PHAR 753
Pharmacology/Medicinal Chemistry Series

Arachidonic Acid Metabolic Pathway and Non-Steroidal Anti Inflammatory Drugs

Winter 2007

Taifo Mahmud
Maybe I shouldn't have taken that anti-inflammatory.
Introduction

The term eicosanoid refers to biologically active lipid mediators (C20 fatty acids and their metabolites), such as prostaglandins, thromboxanes, leukotrienes, and other oxygenated derivatives, which are produced primarily by three classes of enzymes, cyclooxygenases, lipoxygenases, and cytochrome P450 epoxygenase. Arachidonic acid is one of the key precursor fatty acids involved in the biosynthesis of lipid mediators. It is an essential fatty acid that cannot be synthesized de novo in mammals. It is derived from linoleic acid that is found in the diet. Arachidonic acid is a major component of phospholipids, especially of phosphatidylinositol, so that it is important for the integrity of cellular membranes.

Diacylglycerols, enriched in arachidonic acid and derived from phosphatidylinositol, are important cellular messengers.

Anandamide (N-arachidonylethanolamine) is an endogenous cannabinoid or ‘endocannabinoid’, which produces neurobehavioral effects similar to those induced by cannabis and may have important signaling roles in the central nervous system, especially in the perception of pain and in control of appetite.

There are now suggestions that arachidonic acid per se may have some biological important in animal tissues; for example, the cellular level of free arachidonic acid may be a general mechanism by which apoptosis is regulated.

The oxygenated metabolites derived from arachidonic acid and related fatty acids are produced through a series of complex interrelated biosynthetic pathways. There are so numerous and have such a wide range of biological activities that they must be involved in regulations related to the survival and well-being of human being and animals. The natural eicosanoids are synthesized with great stereochemistry precision, and this is important for their biological functions. They are highly active in the nanomolar range in vitro, especially those in relation to inflammatory responses, pain, and fever.
Phospholipase A2

Figure. Role of cytosolic phospholipase A2 in the inflammation signaling pathway

PLA2 (MW ~ 60 – 100 kDa) is regulated by phosphorylation and in the presence of Ca\(^{2+}\) is translocated from the cytosol to the membranes of the nucleus and endoplasmic reticulum, where the precursor phospholipids and the key enzymes of eicosanoid biosynthesis are situated. In addition to control via transcriptional regulation, the activity of the cytosolic Ca\(^{2+}\)-dependent PLA2 responds to various stimuli, including hormones, cytokines, neurotransmitters, etc.
Free arachidonic acid

\[ \text{COX} \]

\[ \text{PGG}_2 \] → \[ \text{PGH}_2 \]

\[ \text{PGD}_2 \] (erythema, edema, pain, platelet aggreg'n)

\[ \text{PGE}_2 \] (erythema, edema, pain, fever)

\[ \text{PGF}_2 \] (vasoconst, uterine)

Prostacyclin

\[ \text{PGI}_2 \] (vasodilatation, platelet aggreg'n)

\[ \text{TXA}_2 \] (vasoconst, platelet aggreg'n)
Cyclooxygenases-1 and -2 (COX-1 and -2) – also known as Prostaglandin H$_2$ synthases-1 and –2
- catalyze the committed step in prostaglandin biosynthesis.
- bifunctional – have cyclooxygenase and peroxidase activity
- membrane-bound enzymes
- present on the luminal surface of the endoplasmic reticulum and of the inner and outer membranes of the nuclear envelope.
- heme-dependent enzymes
- both enzymes catalyzed the same two reactions at different sites. Thus, each carries out a cyclooxygenase reaction in which two molecules of oxygen are added to arachidonic acid to form a bicyclic endoperoxide with a further hydroperoxy group in position 15 to form PGG2. These reactions occur at a hydrophobic channel in the center of the enzyme. The hydroperoxide is then reduced by a peroxidase reaction to form PGH2, a reaction that occurs at a heme-containing site on the surface of the enzyme. Although the reactions occur at different sites, they are functionally coupled.
- the major targets of NSAIDs
- have direct roles in many human pathologies, e.g., thrombosis, inflammation, pain, fever, various cancers, and neurological disorders such as Alzheimer’s and Parkinson’s diseases.

**COX activity**
COX-1 and –2 synthesize primarily PGH$_2$ through PGG$_2$ from arachidonic acid, however they also produce small but significant amounts of other products: 11R-hydroperoxy-(5Z,8Z,12E,13Z)-eicosatetraenoic acid (11R-HPETE) and 15-hydroperoxy-(5Z,8Z,11Z,13E)-eicosatetraenoic acid (15R-HPETE and 15S-HPETE). COX-1 and –2 also catalyze the oxygenation of other polyunsaturated fatty acids into bioactive compounds, such as the series-1 prostaglandin precursor PGH$_1$ from dihomo-$\gamma$-linoleic acid (DHLA; 20:3n-6) and the series-3 prostaglandin precursor PGH$_3$ from eicosapentaenoic acid (EPA; 20:5n-3), a dietary $\omega$-3 fatty acid that has been linked to reduce cardiovascular disease. They also convert linolenic acid (LA; 18:2n-6) to 9- and 13-hydroxyoctadecadienoic acids.

**COX structures**
- primary structures of COX-1 and –2 are 600-602 and 604 amino acids, respectively
- the two isomers have about 60% homology in their amino acids
- both isoforms are processed into mature forms by removal of signal peptides
- both isoforms are homodimers in solution
- both isoforms contain an epidermal growth factor (EGF)-like domain just C-terminal to the signal peptide. (function is unclear)
-COX-1 is uniformly glycosylated at three sites (Asn68, Asn144, and Asn410)
-COX-2 is heterogenously glycosylated at an additional site (Asn588)
-N-glycosylation may play a role in maturation of COXs.

Very recently, it was shown that inducible nitric oxide synthase (iNOS) specifically binds to COX-2 and S-nitrosylates it, enhancing COX-2 catalytic activity. (Science, vol 310, 23 December 2005)

The crystal structures of COX isoforms are structurally homologous and quite superimposable.
The COX monomer consists of three structural domains:

1. the N-terminal EGF domain,
2. a membrane binding domain of about 48 aa in length, and
3. a large C-terminal globular catalytic domain containing the heme binding site.

**Epidermal Growth Factor Domain**
EGF domains create a substantial portion of the dimer interface.
EGF domains may play a role in the insertion of COX into the lipid bilayer.

**Membrane binding domain**
Consists of four α-helices. Hydrophobic and aromatic residues protrude from these helices to create a hydrophobic surface that would interact with only one face of the lipid bilayer.

**Catalytic domain**
The entrance to the COX active site is between the helices of the membrane-binding domain. The hydrophobic channel extends from the membrane-binding domain into the catalytic domain, a distance of about 25 Å. The peroxidase active site is in a large groove on the opposite site of the membrane-binding domain.
Other differences between COX-1 and COX-2

COX-1
- constitutively expressed (although COX-1 can also be induced)
- involved in many aspects of physiological homeostasis

COX-2
- inducible (but, the CNS, the kidney, and the seminal vesicle contain constitutively high level of COX-2)
- expression in a select number of cells is triggered by specific cellular events.
- COX-2 protein level in primary human cells such as endothelial cells, fibroblasts and synovial cells is undetectable or barely detectable. But, it can be induced by diverse agonists including phorbol-12-myristate 13-acetate (PMA), IL-1, LPS, and TNF-α.

Active sites of COX-1

Arg120: the guanidium group of Arg120 interacts with the carboxylate of the fatty acid
Ser530: carbon 7 through 14 of AA form an S-shape that weaves the substrate chain around the side chain of Ser530, the residue acetylated by aspirin. While not essential for catalysis, it may help optimally align the substrate with respect to the Tyr385 for hydrogen abstraction at carbon 13, as well as for subsequent oxygen addition at carbon 11. Acetylation of Ser530 by aspirin blocks the binding of AA and completely inactivates the enzyme.

Tyr385: AA is positioned such that C-13 is oriented near the phenolic oxygen of Tyr385, where the pro-S hydrogen can be abstracted to initiate the COX reaction.

Phe205, Phe209, Val344, Phe381, and Leu534 form a hydrophobic groove above Ser530 and stabilize the conformation of ω-end of AA (carbon 14 through 20).
**Active sites of COX-2**
In general almost the same as those of COX-1 except the COX active site in COX-2 is larger than in COX-1. When aspirin acetylates Ser530 of human COX-2, substrate turnover still occurs, but 15R-HPETE is produced instead of PGG$_2$. 
Comparison of the accessible volume of the sheep COX-1 and human COX-2 hydrophobic substrate binding pockets.

Proposed scheme for radical mechanism of cyclooxygenase activity
**Inhibition mechanism**
Most NSAIDs compete with arachidonate for the COX active site, but each substance can be classified by one of three general modes of actions:

1) rapid, reversible competitive inhibition, e.g., ibuprofen
2) rapid, reversible binding followed by covalent modification, e.g., aspirin
3) rapid, lower-affinity competitive inhibition followed by a time-dependent transition to a high-affinity poorly reversible inhibitory mode, e.g., flurbiprofen, indomethacin, celecoxib, rofecoxib.
A. Salicylic Acid Derivatives

1. History

Willow bark → salicin → glucose + salicylic alcohol → salicylic acid
Oil of Wintergreen contains methyl salicylate
In plants, salicylic acid is synthesized from trans-cinnamic acid by decarboxylation to benzoic acid and further 2-hydroxylation of benzoic acid.

Salicylates modified at the Carboxyl Group

- Sodium salicylate
- Magnesium salicylate
- Choline salicylate
- Methyl salicylate
- Phenyl salicylate
- Salicylamide
- Salsalate
- Cabethyl salicylate
a. Sodium Salicylate

There is still no common agreement about its mechanisms of action. In purified preparations of COX-1 and -2, sodium salicylate is inactive up to 6.25 mM. In intact cells, it was found that sodium salicylate is a weak inhibitor of COX-1 and COX-2.

There are a number of studies showing interference by salicylates with intracellular signaling pathways:
- high concentrations of salicylates have been shown to interfere with kinases, including the mitogen-activated protein kinase (MAPK) cascase. (inhibit most MAPK but activate p38 MAPK).
- Inhibitory effects of salicylates have been observed on several nuclear transcription factors, such as NF-κB and AP-1. However, it remains doubtful to which extent these effects contribute to the anti-inflammatory activity of salicylates. In fact, experimental conducted in mice deficient in p105 (the precursor of p50 component of NF-κB), aspirin and sodium salicylate retained their anti-inflammatory efficacy, while the effect of dexamethasone, which is known to inhibit the activation of NF-κB, was abolished.
- In 2003, it was reported that salicylates at pharmacological concentrations suppress COX-2 and inducible nitric oxide synthase (iNOS) transcription via a C/EBP-dependent pathway (suppresses C/EBPβ binding to COX-2 promoter). Aspirin and sodium salicylate equipotently inhibit COX-2 expression in human cells. [C/EBP = CCAAT/enhancer binding protein]

Stimulation of iNOS by bradykinin and LPS plus interferon-γ enhances prostaglandin formation. NOS inhibitors prevent prostaglandin formation.

Carbethyl Salicylate
An ester of ethylsalicylate and carbonic acid. Insoluble in water and dilute HCl solution. Therefore, it tends to prevent gastric irritation and make it tasteless.

\[
\text{Carbethyl salicylate is metabolized to salicylic acid.}
\]

Salsalate (Disalcid, Amigesic)
- An ester formed between two salicylic acid molecules. It is hydrolyzed to salicylic acid following absorption.
- less gastric upset than aspirin because it is relatively insoluble in the stomach and is not absorbed until it reaches the small intestine.
Salicylamide

Readily prepared from salicyl chloride and ammonia. It is not hydrolyzed to salicylic acid. It is used in place of salicylates in the case of patients sensitive to salicylates.

**Salicylates Modified at the Hydroxyl Group**

**Acetylsalicylic Acid (ASPIRIN, ASPRO)**

Acetylsalicylic acid (Aspirin) was first synthesized in 1898 and introduced as a pain reliever in 1899. Prepared from salicylic acid and acetic anhydride

**Mechanism of action:** irreversibly acetylates cyclooxygenase. However, since aspirin is rapidly deacetylated to salicylate, it has been assumed that anti-inflammatory effects of aspirin are largely mediated by salicylate. This assumption is supported by experimental evidence that in vivo, salicylate and aspirin exhibit similar anti-inflammatory potencies. However, aspirin inhibits COX in platelets as well as in the
vascular wall. In contrast, sodium salicylate does not prevent thromboxane A₂ formation in platelet-rich plasma.

Indications: inflammation, fever, pain. Also, in a smaller dose it is used for preventing myocardial infarction and ischemia stroke.

Aspirin is metabolized to salicylic acid. Salicylic acid is then metabolized primarily to two major inactive metabolites: salicyluric acid and gentisic acid. Both are excreted in the urine.
Other Salicylate Analogs

*Flufenisal*

- 2X potency of ASA

*Diflunisal*

- 3X potency of ASA

Flufenisal and Diflunisal (DOLOBID)

Two to three time as effective as aspirin with twice the duration of action and a lower incidence of gastric irritation

**B. Aniline and p-Aminophenol Derivatives**

*Aniline*  

*Acetanilide*  

*p-aminophenol*  

*Acetaminophen (Tylenol, Datril)*

Acetaminophen (Datril, Tylenol)

Acetaminophen is a very weak anti-inflammatory drug but is effective as an antipyretic and analgesic agent. It lacks certain side effects of NSAIDs, e.g., gastric ulceration, blockade of platelet aggregation.
In acute overdose, acetaminophen becomes a particularly toxic drug. Normally, acetaminophen is metabolized by N-deacetylation and glucuronidation. However, in acute overdose and the normal pathway is saturated, a second metabolic pathway takes over to give rise to a reactive species called ‘arylating intermediate’. This intermediate reacts rapidly with GSH, causing a complete depletion of glutathione, which leave the liver defenseless against reactive intermediates produced by the mixed function oxidases and against other reactive compounds. The process may be reversed by administration of acetylcysteine, which preferentially reacts with reactive species.
C. N-Arylanthranilic Acids (The Fenamates)

1. Mefenamic Acid (Ponstel)
2. Flufenamic acid (Arlef)
3. Meclofenamic acid (Meclomen)
C. Indole-3-acetic Acid Derivatives

1. Indomethacin (Indocin, Indocin SR, Indo-Lemmon, Indomethegan)

![Indomethacin structure]

Indomethacin was developed in 1965. It is one of the more potent NSAIDs (about 2-3 times of phenylbutazone). It is a potent anti-inflammatory and antipyretic drug. GI side effect is about equal to aspirin, but does cause headache and vertigo in some patients.

A time-dependent, functionally irreversible inhibitor of COX. In the crystal structure of indomethacin bound to COX-2, a small hydrophobic pocket was identified that surrounds the 2'-methyl group of indomethacin. The pocket is formed by Ala-527, Val-349, Ser-530, and Leu-531. Binding of the 2'-methyl of indomethacin in the hydrophobic pocket is important for its time-dependent inhibition of COX enzymes. Without the 2'-methyl group anchoring desmethyl-indomethacin in the active site, the compound was readily competed off of the enzyme by arachidonic acid.

About 30 years ago, Rome and Lands demonstrated that converting the carboxyl group of indomethacin into a methyl ester renders it a reversible, competitive inhibitor of COX-1, but the same and similar transformations do not abolish time-dependent inhibition of COX-2.
D. Arylacetic and Arylpropionic Acid Derivatives

Mechanism of action for organic acids: compete with arachidonic acid at the active site of cyclooxygenase.

**Sulindac (Clinoril)**

Sulindac is an arylacetic acid with a pKa of 4.7. Structurally, it is very similar with indomethacin. The double-bond (called indene isostere) has the same electronic character as the lone pair of the indole nitrogen and also permanently set the aracyl substituent in the cis conformation. The electron withdrawing p-methylsulfoxide increases potency and also increases solubility of the drug.
These drugs generally have three structural features of indomethacin:
- an acidic carboxyl group
- a nitrogen-containing ring system, except diclofenac potassium
- an out-of-plane phenyl system, except etodolac

**Tolmetin Sodium** (Tolectin, Tolectin DS, Tolectin 600)

**Diclofenac** potassium and sodium (Cataflam, Voltaren, Arthrotec [combination with misoprostol])

Binds to COX-2 in an inverted conformation with its carboxylate group hydrogen-bonded to Tyr-385 and Serine-530.

**Ketorolac Tromethamine** (Toradol)

**Etodolac** (Lodine, Lodine XL)
Arylpropionic Acid Analogs

Ibuprofen

Naproxen

Fenoprofen (Nalfon)

Ketoprofen

Flurbiprofen (Ansaid, Ocu fen)

These drugs have an alpha methyl group. In general, the S(+) isomer is the active form, and the R(-) isomer is inactive. However, some of them are administered as racemates. Fortunately, the R(-) isomer of most analogs is converted to the corresponding S(+) isomer in vivo. However, the R(-) isomer of naproxen has been reported to be a liver toxin.

Ibuprofen (Motrin, Advil, Nuprin, Medipren, Cap-Profen, etc)

It is administered as a racemate. Indicated for mild to moderate pain and inflammation.
Naproxen (Aleve [the S(+) isomer 97% ee], Anaprox [the S (+) isomer], Naprosyn [the S(+) isomer], Naprelan)

![Naproxen](image)

Fenoprofen (Nalfon)

![Fenoprofen](image)

Ketoprofen (Oruvail, Orudis, Actron)

![Ketoprofen](image)

Flurbiprofen (Ansaid, Ocufen)

![Flurbiprofen](image)
Oxaprozin (DayPro)

\[
\begin{align*}
\text{Oxaprozin (DayPro)} & \\
\text{N} & \\
\text{O} & \\
\text{C} & \\
\text{O} & \\
\text{H} & \\
\text{x} & \\
\text{a} & \\
\text{p} & \\
\text{r} & \\
\text{o} & \\
\text{z} & \\
\text{i} & \\
\text{n} & \\
\text{a} & \\
\text{b} & \\
\text{l} & \\
\text{e} & \\
\end{align*}
\]

Nabumetone (Relafen)

\[
\begin{align*}
\text{Nabumetone} & \\
\text{H}_3\text{CO} & \\
\end{align*}
\]

Introduced in the US in 1992. Contains no acidic functional group. It is converted by oxidation to the corresponding arylacetic acid. It potentially has less stomach upset. It has a very long life, therefore, can be dosed once a day.
E. Oxicams (Enolic Acids)

Oxicams have extremely long half-lives, and thus are usually administrated once a day. Oxicams are indicated for RA and osteoarthritis. They have analgesic, anti-inflammatory and antipyretic activities.

1. Meloxicam (Mobic)

\[
\text{Meloxicam (Mobic)}
\]

2. Piroxicam (Feldene)

\[
\text{Piroxicam (Feldane)}
\]

The activity based on the enolic hydroxyl group, which has a pKa of 4.3. This hydroxyl group presumably interacts with Tyr-385 and Serine-530 of COX-2 and important in its time-dependent inhibition.

F. Pyrazolidinediones

1. Phenylbutazone
G. COX-2 Selective Inhibitors

1. Celecoxib (Celebrex, Pfizer)

Celecoxib is the only COX-2 inhibitor currently available on the market. However, based on emerging information, including preliminary reports from NIH, the risk of cardiovascular events may be increased in patients receiving Celebrex. Recently, Pfizer is placing a $100 million bet on a 20,000-person, international trial, focusing on patients with hearth disease, including those who recently underwent bypass surgery and those at risk of cardiac problems. The trial will end after 715 “events” – hearth attacks, strokes, or deaths – have occurred.

2. Rofecoxib (Vioxx, Merck)

Merck voluntarily withdrew Vioxx from the U.S. and worldwide market due to safety concerns of an increased risk of cardiovascular events (including heart attack and stroke) in patients on Vioxx. Vioxx is a prescription COX-2 selective, NSAID that was approved by FDA in May 1999 for the relief of the signs and symptoms of osteoarthritis, for the management of acute pain in adults, and for the treatment of menstrual symptoms.
3. Valdecoxib (Bextra, Pfizer)

On April 7, 2005, the FDA asked Pfizer to voluntarily remove Bextra from the market.

For many years, COX-2 has been the favorite target for anti-inflammatory drug development. However, recent discovery of the adverse effects of selective COX-2 inhibitors have stimulate intense debate. Interestingly, in the early phase of inflammation, COX-2 facilitates inflammatory PG production, however, in the late phase it has anti inflammatory effects. Moreover, although some PGs are proinflammatory, others have anti-inflammatory effects. Thus, it is likely that PGs with opposing effects maintain homeostasis, although the molecular mechanism remains unclear. It was reported that a proinflammatory PG, PGD2, via its receptor, mediates the activation of NF-kB stimulating COX-2 gene expression. On the other hand, the anti-inflammation PGA1 suppresses NF-κB activation and inhibits COX-2 gene expression. Therefore, selective COX-2 inhibitors may disrupt the balancing act of PGs, which resulted in reported adverse effects.

4. Lumiracoxib (Prexige, Novartis)

This COX-2 inhibitor has been launched across Europe, Canada, Australia and other countries in 2006. However, until recently, the FDA has not yet granted approval for its sale in the US. Structurally, lumiracoxib is more closely resembles the structure of diclofenac, making it a member of the arylacetic acid family of NSAIDs. It has extremely high COX-2 selectivity and binds to a different site on the COX-2 receptor than the standard COX-2 inhibitors. Lumiracoxib has a significantly lower GI side effect compared with ibuprofen and naproxen and has not been associated with any increased cardiovascular risk.