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Nonsteroidal Antiinflammatory Drugs: A New Generation of Cyclooxygenase Inhibitors**

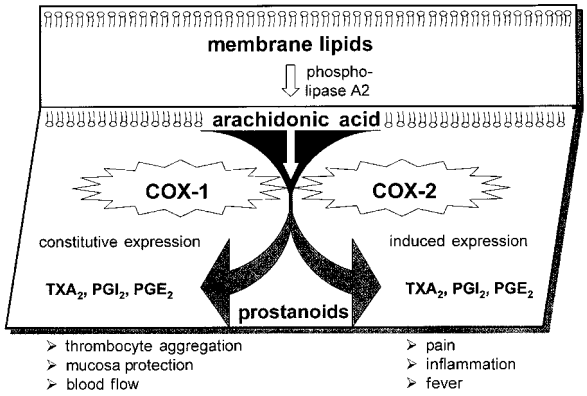
Martin Beuck*

Aspirin®—or acetylsalicylic acid—is synonymous for first aid relief of pain, fever, and inflammation. The 100-year-old and most popular drug is facing new competition. Not simply another compound, but a whole new class with a different spectrum of activities give potential access to indications outside of pain and inflammation.

Therapeutic Basis

The therapeutic effect of acetylsalicylic acid is based on a covalent modification of cyclooxygenase and the inhibition of the first step of prostaglandin synthesis, shown first by Sir John Robert Vane.[1] Cyclooxygenase (COX) exists in two isoforms, COX-1 and COX-2. Serine residue 530 of COX-1 and serine residue 516 of COX-2 are modified by acetylation. Inhibition of COX-1 or COX-2 leads to very different pharmacological effects. The COX-1 inhibition is predominantly responsible for anti-thrombotic effects, while anti-inflammatory effects are mediated mainly through COX-2.

The cyclooxygenase COX-1 is expressed constitutively in all tissues, and is thus always present and active. As far as it is known, this is the case for COX-2 only in kidney, brain, and ovaries. During inflammatory processes COX-2 is increasingly expressed in affected tissues, and consequently production of the pain-mediating prostaglandins is also increased (Scheme 1).



Scheme 1. Role of COX-1 and COX-2 in arachidonic acid metabolism. COX-1/2: cyclooxygenase-1/2; TXA_2: thromboxan A_2; PGI_2: prostacyclin; PGE_2: prostaglandin E_2.

In contrast to self medication of mild headaches and malaise, therapy of pain, for example that caused by rheumatoid arthritis, was treated with high doses of acetylsalicylic acid in the past. This led to undesirable gastrointestinal side effects. These effects in the gastrointestinal tract were mainly mediated by COX-1. Inhibition of COX-1 impairs the synthesis of prostanoids, which have a protective effect on gastric mucosa.

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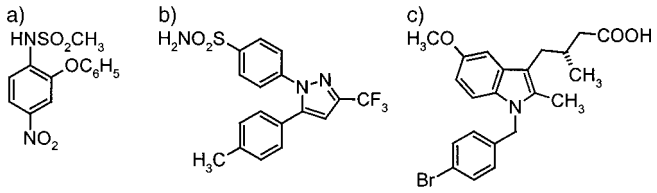
Consequently, a strategy to change selectivity towards COX-2-specific inhibitors was followed, leading to novel pain killers without the risk of the known side effects.

New Generations of COX Inhibitors

The currently known COX-2 inhibitors can be divided into three categories:[2] 1) methanesulfoanilides (e.g. Nimesulide, Scheme 2a), 2) methylsulfonyl/sulfonamide-substituted tricycles (e.g. Celecoxib, Scheme 2b), 3) analogues based on nonselective inhibitors (e.g. analogues of indomethacine, Scheme 2c).



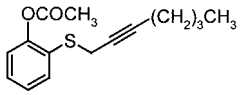
Scheme 2. a) Nimesulide: a first-generation COX-2 inhibitor; b) Celecoxib: a second-generation COX-2 inhibitor; c) the indomethacin analogue L-761,066: a nonselective COX-2 inhibitor.

First concepts for the development of highly specific COX-2 inhibitors for the treatment of rheumatoid arthritis and osteoarthritis are currently being pursued by Monsanto and Merck & Co, and are in advanced stages of clinical development (Table 1). On the market are Meloxicam and in some countries Nimesulide, a COX-2 inhibitor of the first generation which has about a 10- to 20-fold higher affinity for COX-2. The second-generation compounds in clinical development show a 300- to 400-fold (Vioxx/Celebra) higher affinity for COX-2. It is expected that the late followers of second-generation products will have a further 10-fold increased selectivity.

So far clinical studies for arthritis seem to show an equivalent effect. Gastrointestinal side effects were not observed, at least within the very short period of investigation of about a week.

Compounds in clinical development are selective and generally reversibly binding inhibitors of COX-2. Reversibility, however, is limited by the dissociation kinetics of the COX-2 enzyme – inhibitor complex.[3] After initial binding of the inhibitors to the enzyme a change of conformation in the COX-2 enzyme seems to slow down dissociation of the inhibitor from the enzyme, leading to apparently irreversible binding characteristics.

The first selective COX-2 inhibitors to show irreversible binding were presented recently by Kalgutkar et al.[4] They describe a new series of compounds with selectivity for COX-2 in combination with a covalent modification of the enzyme, as observed for acetylsalicylic acid. Chemically these compounds belong to a group of acetoxybenzenes that are substituted with alkyl sulfides. The result of the work is significant, as the best compound shows a 20-fold higher selectivity for COX-2 than for COX-1. This is about the level of first-generation noncovalently binding COX-2 inhibitors. Systematic variation led to *o*-(acetoxyphenyl)heptynyl sulfide (APHS, Scheme 3), the most potent compound of this series. With an IC_{50} value of 0.8 μM for COX-2 and 17 μM for COX-1, AHPS is not yet a highly specific and selective inhibitor. However, it is a starting point, and first results from in vitro and in vivo models confirm this. In a rat inflammation model APHS reduced synthesis of prostaglandin E2 by 95% at 5 mg kg^{-1}, while COX-1-mediated synthesis of thromboxan B2 was not affected.



Scheme 3. *o*-(Acetoxyphenyl)hept-2-ynyl sulfide (APHS):[4] the first covalently binding COX-2 inhibitor.

New Indications for COX-2 Inhibitors

New studies from the last two years revealed that in addition to arthritis and pain, cancer and neurodegenerative diseases like Alzheimer's disease could potentially be treated with COX-2 inhibitors. In rats[5] it could be shown that Celecoxib (Monsanto) reduced azoxymethan-induced colon cancer by more than 90% within the observation period of about a year (50 weeks). Induction of COX-2 expression in these cells was already clear from preceding in vitro experiments.

The molecular mechanism currently under discussion is based on the assumption that COX-2 inhibition leads to a shift in the ratio of cell proliferation and apoptosis. Apoptosis is controlled cell death and can be initiated by *N*-acylsphingosine, which is produced from sphingosine and arachidonic acid. Inhibition of COX-2 results in an enlarged pool of arachidonic acid, thus favoring apoptosis.

Consequently, current studies are designed to show whether Celecoxib is able to reduce formation of colon cancer in humans or to stop further progression of preneoplastic lesions to cancer. If this is a class effect based on the mechanism, one would expect similar results with other COX-2 inhibitors in clinical development. Furthermore, epidemiological studies

Table 1. Inhibitors of COX-2 in clinical development.[a]

COX-2 inhibitor	Company	Status	Indications
Meloxicam	Boehringer Ingelheim	marketed	rheumatoid arthritis, osteoarthritis, spondylitis ankylosans
Nimesulide	Helsinn	marketed	inflammation, fever, rheumatoid arthritis, pain
Celecoxib (Celebra)	Monsanto	phase III	rheumatoid arthritis, osteoarthritis
MK966 (Vioxx)	Merck & Co.	phase III	rheumatoid arthritis, osteoarthritis
JTE522	Japan Tobacco	phase II	rheumatoid arthritis, osteoarthritis
T-614	Toyama	phase II	rheumatoid arthritis
SC-57666	Monsanto	phase I	rheumatoid arthritis
S-2474	Shionogi	phase I	arthritis
GR253035	Glaxo Wellcome	phase I	Alzheimer's disease, chronic inflammatory pain caused by osteoporosis, rheumatoid arthritis

[a] Source: *Pharmaprojects*, PJB Publications, Richmond, Surrey (UK).

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suggest that acetylsalicylic acid could also be effective in this indication.[6]

The relevance of COX-2 in the progression of Alzheimer's disease is also currently being discussed. It is believed that neuronal cells show increased expression of COX-2 upon stress,[7] and subsequently start the apoptotic process in concert with other highly regulated genes (*c-jun*, *c-fos*, and *fos-B*). It may also be that the effect is mediated through inhibition of other inflammatory components (platelet-activating factor, interleukin-1β), which are believed to play a role in the development of Alzheimer's disease.

In conclusion, the present models describing the profile of COX-2 inhibitors are not complete. On one hand (cancer) apoptosis is induced by COX-2 inhibition, and on the other hand apoptosis is prevented, like in neuronal cells. Irrespective of the final outcome of molecular pharmacology studies, first of all it is important that this new therapeutic approach gives rise to new prospects for patients. The therapeutical value now needs to be shown in clinical studies.

Conclusion

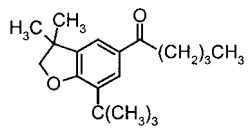
The current paper is focused less on the specific effect of COX-2 inhibitors, but rather on the expectations from this new drug class to achieve a significantly improved profile of side effects. Up to now the clinical results seem to confirm these expectations, at least with respect to the protection of the gastrointestinal mucosa. An exception is treatment of patients with preexisting ulcers. Their healing process is worsened under therapy with COX-2 inhibitors.

We will have to wait and see whether other side effects will show up after long-term therapy. Because COX-2 is expressed constitutively in kidney, brain, and ovaries, toxic effects on kidney, central nervous system (CNS) side effects, and fertility interferences cannot be excluded.

At this point in time it is hard to predict which other additional therapeutic advantages the covalent binding of inhibitors from Kalgutkar et al. might have. This is particularly difficult because of the virtually irreversible binding characteristics of the current COX-2 inhibitors.

Alternative Approaches

Other than COX-2 inhibition there are currently two approaches pursued in order to better control side effects. Procter & Gamble[8] is following a strategy of dual inhibitors for cyclooxygenase and lipoxygenase. The rationale is that the component with the detrimental effect on gastric mucosa is leukotriene B4, which is a product of arachidonic acid and 5-lipoxygenase. The lead structures identified belong to a series of 5-substituted dihydromethylbenzofurans (Scheme 4). They represent a new class of antiinflammatory and analgesically active compounds. Inhibitory constants for cyclooxygenase are in the sub-micromolar range; the value for 5-lipoxygenase is still in the range of 3 – 15 μM. To test the pharmacological concept, improvements of about 10- to 100-fold are necessary.



Scheme 4. 7-*tert*-Butyl-2,3-dihydro-3,3-dimethyl-5-pentanoylbenzofuran:[2] a dual COX-2 and 5-lipoxygenase inhibitor.

A second strategy envisages synthesis of NO-acetylsalicylic acid; that is, the acetylsalicylic acid is further substituted with a NO donor group. Release of NO in the gastrointestinal tract would lead to a local relaxation of the blood vessel with lower adhesion of leukocytes. This would improve protection of the mucosa and reduce haemorrhagic efffects.[9] Positive in vitro and in vivo results are available. Further preclinical and clinical investigations will demonstrate the therapeutic benefits, or lack thereof, compared to acetylsalicylic acid.

Outlook

Are COX-2 inhibitors better than Aspirin? The question suggests that these compounds are comparable. However, this is only partially true. Inhibitors of COX-2 represent a new therapeutic principle with prospects for the treatment of severe pain caused by rheumatic diseases or osteoarthritis. They are expected to take a significant part of this market. Already today acetylsalicylic acid is mainly used for myocardial infarct prophylaxis and moderate pain, such as that caused by headache. Dosage is low and gastrointestinal side effects do not play a major role for either indication. The spectrum of effects for acetylsalicylic acid is broader than just inhibition of COX-2. Other indications will potentially be added in the future, like prophylaxis and/or treatment of colon cancer or Alzheimer's disease. Acetylsalicylic acid has a fair chance to compete with COX-2 inhibitors. The advancement of NO-acetylsalicylic acid through clinical studies would be a welcome development indeed. The unique strength of acetylsalicylic acid is its pharmacologically nonselective behavior and lack of specificity for a single target. It is the portfolio of different properties that makes acetylsalicylic acid such a unique drug. In contrast COX-2 inhibitors cover only part of this spectrum and are rather considered to be experts in their respective field.

It is tempting to compare this situation with sports: acetylsalicylic acid would be the favorite in the decathlon, while COX-2 inhibitors would be competitive in two or three disciplines.

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Keywords: acetylsalicylic acid · arthritis · cyclooxygenases · rheumatism

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