EICOSANOIDS AND INFLAMMATION

Learning Objectives

(1) Summarize the biochemical foundations of the inflammatory response and the participation of eicosanoids in this process.
(2) Describe the conversion of arachidonic acid to eicosanoids and name the key enzymes.
(3) Comment on the physiological roles of these bioactive lipids.
(4) Discuss the biochemical basis of selected pharmacological agents used to treat inflammation and other eicosanoid-related health problems.

I. Case Study of Rheumatoid Arthritis

A. History and physical

CC is a 45-yr-old, 52-kg female who has been referred to a rheumatology clinic with the chief complaints of chronic joint pain and fatigue during the past four months. The patient is pre-menopausal, has no recent history of infection or inoculation, and is taking aspirin twice daily for symptomatic relief. Joint and muscle stiffness are primarily in the morning and persist for several hours and occasionally all day. CC is afebrile but reports anorexia and a recent weight loss of 4 kg. Her symptoms have been much worse during the past month, and she has been forced to limit her physical activities. She is no longer able to wear her wedding band and has difficulty removing lids from jars.

Physical examination reveals varying degrees of swelling (synovial thickening), tenderness, and warmth of the metacarpophalangeal (MCP) and proximal interphalangeal (PIP) joints of both hands. Her wrist joints and elbows are also involved. There are small, non-tender nodules on the hands and left forearm. No skin rashes or lesions are observed.

Pertinent laboratory findings include: Westergren erythrocyte sedimentation rate (ESR), 42 mm/hr (normal, 0-20); hemoglobin, 106 gAL^{-1} (normal,120-160), with microcytosis; hematocrit, 33% (normal, 36-46); platelet count, $480\times10^{3}Amm^{-3}$; albumin, 38 gAL^{-1} (normal, 31-43); high-specificity C-reactive protein, 27 $mgAL^{-1}$ (normal, 1-3); serum iron, 400 : gAL^{-1} (normal, 500-1600); iron-binding capacity, 2.75 $mgAL^{-1}$; and rheumatoid factor (RF) that is seropositive at a dilution of 1:320. Tests for systemic lupus erythematosus, antinuclear antibodies, and tuberculin are negative. X-ray films of the hands and arms show soft tissue swelling, with no indication of periarticular osteopenia. Other routine laboratory and physical findings are normal.

B. Diagnosis

The initial clinical impression is that of a patient with rheumatoid arthritis (RA). RA is the most common chronic inflammatory arthritis, affecting about 1% of adults, with a 2-3-fold greater prevalence in females. Susceptibility to RA appears to be polygenic. While there is no specific test for the diagnosis of RA, CC presents with several features of early progressive disease: active synovitis, elevated ESR and RF, and rheumatoid nodules. An increase in ESR is often seen in acute and chronic inflammatory states but may also be elevated in malignancy. Similarly, the elevated platelet count is suggestive of an inflammatory response. The mild anemia is generally consistent with a chronic disease and probably would not respond to iron therapy. The normal serum uric acid concentration is inconsistent with gout as the cause of joint inflammation. RF is present in the serum of 65-85% of individuals with RA; it is an immunoglobulin formed from abnormal IgG molecules produced by lymphocytes in the synovial fluid.

C. Treatment

CC began taking 975 mg aspirin (3 tablets) three times daily with meals and water, in conjunction with physical therapy and rest. A week later, her symptoms were only marginally improved. Her physician then prescribed an increased dosage of aspirin to 4 tablets three times daily. This treatment was well tolerated and provided excellent analgesic and anti-inflammatory results.

II. Synthesis of Eicosanoids

Eicosanoids are oxygenated derivatives of polyunsaturated fatty acid found in a wide range of microorganisms, plants, and animals. Eicosanoids represent a broad spectrum of bioactive lipids that include prostaglandins, prostacyclins, thromboxanes, and leukotrienes. In humans, eicosanoids [Greek *eikosi*, twenty] are produced by the oxidation of 20-carbon fatty acids that are derived from the two *essential fatty acids*, *linoleate* and *linolenate*. Eicosanoids are potent local hormones that are released by most cells, act on that same cell or nearby cells, and then are rapidly inactivated. In **Table 1** are summarized some functions of prostaglandins and other eicosanoids in the human body and the pathologic consequences that occur if their metabolism is altered. For many of these functions, different eicosanoids exhibit opposite actions to achieve homeostatic balance.

The biosynthesis of eicosanoids begins with the interaction of certain hormones, growth factors, or cytokines with cell-surface receptors and the stimulation of one or more signal transduction pathways. Among the downstream cellular signaling events is the activation of a phospholipase A_2 (**Figure 1**). Arachidonic acid, the most common precursor to eicosanoids, is esterified at the *sn*-2 position of cell membrane phospholipids; activated phospholipase A_2 catalyzes ester hydrolysis and the release of the free acid. This hydrolysis appears to be the rate limiting step for eicosanoid formation. Arachidonic acid can be subsequently transformed by several pathways (some major, others minor). ***The metabolic fate of arachidonic acid depends on the cell type and its specific complement of enzymes.***

Table 1. General Functions of Prostaglandins and Other Eicosanoids

Physiological Functions

- Vasoconstriction and vasodilation
- Regulation of hemostasis
- Bronchoconstriction and bronchodilation
- Cytoprotection of gastrointestinal tract; secretion of mucus, fluids, and duodenal bicarbonate
- Renal blood flow, Na^+ and water reabsorption, renin release
- Female reproduction activities (ovulation, implantation, uterine muscle tone, and contractility)
- Central nervous system regulation of temperature, pain, sleep, and cerebral blood flow
- Smooth muscle contraction
- Chemotaxis

Pathological Effects (excess or deficiency)
Gastric bleeding, ulceration, and perforation; inflammatory bowel disease; arthritis; asthma; infertility; colorectal adenocarcinoma; hypercalcemia

Therapeutic Applications
Treatment of gastric ulceration, pulmonary hypertension, and erectile dysfunction; abortifacient

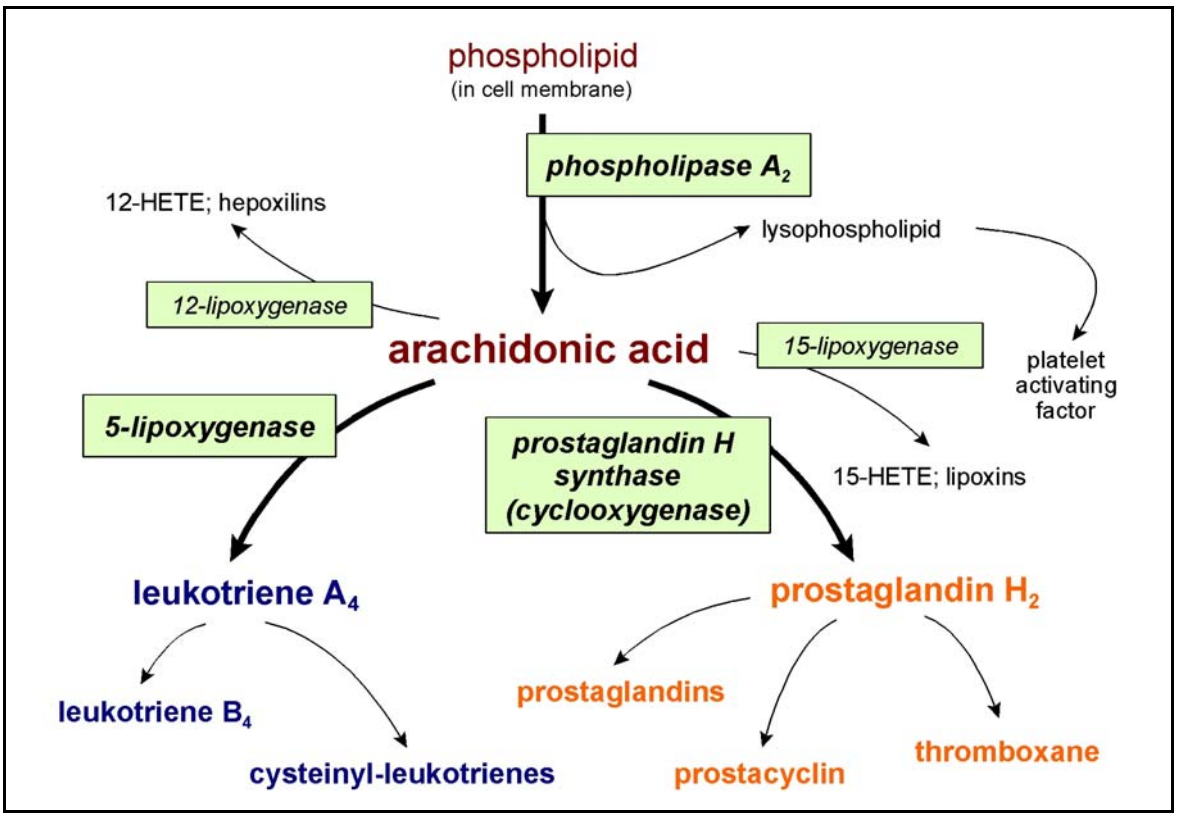

Figure 1. Biosynthesis of Eicosanoids from Arachidonic Acid

Phospholipase A_2, prostaglandin H synthase (PGHS), and 5-lipoxygenase are three important enzymes regulating eicosanoid synthesis. Both PGHS and 5-lipoxygenase utilize molecular oxygen (O_2) as one substrate in their catalytic mechanisms. It is important to observe that the metabolism of arachidonic acid is a highly branched pathway (**Figure 1**). Later sections in this chapter will summarize findings that the class of eicosanoid is specific to each cell and that arachidonic acid metabolites possess different half-lives and, in their physiological actions, may be complementary, additive, or even antagonistic! Interestingly, the action of phospholipase A_2 on a phospholipid such as phosphatidylcholine produces a lysophospholipid that may be directly converted, as shown in **Figure 1**, to platelet activating factor (PAF), another important mediator of the inflammatory response.

A. Prostaglandin H synthase

Prostaglandin H synthase, the first enzyme unique to the synthesis of prostaglandins, prostacyclins, and thromboxanes, is better known by its more popular name, cyclooxygenase (COX). Prostaglandin H synthase catalyzes two separate reactions: (1) the *bis*-oxygenation of arachidonic acid (**Figure 2B**) at carbons 11 and 15 (cyclooxygenation activity) and (2) the subsequent two-electron reduction (peroxidation) to produce prostaglandin H_2 (PGH_2), an intermediate common to several synthetic pathways (**Figure 1**). PGHSs are homodimeric, heme-containing glycoproteins that are localized to the endoplasmic reticulum membrane. Non-steroidal anti-inflammatory drugs (NSAIDs) compete with arachidonic acid for binding to the cyclooxygenation catalytic site (discussed further in **Section V**); these compounds, however, have no appreciable effects on the peroxidation activity.

B. Cyclooxygenase isoforms

There is now solid genetic evidence for two PGHS or COX isoforms (COX-1, COX-2), each having specific catalytic, regulatory, and tissue distribution properties (**Table 2**). The overall catalytic process is designed to achieve (1) the stereospecific extraction of one hydro-

gen from arachidonic acid and (2) the control of the subsequent oxygenations, producing PGH_2, 1 of 64 possible reaction conformers (**Figure 2**). This is accomplished in a substrate binding channel that appropriately constrains arachidonic acid (**Figure 2**). Substrate preferences led to the suggestion that the COX-2 active site was somewhat larger than that of COX-1, a difference confirmed by crystallographic analysis and exploited by pharmaceutical design (**Section V**).

C. Lipoxygenases; LTC_4 synthase

In contrast to its metabolism in the presence of PGHS, arachidonic acid may face different fates in other cell environments. Catalytic action on arachidonic acid by ***5-lipoxygenase*** yields the acyclic hydroxyeicosatetraenoic acid (HETE) or leukotriene A_4 (LTA_4). Eventual products of this pathway include the other major class of eicosanoids known as leukotrienes (**Figure 1**). 5-Lipoxygenase is a soluble, non-heme-iron protein and, like PGHS, is expressed as distinct isoforms. Tissue distribution of 5-lipoxygenase includes cells of myeloid lineage (neutrophils, monocytes, macrophages, B lymphocytes), lung, intestine, and placenta. In the presence of ***LTC_4 synthase***, a perinuclear membrane enzyme, the reduced glutathione ((-Glu-Cys-Gly) conjugate precursor of the cysteinyl-leukotrienes (or peptidyl-leukotrienes) is formed.

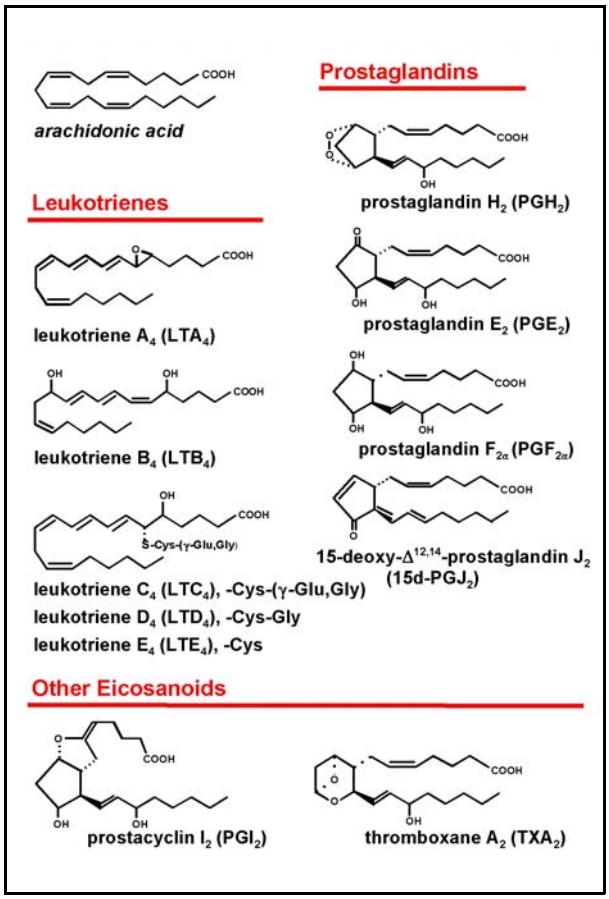

Figure 3. Structures of Arachidonic Acid and Its Principal Eicosanoid Derivatives

In humans, the closely related enzymes, 12-lipoxygenase and 15-lipoxygenase, react with arachidonic acid to generate still other oxygenated metabolites, including the recently recognized family of lipoxins, some of which are potent anti-inflammatory regulators of leukocyte activity. Interestingly, lipoxygenases are found in most plants, fungi, and animals.

D. Nomenclature of eicosanoids

Arachidonic acid is the precursor molecule from which most physiologically important eicosanoids are synthesized. **Figure 3** shows the structures of this polyunsaturated fatty acid and its prostaglandin, prostacyclin, thromboxane, and leukotriene metabolites. Several important points to note in the structures of these arachidonic acid-derived eicosanoids are (1) all contain 20 carbon atoms; (2) all contain additional oxygen atoms; (3) prostaglandins are cyclic compounds with two double bonds; (4) formation of the ring systems consumes two double bonds; and (5) leukotrienes are non-cyclic compounds with four double bonds and may be modified by glutathione-derived amino acids. The common abbreviations of eicosanoids are based primarily upon the type of ring structure (*e.g.*, compare PGE_2 and PGI_2), the nature and orientation of ring substituents (*e.g.*, compare PGE_2 and $PGF_{2\alpha}$), and the extent of modification the by amino acid components of glutathione (*e.g.*, compare LTC_4 and LTE_4).

Another important piece of information in eicosanoid nomenclature is the numerical subscript, which identifies the polyunsaturated fatty acid from which the molecule is derived.

Eicosanoids synthesized from arachidonic acid, 20:4(5,8,11,14), include PGE_2, TXA_2, and LTD_4. Similarly, but less commonly, those derived from eicosatrienoic acid, 20:3(5,8,11), or eicosapentaenoic acid, 20:5(5,8,11,14,17), contain one less or one more double bond and include, respectively, PGE_1 and TXA_3. Leukotrienes, in contrast, retain the same number of double bonds as their precursor fatty acids; thus, LTB_4 is produced from arachidonic acid. Further metabolism of an eicosanoid, however, may lead to exceptions to these nomenclature guidelines (*e.g.*, 15d-PGJ_2).

III. Biological Activity of Eicosanoids

In **Table 3** are listed some specific actions of eicosanoids derived from arachidonic acid. Some of these compounds show dramatic opposing effects: PGI_2 is made by blood vessel endothelial cells and inhibits platelet aggregation; TXA_2 is made by platelets and promotes platelet aggregation. While the roles of prostaglandins in tissue function and physiology have been extensively examined, the role of lipoxygenase metabolites has only recently been addressed. Several disease conditions are now associated with altered lipoxygenase metabolism of arachidonic acid. The sulfidopeptide leukotrienes, LTC_4 and LTD_4 are secreted by mast cells and eosinophils. They are responsible for the bronchial smooth muscle constriction and bronchospasms associated with asthma.

Themselves the products of one set of signal transduction pathways (**Section II**), the short-lived, hormone-like eicosanoids act in an autocrine or paracrine manner on their target cells. Early observations noted that many eicosanoids elicited changes in cellular cyclic-AMP levels, Ca^{2+} distribution, and phospholipid turnover. These classic signal transduction cascades are initiated by means of binding to cellular receptors. Our current state of knowledge suggests that the majority of eicosanoid receptors are members of the G protein-coupled receptor superfamily. These cell-surface membrane proteins interact with several heterotrimeric guanine nucleotide-binding proteins (G_s, G_i, G_q) and ultimately communicate with adenylyl cyclase, phospholipases, or other intracellular targets. Ligands for these receptors include prostaglandins D, E, and F, prostacyclin, thromboxane, leukotriene B, and the cysteinyl-leukotrienes. Antagonists to the Cys-LT_1 receptor are currently employed in the treatment of asthma (**Section V**).

Other eicosanoids utilize different signaling pathways. Those eicosanoids that are synthesized on nuclear membranes appear to bind to members of the nuclear receptor superfamily of transcription factors. The best understood is 15d-PDJ_2, a PGD_2 metabolite that binds to peroxisome proliferator-activated receptors of the (subtype (PPAR(). Initiation of this signaling pathway leads to adipocyte differentiation or endothelial cell apoptosis. In addition to interacting with a cell-surface G protein-coupled receptor, leukotriene LTB_4 also binds to the liver-specific PPAR", with subsequent regulation of fatty acid degradation. Like LTB_4, PGI_2 similarly employs a dual receptor signaling system, binding not only to a cell-surface G protein-coupled receptor in many tissues but also to the nuclear-localized PPAR* in the uterus.

Table 3. Specific Actions of Some Prostaglandins and Leukotrienes

PGD_2	promotion of sleep
PGE_2	smooth muscle contraction; pain and fever modulation; bronchoconstriction
$PGF_{2"}$	uterine smooth muscle contraction
PGI_2	inhibition of platelet aggregation; vasodilation; embryo implantation
TXA_2	stimulation of platelet aggregation; vasoconstriction
15d-PGJ_2	adipocyte differentiation
LTB_4	leukocyte chemotaxis
Cys-LTs	bronchial smooth muscle contraction (asthma)

IV. Nature of the Inflammatory Response

You are now familiar with the metabolism, structures, and actions of the major eicosanoids in the human body. This section presents a brief description of inflammation, with an emphasis on those points relevant to arachidonic acid metabolism and its regulation.

Inflammation covers a wide range of pathophysiological events and presents in many different settings: acute or chronic; organ-specific, such as arthritis or asthma; reversible or irreversible. The inflammatory response seen in many chronic diseases or following acute injury is initiated when irritated or damaged blood vessels (primarily arterioles) expose subendothelial surfaces and cartilage to circulating platelets. Platelets adhere to these surfaces, aggregate at the openings in the damaged blood vessels, and release the contents of their granules into the extracellular space. Other cells, including vascular endothelial cells, leukocytes, mast cells, and macrophages, are recruited by chemotactic processes and become involved in the further development of inflammation.

One common denominator in these inflammatory reactions is the role of ***mediators***. These molecules act as endogenous hormones and regulatory substances at the site of injury; some examples of mediators are presented in **Table 4**. It should be stressed that not all mediators participate in all settings of inflammation. For example, based on the efficacy of antihistamine pharmacological agents, histamine may be invoked in certain bronchial and cutaneous allergic inflammatory responses, yet this biologic amine plays only a minor role in rheumatoid arthritis or asthma.

Table 4. Selected Mediators of Inflammation

amines	histamine serotonin catecholamines
peptides / proteins	bradykinin interleukin-1 tumor necrosis factor
lipids	platelet activating factor prostaglandins thromboxanes leukotrienes

Among the compounds released from platelet granules are serotonin, adenosine diphosphate (ADP), and platelet activating factor (PAF). Serotonin, a vasoconstrictor, helps close off the damaged blood vessels; ADP and PAF promote further platelet aggregation and granular release. The chemically-induced aggregation is accompanied by activation of phospholipases within the platelets and the release of arachidonic acid from platelet membrane phospholipids. In these cells arachidonic acid is rapidly converted to thromboxane A_2 (TXA_2), a strong promoter of platelet aggregation. Another critical arachidonic acid-derived product is prostaglandin E_2 (PGE_2), the predominant lipid mediator detected in inflammatory conditions ranging from experimental acute edema to sunburn and chronic rheuma-

toid arthritis. ***An important piece of evidence suggesting that arachidonic acid metabolites play a fundamental role in inflammation was the finding that aspirin inhibited a key enzyme required for prostaglandin synthesis.*** Actions of anti-inflammatory agents are discussed in **Section V**.

While the participation of eicosanoids as mediators of these chronic pathologies is well documented, it is now clear that cytokines also play a critical role. These small extracellular proteins are characteristically produced by immune and inflammatory cells, function as short-distance messengers between cells of the immune system, and, like the eicosanoids, exert a variety of synergistic and antagonistic effects on target tissues. Cytokines include interleukins, interferons, and tumor necrosis factor. They bind to cell-surface receptors to trigger signaling cascades that often result in altered gene transcription.

Exposure to irritant plants or stinging insects often gives rise to a classic inflammatory response. The vasoconstriction that occurs immediately after injury is followed by a prolonged period of vasodilation, mediated primarily by PGE_2 and prostacyclin I_2 (PGI_2) and more weakly by leukotriene B_4 (LTB_4) and PAF. The resultant increased blood flow and consequent capillary congestion brings about ***erythema*** (redness) at the inflamed site. This is the first symptom of the inflammatory response. Blood vessel endothelial cells produce PGI_2 ($t_{1/2}$ = 5-10 min). TXA_2 is an extremely short-lived ($t_{1/2}$ = 30-40 sec) but potent vasoconstrictor of most vascular smooth muscle. TXA_2 appears to mediate the release of PAF, PGE_2, and LTB_4.

Dilation of the arterioles by these pro-inflammatory mediators leads to elevated hydrostatic pressure in the venules and an increase in the vascular wall area. The net result is the development of vascular permeability. The exudation of plasma from the permeabilized blood vessels into the connective tissue produces ***edema***, the second symptom of inflammation. LTB_4 and PAF are produced in leukocytes and act with histamine (decarboxylated histidine, released by mast cells) and bradykinin (a small peptide hydrolyzed from a high-molecular-weight plasma kininogen precursor) to induce permeability in the dilated venules. Another important function of LTB_4, PAF, histamine, and bradykinin is to attract phagocytic cells. Collectively, these mediators act as chemotactic agents and promote the recruitment of granulocytes (neutrophils, eosinophils) and monocytes to the site of injury or infection. The much larger macrophages (tissue-resident monocytes) are slower to appear; they ultimately engulf bacteria and dispose of cellular and tissue debris.

The third symptom of inflammation is ***hyperalgesia*** (pain). In response to pro-inflammatory cytokines, COX-2 activity increases to produce elevated levels of PGE_2 in endothelial, cutaneous, and synovial cells. This eicosanoid, acting alone or in concert with bradykinin or other stimuli, may then sensitize polymodal nociceptors and other afferent pain neurons. Synthesis of PGE_2 within the central nervous system and a high level of PGE_2 receptors in the circumventricular region of the brain are consistent with a role for this eicosanoid as an endogenous pyrogen and activator of neuronal pathways associated with sensitivity to pain. Lastly, PGE_2 is a potent pyretic agent and is involved in development of ***heat*** or ***fever***, the fourth symptom of inflammation; its production in bacterial and viral infections appears to be stimulated by certain cytokines, *e.g.*, interleukin-1.

Many chronic, debilitating diseases, such as bronchial asthma and rheumatoid arthritis, may be described as inflammatory processes. The hallmark of asthma is a variable airway obstruction, accompanied by airway hyperresponsiveness to a variety of stimuli. Prominent clinical manifestations include wheezing and shortness of breath. Criteria used in the diagnosis of rheumatoid arthritis were presented in the Case Study (**Section I**). Asthma and arthritis share many of the same characteristics of acute inflammation that were presented in the preceding paragraphs, including a triggering (immunologic) stimulus, cell migration and activation, and tissue stimulation and/or damage. Indeed, in the arthritic patient the affected joints are generally swollen, warm, and painful, and because synovial vessels, not

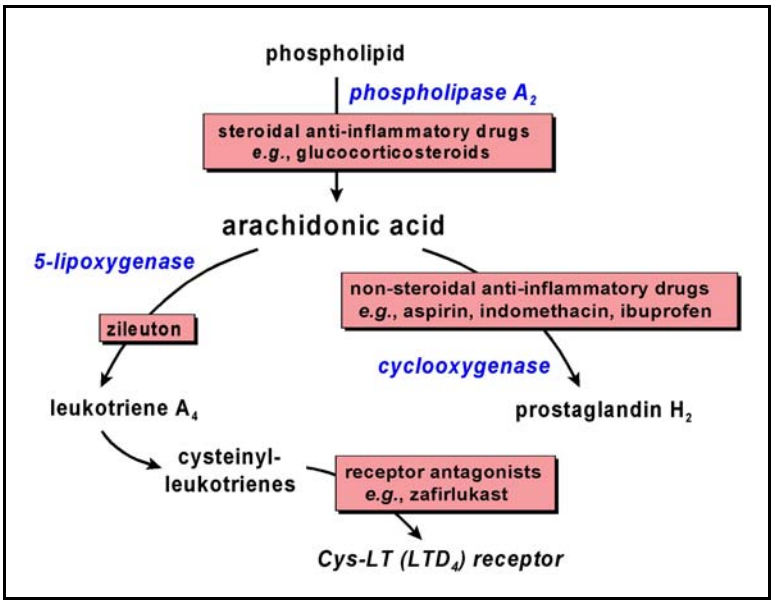

Figure 4. Targets of Common Anti-Inflammatory Pharmaceutical Agents

superficial vessels, are dilated, the skin is rarely red.

V. Effects of Anti-Inflammatory Drugs on Arachidonic Acid Metabolism

A. Non-steroidal anti-inflammatory drugs

With the link between eicosanoids and inflammation firmly established, the development of anti-inflammatory pharmacological agents has been rapid and competitive. Non-steroidal anti-inflammatory agents (NSAIDs) act by inhibiting the COX enzymes in cells of the tissues at the inflamed site (**Figure 4**). Aspirin is the best-known example of an NSAID. The result is a block in the conversion of arachidonic acid, liberated from membrane phospholipids, into prostaglandins, prostacyclin, and thromboxane. Among the mediators of inflammation whose synthesis is blocked are PGE_2, PGI_2, and TXA_2.

The anti-inflammatory properties of aspirin were first documented in the late 1940's, although successful treatment of acute rheumatoid arthritis with aspirin had been reported in 1876. It was not until 1971 that the link between aspirin and prostaglandins was established. ***Aspirin inhibits the cyclooxygenase activity of prostaglandin H synthase by acetylating the hydroxyl group of a unique serine residue; this inhibition is irreversible.***

The structure of aspirin is shown in **Figure 5**; the acetyl group is depicted in the highlighted box. Interestingly, the salicylate product that is generated after transfer of the acetyl group has therapeutic properties independent of those caused by the acetylation reaction. Nevertheless, the primary action of aspirin still relates to suppression of prostaglandin synthesis. Because enzyme acetylation is irreversible, new protein synthesis is required for the recovery of prostaglandin synthesis. This effect, in fact, forms the rationale for the daily ingestion of aspirin (325 mg) to reduce the risk of thrombosis in patients at risk for myocardial infarction and other cardiovascular disease. While both PGI_2 and TXA_2 require PGHS activity for their synthesis, the platelet has no nucleus and a life span of 8 days in circulation. Consequently, platelet-dependent synthesis of TXA_2 is more sensitive to aspirin inhibition.

Most other NSAIDs (see **Figure 5** for some common agents) act by different mechanisms. Ibuprofen is a classical reversible inhibitor of PGHS activity, while indomethacin exhibits a more complex, time-dependent inhibition. Thus, with these NSAIDs, enzyme activity should return to normal as the drugs dissociate from the protein and are cleared by metabolism and excretion.

Although NSAIDs act by inhibiting PGHS, their relative effectiveness as anti-inflammatory, analgesic, and antipyretic agents differs markedly. Generally, the anti-inflammatory effect of an NSAID is positively correlated with its ability to inhibit PGHS. The relationship of this property to analgesic and antipyretic activity remains to be elucidated. Ibuprofen (Motrin®, Advil®), is more effective than aspirin for the relief of the pain accompanying dysmenorrhea. Naproxen (Aleve®) has a significantly longer half-life in the human

body compared to most other NSAIDs. Ketorolac (Toradol®), has the clinically useful property of both oral and parenteral (intramuscular) routes of administration and is especially effective in pain relief and reduction in narcotic requirements.

Acetaminophen, a common active ingredient in many aspirin substitutes (*e.g.*, Tylenol®), is an effective analgesic and antipyretic agent but is only minimally anti-inflammatory. Consequently, it is usually not described as an NSAID. Recent evidence suggests that acetaminophen and related drugs interact with a third COX isoform that is expressed in the brain, where analgesia and the ability to reduce fever are regulated. The genetic and structural relationship of COX-3 to COX-1 and COX-2 remains to be established.

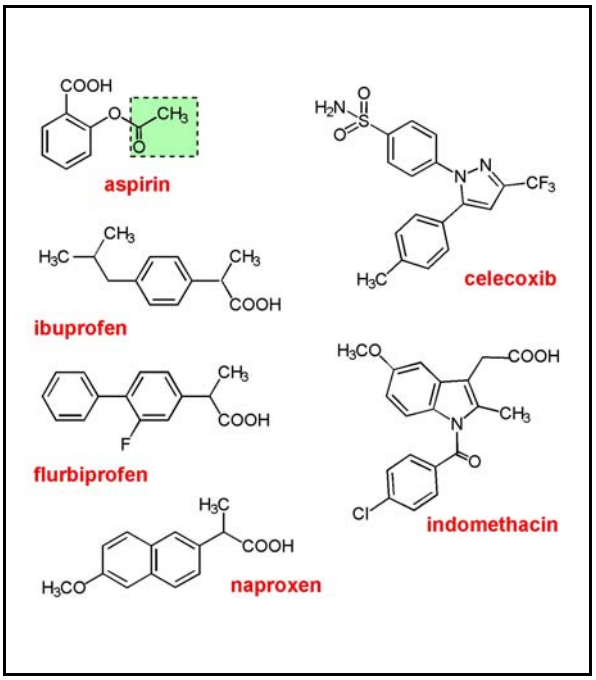

Figure 5. Structures of Some Common Non-Steroidal Anti-Inflammatory Agents (NSAIDs)

B. Adverse effects of NSAID treatment

Adverse effects of NSAIDs may result from inhibition of prostaglandin synthesis in areas where they serve important physiological and homeostatic functions (**Table 2**). The most common problems associated with NSAIDs are gastrointestinal, with bleeding, ulceration, and perforation. Prostaglandins play an important role in protecting the gastric mucosa. Thus, NSAIDs are contraindicated for patients with a history of peptic ulcer disease or other gastrointestinal disturbances. Other risk factors for the development of NSAID-associated ulcers are advanced age and concomitant use of corticosteroids and/or anticoagulants. Renal toxicity is another well-documented side effect of NSAID treatment, particularly in individuals where renal function is already compromised. Dizziness, headaches, drowsiness, and other central nervous system-associated reactions have also been reported.

Acute episodes of bronchial hypersensitivity are observed in some patients as a consequence of NSAID therapy. The development of these symptoms (lacrimation, nasal congestion, and wheezing) occurs in approximately 10% of adults with asthma and may result from a shunting of released arachidonic acid to a lipoxygenase-catalyzed path of metabolism, with a consequent elevation of leukotrienes and related compounds (**Figure 1**). The presence of these adverse reactions (often referred to as “aspirin-induced asthma”) may sometimes make it desirable to consider specificity within the class of NSAIDs. A common substitution for aspirin is acetaminophen, useful for treatment of pain and fever in patients who cannot tolerate aspirin (*e.g.*, individuals with peptic ulcers or for whom prolongation of bleeding time is particularly problematic). As noted earlier, this drug has little anti-inflammatory action.

C. Specific cycloxygenase inhibitors

With the recognition that COX isoforms participate in different physiological and pathological reactions, it became clear that the development of specific COX-2 inhibitors could potentially impact the inflammatory response

while sparing the COX-1-derived eicosanoids

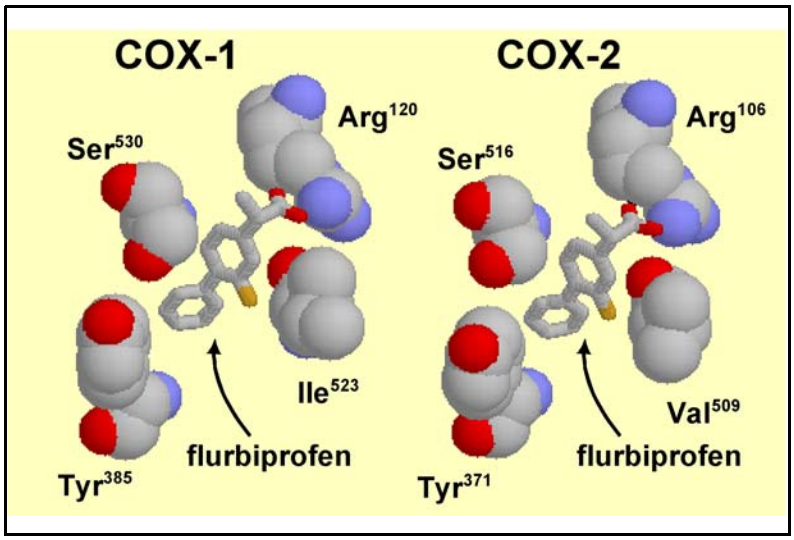

Figure 6. Comparison of NSAID Binding to Cyclooxygenase Isoforms

required for normal gastrointestinal function. Such drugs could conceivably be used to avoid the effects of NSAID toxicity. Examination of the cyclooxygenase active site reveals a long, narrow channel with a hairpin bend. Important functional groups include a Tyr residue and heme at the apex, a Ser residue on one side, and an Arg residue near the entrance (**Figure 2**). A hydrophobic amino acid, opposite Ser, directs the stability and orientation of substrate-enzyme interactions. This critical residue is different in the COX-1 and COX-2 isoforms (**Figure 6**): in humans Ile^{523} is found in COX-1, while the comparable residue Val^{509} is found in COX-2. The nearly complete occupation of the active sites by the relatively non-selective NSAID flurbiprofen (**Figure 5**) blocks access of substrates. It is apparent from the cyclooxygenase structures how acetylation by aspirin of the active-site Ser alters the topology at the entrance to the substrate channel.

An early example of isoform selectivity is meloxicam, an NSAID recently introduced in European countries. It has a preferential selectivity (up to 80-fold) toward the COX-2 isoform compared to the COX-1 enzyme and is prescribed for rheumatoid arthritis and osteoarthritis. More recently, COX-2-specific inhibitors have been designed with isoform selectivities in the range 375 to more than 800-fold. Thus far, three have been approved for clinical use: celecoxib (Celebrex™; **Figure 5**), rofecoxib (Vioxx™), and valdecoxib (Bextra™). In early trials these "coxibs" were shown to provide analgesic and antipyretic benefits comparable to ibuprofen, naproxen, or other non-selective NSAIDs and, more importantly, to reduce significantly the incidence of gastric and duodenal ulceration.

D. Steroidal anti-inflammatory drugs

These common drugs are natural and synthetic steroids with glucocorticosteroid agonist characteristics. Corticosteroids, like other members of the steroid and steroid-like hormones, bind to cytosolic or nuclear receptors and regulate the transcription of target genes. They exhibit profound anti-inflammatory actions, both physiologically and pharmacologically. In response to injury, infection, or stress, the release of these hormones (*e.g.*, cortisol) is thought to modulate leukocyte function. It is likely that one action of corticosteroids is the inhibition of phospholipase A_2. This occurs indirectly through changes in the level of regulatory proteins that are synthesized in alveolar macrophages and control the availability of phospholipid substrate to the enzyme. This inhibition will prevent the mobilization of arachidonic acid and other pro-inflammatory eicosanoid precursor fatty acids (**Figure 4**). As a result, the synthesis of all eicosanoids will be blocked. In particular, inhibition of leukotriene synthesis could account for the failure of leukocyte and macrophage chemotaxis in patients treated with steroidal anti-inflammatory drugs. Corticosteroids also down regulate the expression of the gene encoding cyclooxygenase-2 **(Table 2**).

Examples of synthetic steroidal anti-inflammatory drugs are beclomethasone, budesonide, and dexamethasone. In the treatment

of asthma, these agents are inhaled. Recent biopsy studies in asthmatic patients have provided direct evidence for the anti-inflammatory effect of corticosteroids. Following several months of inhalation therapy, individuals had many fewer mast cells, eosinophils, and lymphocytes; with milder forms of asthma, there was complete resolution. A major action of corticosteroids in the lung is a reduction in the synthesis of cytokines, such as the interleukins, that modulate the growth of specific lung-resident cell populations.

E. Other anti-inflammatory therapeutic strategies

With the awareness that products of the 5-lipoxygenase pathway of arachidonic acid metabolism contribute directly to inflammation, newer strategies are being developed to alter this branch of eicosanoid synthesis. Specific inhibition of 5-lipoxygenase is a major pharmacologic goal (**Figure 4**) and currently can be achieved with zileuton (Zyflo™) and related *N*-hydroxyurea compounds. In clinical trials zileuton significantly improved airway function and relieved symptoms associated with asthma; the amelioration of bronchospasm by zileuton was similar to that reported in previous studies of asthmatic patients treated with inhaled corticosteroids. Another approach focuses on the use of antagonists directed against receptors for the cysteinyl-leukotrienes, LTC_4, LTD_4, and LTE_4. Examples of these newly developed agents are zafirlukast (Accolate®) and montelukast (Singulair®).

An alternative to specific COX-2 inhibitors in the treatment of inflammation is the NO-NSAID class of pharmacological agents based on the observations that nitric oxide (NO), like certain prostaglandins, protects the gastric mucosa and that COX-1-deficient mice do not develop gastric ulcers, an NO precursor was linked to a conventional NSAID. Such a drug would, in principle, release NO to the gastric mucosa to compensate, in part, the NSAID-generated reduction in eicosanoid synthesis. Current experimental compounds include NO-releasing derivatives of aspirin, ibuprofen, and naproxen.

Inflammation is a complex and multi-step process that is an integral component in pathologies ranging from simple exercise-related stress to arthritis, atherosclerosis, and cancer. Eicosanoids exhibit a truly remarkable spectrum of both pro- and anti-inflammatory roles. Consequently, the demand for pharmacological interventions and other treatment strategies that are capable of specificity and selectivity is challenging.

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Table 2. Properties of Human Cyclooxygenase Isoforms

	COX-1	COX-2
gene location and size	chromosome 9q32-33.3 22 kB	chromosome 1q25.2-25.3 8.3 kB
molecular mass	72 kDa (599 amino acids); glycosylated	72 kDa (604 amino acids); glycosylated
tissue expression	**constitutive** in most tissues, in particular, stomach, kidney, monocyte/macrophage, platelet	**inducible** in endothelial cell, synoviocyte, chondrocyte, fibroblast, smooth muscle, reproductive tract **constitutive** in central nervous system, kidney, pancreas $-cells
endogenous inducers	(not applicable)	pro-inflammatory cytokines, growth factors, IgE receptor aggregation, oncogene expression
endogenous inhibitors	unknown	glucocorticosteroids; interleukin-1
intracellular location	luminal surface of endoplasmic reticulum	luminal surface of endoplasmic reticulum

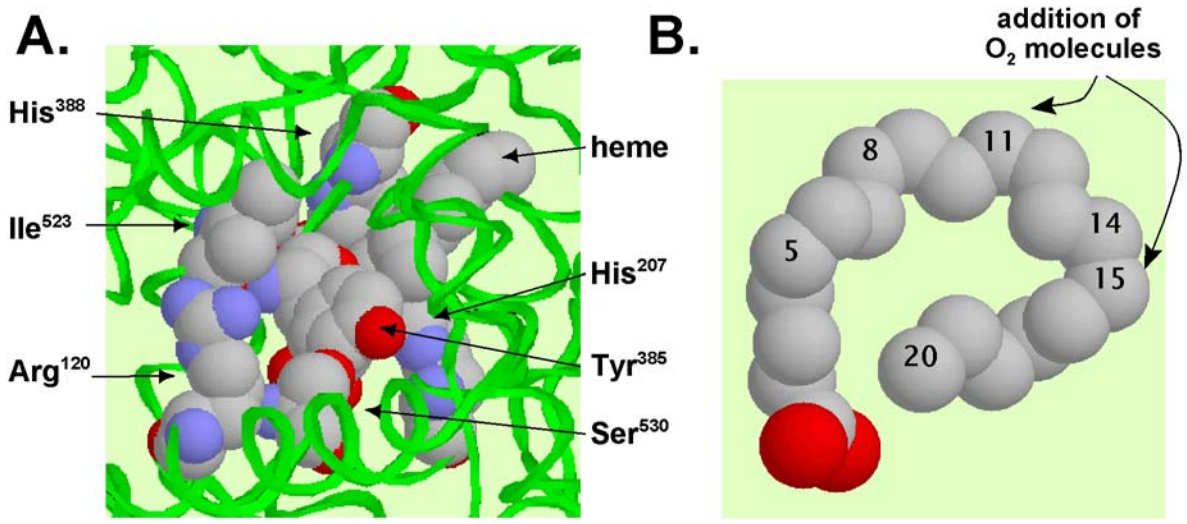

Figure 2. Active Site of Cyclooxygenase

In Panel **A** are shown the critical amino acid residues in the substrate binding channel of sheep cyclooxygenase-1. His^{207} and His^{388} serve as distal and proximal ligands, respectively, of the heme group; Arg^{120} interacts with the carboxylate function of the fatty acid substrate and Ile^{523} participates in hydrophobic bonding; Tyr^{385} plays a role in free radical chemistry; and Ser^{530} is the target of acetylation by aspirin (see **Figure 5**). In Panel **B** is depicted the likely orientation of arachidonic acid in the binding channel. The carbons at which molecular oxygen is introduced are indicated.