methanesulfonamide (R-805): a chemically novel anti-inflammatory agent. *Arch Int Pharmacodyn Ther* 1976; 221: 131-9
120. Gans KR, Galbraith W, Roman RJ, et al. Anti-inflammatory and safety profile of DuP 697, a novel orally effective prostaglandin synthesis inhibitor. *J Pharmacol Exp Ther* 1990; 254: 180-7
121. Wong S, Lee SJ, Frierson MR, et al. Antiarthritic profile of BF-389. A novel anti-inflammatory agent with low ulcerogenic liability. *Agents Actions* 1992; 37: 90-8
122. Vane JR, Botting RM. New insights into the mode of action of anti-inflammatory drugs. *Inflamm Res* 1995; 44: 1-10
123. Prasit P, Black WC, Chan CC, et al. L745,337: a selective cyclooxygenase-2 inhibitor. *Med Chem Res* 1995; 5: 364-74
124. Tanaka K, Makino S, Shimotori T, et al. Pharmacological studies of the new anti-inflammatory agent 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one. 2nd communication: effect on the arachidonic acid cascades. *Arzneimittel Forschung* 1992; 42: 945-50
125. Reitz DB, Li JJ, Norton MB, et al. Selective cyclooxygenase inhibitors: novel 1,2-diarylcyclopentenones are potent and orally active COX-2 inhibitors. *J Med Chem* 1994; 37: 3878-81
126. Reitz DB, Li JJ, Norton MB, et al. Novel 1,2-diarylcyclopentenones are selective, potent, and orally active cyclooxygenase inhibitors. *Med Chem Res* 1995; 5: 351-63
127. Hubbard RC, Koepf RJ, Yu S, et al. SC-58635 (celecoxib), a novel COX-2 selective inhibitor, is effective as a treatment for osteoarthritis (OA) in a short-term pilot study [abstract]. *Arthritis Rheum* 1996; 39 Suppl. 9: S 226
128. Tanaka K, Kawasaki H, Kurata K, et al. T-614, a novel anti-rheumatic drug, inhibits both the activity and induction of cyclooxygenase-2 (COX-2) in cultured fibroblasts. *Jpn J Pharmacol* 1995; 67: 305-14
129. Anderson GD, Hauser SD, McGarity KL, et al. Selective inhibition of cyclooxygenase (COX-2) reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. *J Clin Invest* 1996; 97: 2672-9
130. Tavares IA, Bishai PM, Bennett A. Activity of nimesulide on constitutive and inducible cyclooxygenases. *Arzneimittel Forschung* 1995; 45 (II): 1093-5
131. Taniguchi Y, Ikesue A, Yokoyama K, et al. Selective inhibition by nimesulide, a novel non steroidal anti-inflammatory drug, with prostaglandin endoperoxide synthase-2 activity *in vitro*. *Pharm Res* 1995; 1: 173-5
132. Vojnovic I, Amirmansour C, Warner TD, et al. Nimesulide: selectivity for COX-2 over COX-1 [abstract P4]. William Harvey Research Conferences on improved non-steroid anti-inflammatory drugs; COX-2 enzyme inhibitors; 1995 Oct 10-11: London
133. Engelhardt G, Homma D, Schlegel K, et al. Anti-inflammatory, analgesic, antipyretic and related properties of meloxicam, a new non-steroidal anti-inflammatory agent with favourable gastrointestinal tolerance. *Inflamm Res* 1995; 44: 423-33
134. Futaki N, Arai I, Hamasaka Y, et al. Selective inhibition of NS-398 on prostanoid production in inflamed tissue in rat carrageenan-air-pouch inflammation. *J Pharm Pharmacol* 1993; 45: 753-5
135. Davis R, Brogden RN. Nimesulide: an update of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy. *Drugs* 1994; 48: 431-54
136. Kankaanranta H, Moilanen E, Vapaatalo H. Effects of non-steroidal anti-inflammatory drugs on polymorphonuclear leukocytes functions *in vitro*: focus on fenamates. *Arch Pharmacol* 1994; 350: 685-91
137. Tanaka K, Shimotori T, Makino S, et al. Pharmacological studies of the new anti-inflammatory agent 3-formylamino-7-methylsulfonylamino-6-phenoxy-4H-1-benzopyran-4-one. 1st communication: Anti-inflammatory, analgesic and other related properties. *Arzneimittel Forschung* 1992; 42: 935-44
138. Magni E. The effect of nimesulide on prostanoid formation. *Drugs* 1993; 46 Suppl. 1: 10-4
139. Pairet M, Engelhardt E. Distinct isoforms (COX-1 and COX-2) of cyclooxygenase: possible physiological and therapeutic implications. *Fund Clin Pharmacol* 1996; 10: 1-15
140. Frölich JC. Prostaglandin endoperoxide synthase isoenzymes: the clinical relevance of selective inhibition. *Ann Rheum Dis* 1995; 54: 942-3
141. Noble S, Balfour JA. Meloxicam. *Drugs* 1996; 51: 424-30
142. Furst DE. Are there differences among nonsteroidal anti-inflammatory drugs? *Arthritis Rheum* 1994; 37: 1-9
143. Lanza FL. Gastrointestinal toxicity of newer NSAIDs. *Am J Gastroenterol* 1993; 88: 1318-23
144. Lichtenstein DR, Syngal S, Wolfe M. Nonsteroidal anti-inflammatory drugs and the gastrointestinal tract. *Arthritis Rheum* 1995; 38: 5-18
145. Roth SH. NSAID gastropathy. A new understanding. *Arch Intern Med* 1996; 156: 1623-8
146. Fries JF, Miller SR, Williams CA, et al. Toward an epidemiology of gastropathy associated with nonsteroidal anti-inflammatory drug use. *Gastroenterology* 1989; 96: 647-55
147. Schoen RT, Vender RJ. Mechanisms of nonsteroidal anti-inflammatory drug-induced gastric damage. *Am J Med* 1989; 86: 449-58
148. Price AH, Fletcher M. Mechanisms of NSAID-induced gastroenteropathy. *Drugs* 1990; 40 Suppl. 5: 1-11
149. Gabriel SE, Jaakkimainen L, Bombardier C. Risk for serious gastrointestinal complications related to use of nonsteroidal anti-inflammatory drugs. A meta-analysis. *Ann Intern Med* 1991; 115: 787-96
150. Polissou R. Nonsteroidal anti-inflammatory drugs: practical and theoretical considerations in their selection. *Am J Med* 1996; 100 Suppl. 2A: 31S-6S
151. Lichtenberger LM, Graziani LA, Dial EJ, et al. Role of surface-active phospholipids in gastric cytoprotection. *Science* 1983; 219: 1327-9
152. Lichtenberger LM, Wang Z-M, Romero JJ, et al. Non-steroidal anti-inflammatory drugs (NSAIDs) associated with zwitterionic phospholipids: insight into the mechanism and reversal of NSAID-induced gastrointestinal injury. *Nature Med* 1995; 1: 154-8
153. Somasundaram S, Hayllar H, Rafi S, et al. The biochemical basis of non-steroidal anti-inflammatory drug induced damage to the gastrointestinal tract: a review and a hypothesis. *Scand J Gastroenterol* 1995; 30: 289-99
154. Mahmud T, Scott DL, Bjarnason I. A unifying hypothesis for the mechanism of NSAID related gastrointestinal toxicity. *Ann Rheum Dis* 1996; 55: 211-3

155. Rainsford KD. Mechanisms of gastrointestinal toxicity of non-steroidal anti-inflammatory drugs. *Scand J Gastroenterol* 1989; 24 Suppl. 163: 9-16
156. Scarpignato C. Nonsteroidal anti-inflammatory drugs: How do they damage gastroduodenal mucosa? *Dig Dis* 1995; 13 Suppl. 1: 9-39
157. Wallace JL, Keenan CM, Granger DN. Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. *Am J Physiol* 1990; 259: G462-7
158. Wallace JL, McKnight W, Miyasaka M, et al. Role of endothelial adhesion molecules in NSAID-induced gastro-mucosal injury. *Am J Physiol* 1993; 265: G993-8
159. Santucci L, Fiorucci S, Giansanti M, et al. Pentoxifylline prevents indomethacin induced acute gastric mucosal damage in rats: role of tumour necrosis factor alpha. *Gut* 1994; 35: 909-15
160. Appleyard CB, McCafferty DM, Tigley AW, et al. Tumor necrosis factor mediation of NSAID-induced gastric damage: role of leukocyte adherence. *Am J Physiol* 1996; 270: G42-8
161. Whittle BJR, Lopez-Belmonte J, Moncada S. Regulation of gastric mucosal integrity by endogenous nitric oxide: interactions with prostanoids and sensory neuropeptides in the rat. *Br J Pharmacol* 1990; 99: 607-11
162. Tepperman BL, Whittle BJR. Endogenous nitric oxide and sensory neuropeptides interact in the modulation of rat gastric microcirculation. *Br J Pharmacol* 1992; 105: 171-5
163. Wallace JL, Cirino G. The development of gastrointestinal-sparing nonsteroidal anti-inflammatory drugs. *Trends Pharmacol Sci* 1994; 15: 405-6
164. Corbett JA, Kwon G, Turk J, et al. IL-1 β induces the coexpression of both nitric oxide synthase and cyclooxygenase by islets of langerhans: activation of cyclooxygenase by nitric oxide. *Biochemistry* 1993; 32: 13767-70
165. Wu KK. Inducible cyclooxygenase and nitric oxide synthase. *Adv Pharmacol* 1995; 33: 179-207
166. Salvemini D, Misko TP, Masferrer JL, et al. Nitric oxide activates cyclooxygenase enzymes. *Proc Natl Acad Sci USA* 1993; 90: 7240-4
167. Salvemini D, Seibert K, Masferrer JL, et al. Endogenous nitric oxide enhances prostaglandin production in a model of renal inflammation. *J Clin Invest* 1994; 93: 1940-7
168. Lee M, Feldman M. Nonessential role of leukotrienes as mediators of acute gastric mucosal injury induced by aspirin. *Dig Dis Sci* 1992; 37: 1282-7
169. Wallace JL, McCafferty DM, Carter L, et al. Tissue-selective inhibition of prostaglandin synthesis in rat by tepoxalin: anti-inflammatory without gastropathy? *Gastroenterology* 1993; 105: 1630-6
170. Rainsford KD. The effects of 5-lipoxygenase inhibitors and leukotriene antagonists on the development of gastric lesions induced by nonsteroidal anti-inflammatory drugs in mice. *Agents Actions* 1987; 21: 316-9
171. Vaananen PM, Keenan CM, Grisham MB, et al. A pharmacological investigation of the role of leukotrienes in the pathogenesis of experimental NSAIDs - gastropathy. *Inflammation* 1992; 16: 227-40
172. Schrier DJ, Baragi VM, Connor DT, et al. The pharmacologic effects of 5-[3,5-bis(1,1-dimethylethyl)-4-hydroxyphenyl]-1,3,4-thiadiazole-2(3H)-thione, choline salt (CI-986), a novel inhibitor of arachidonic acid metabolism in models of inflammation, analgesia and gastric irritation. *Prostaglandins* 1994; 47: 17-30
173. Argentieri DC, Ritchie DM, Ferro MP, et al. Tepoxalin: a dual cyclooxygenase/5-lipoxygenase inhibitor of arachidonic acid metabolism with a potent anti-inflammatory activity and a favorable gastrointestinal profile. *J Pharmacol Exp Ther* 1994; 271: 1399-1408
174. Rainsford KD, Tsang S, Hunt RH, et al. Effects of non-steroidal anti-inflammatory drugs on prostaglandin H synthase isoenzyme 2 (cyclo-oxygenase 2) production by porcine gastric mucosa in organ culture. *Inflammopharmacology* 1995; 3: 299-310
175. Skeith KJ, Wright M, Davis P. Differences in NSAID tolerability profiles: fact or fiction? *Drug Saf* 1994; 10: 183-95
176. Griffin MR, Piper JM, Daugherty JR, et al. Nonsteroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elderly persons. *Ann Intern Med* 1991; 114: 257-63
177. Laporte JR, Carne X, Vidal X, et al. Upper gastrointestinal bleeding in relation to previous use of analgesics and non-steroidal anti-inflammatory drugs. *Lancet* 1991; 337: 85-9
178. Savage RL, Moller PW, Ballantyne CL et al. Variation in the risk of peptic ulcer complications with nonsteroidal anti-inflammatory drug therapy. *Arthritis Rheum* 1993; 36: 84-90
179. Henry D, Dobson A, Turner C. Variability in the risk of major gastrointestinal complications from nonaspirin nonsteroidal anti-inflammatory drugs. *Gastroenterology* 1993; 105: 1078-88
180. Langman MJS, Weil J, Wainwright P, et al. Risk of bleeding peptic ulcer associated with individual non-steroidal anti-inflammatory drugs. *Lancet* 1994; 343: 1075-8
181. Garcia-Rodriguez LA, Jick H. Risk of upper gastrointestinal bleeding and perforation associated with individual nonsteroidal anti-inflammatory drugs. *Lancet* 1994; 343: 769-72
182. Kurata JH, Nagawa AN, Abbey DE, et al. A prospective study of risk for peptic ulcer disease in seventh day adventists. *Gastroenterology* 1992; 102: 902-9
183. Lanza LL, Walker AM, Bortnick EA, et al. Peptic ulcer and gastrointestinal hemorrhage associated with nonsteroidal anti-inflammatory drug use in patients younger than 65 years. *Arch Intern Med* 1995; 155: 1371-7
184. Fries J. Toward an understanding of NSAID-related adverse events: the contribution of longitudinal data. *Scand J Rheumatol* 1996; 25 Suppl. 102: 3-8
185. Committee on Safety of Medicines. Non-steroidal anti-inflammatory drugs and serious gastrointestinal adverse reactions - 2. *BMJ* 1986; 292: 1190-1
186. Bateman DN. NSAIDs: time to re-evaluate gut toxicity. *Lancet* 1994; 343: 1051-2
187. Henry D, Lim LY, Garcia-Rodriguez LA, et al. Variability in risk of gastrointestinal complications with individual non-steroidal anti-inflammatory drugs: results of a collaborative meta-analysis. *BMJ* 1996; 312: 1563-6
188. Rainsford KD, Quadir M. Gastrointestinal damage and bleeding from non-steroidal anti-inflammatory drugs: I. Clinical and epidemiological aspects. *Inflammopharmacology* 1995; 3: 169-90
189. Brune K, Rainsford KD, Wagner K, et al. Inhibition by anti-inflammatory drugs of prostaglandin production in cultured macrophages. *Arch Pharmacol* 1981; 315: 269-76
190. Roth SH. Endoscopy-controlled study of the safety of nabumetone compared with naproxen in arthritis therapy. *Am J Med* 1987; 83: 25-30
191. Lanza F, Rack MF, Lynn M, et al. An endoscopic comparison of the effects of etodolac, indomethacin, ibuprofen, naproxen,

231. Cochran FR, Vitek MP. Neuroinflammatory mechanisms in Alzheimer's disease: new opportunities for drug discovery. *Expert Opin Invest Drugs* 1996; 5: 449-55
232. Fricker J. NSAIDs may reduce risk of Alzheimer's disease [editorial]. *Lancet* 1996; 347: 958
233. Norris SH. Renal eicosanoids. *Semin Nephrol* 1990; 10: 64-88
234. Toto RD. The role of prostaglandins in NSAID induced renal dysfunction. *J Rheumatol* 1991; 18 Suppl. 28: 22-5
235. Shield MJ. Anti-inflammatory drugs and their effects on cartilage synthesis and renal function. *Eur J Rheumatol Inflamm* 1993; 13: 7-16
236. Smith WL, Bell TG. Immunohistochemical localization of the prostaglandin-forming cyclooxygenase in the renal cortex. *Am J Physiol* 1978; 235: F451-7
237. Rzymkiewicz D, Leingang K, Baird N, et al. Regulation of prostaglandin endoperoxide synthase gene expression in rat mesangial cells by interleukin-1 β . *Am J Physiol* 1994; 266: F39-45
238. Hughes AK, Padilla E, Kutchera WA, et al. Endothelin-1 induction of cyclooxygenase-2 expression in rat mesangial cells. *Kidney Int* 1995; 47: 53-61

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