CHAPTER 1

Introduction to inflammation and targets involved in inflammation
1.1. INFLAMMATION

Inflammation is defensive but exaggerated local tissue reaction in response to exogenous and endogenous insult.\(^1\) It is body’s defense reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cell and tissues. The agents causing inflammation are mentioned as under\(^2\):

- Physical agents like heat, cold, radiation, mechanical trauma.
- Chemical agents like organic and inorganic poisons.
- Infective agents like bacteria, viruses and their toxins.
- Immunological agents like cell-mediated and antigen-antibody reaction.

Inflammation involves two basic processes as inflammatory response and later followed by healing. Though both processes generally play protective role against injurious agents, inflammation and healing may cause considerable harm to the body as well like anaphylaxis to bites by insects or reptiles, drugs, toxins, rheumatoid arthritis\(^3\).
Basically inflammation may be acute and chronic inflammation.

1.1.1. ACUTE INFLAMMATION

It is defined as the early inflammatory response of about shorter duration followed by repair. The main features of acute inflammation are accumulation of fluid and plasma at inflammatory sites, activation of platelets and polymorphonuclear neutrophiles as inflammatory cells.

**VASCULAR EVENTS (Figure 1.1.)**

A. Haemodynamic effect

- **Transient vasoconstriction**- It is immediate response by the arterioles by any type of injury. Its duration may be three seconds to five minutes.

- **Progressive vasodilation**- This change is obvious within half an hour of an injury. It result in increased blood volume in microvascular bed of the area, which is responsible for redness and warm that site of inflammation.

- **Local hydrostatic pressure**- Progressive vasodilation, in turn, may elevate local hydrostatic pressure resulting in transduction of fluid in to extracellular space.

- **Slowing or stasis**-slowing attributed to increase permeability of microvasculature that results in increased concentration of red cells and raised blood viscosity.

- **Leukocytic margination**-leukocytes stick to the endothelial cells then move and migrate through gaps between endothelial cells to extravascular space. Attachment of leukocytes to endothelium and move and migrates through gap between endothelial cells in to extravascular space called emigration.

Haemodynamic change characterized by the

- Rubor (redness)
- Tumor (swelling)
- Calor (heat)
- Dolar (pain)

The actual expression of these processes depends on the site of inflammation. For example, a skin abscess may result in the appearance of all of these features. In contrast, pneumonia, because of the inaccessibility of the lung to examination, may manifest only as loss of function (shortness of breath and hypoxia). Nevertheless, similar pathological processes occur in both sites.
B. Altered vascular permeability

*Contraction of endothelial cells* – This is common mechanism of increased leakiness that affects venules and not capillaries and arterioles. This phase mediated by release of histamine, Bradykinin and its end within 15-30 minutes.

*Retraction of endothelial cells* - This phase is mediated by cytokines such as IL-1 and TNFα. This phase takes 4-6 hour after the injury.

*Direct injury to endothelial cells* - It causes cell necrosis and creates physical gaps at the site of detached cells. This phase lasts for several hours to days.

*Endothelial injury mediated by leukocytes* - leukocytes adheres to the endothelial cells and gets activated that cause release of proteolytic enzymes and increase vascular leakiness.

**CELLULAR EVENTS (Figure 1.2.)**

These events of acute inflammation consist of two processes as
A. Exudation of leukocytes

Escape of leukocytes from lumen of microvasculature interstitial tissues is the most important features of inflammatory responses. This type of changes leading to migration of leukocytes as

![Figure 1.2. Cellular events during acute inflammation](image)

**Changes in the formed element of blood**- At normal case there is axial flow which show normal process, but due to slowing and stasis central stream of cells widens and plasma zone become narrower because loss of plasma zone become narrower because loss of plasma by exudation called *Margination*.

**Rolling and adhesion**- Marginated neutrophiles slowly roll over endothelial cells lining. Rolling on vessel wall followed by adhesion between leukocytes and endothelial cells

**Emigration**—After sticking of neutrophiles to endothelium cells escape out to extravascular space called emigration

B. Phagocytosis (Figure 1.3.)

It is the process of engulfment of solid particulate material by the cells that called cell eating. There are two types of phagocytic cells
Figure 1.3. Phagocytosis events during acute inflammation

Polymorphonuclear neutrophiles (PMNS) – Appears in early phase of inflammation and called as microphages. Inflammation is characterized by the immediate infiltration of a specific site or lesion with PMNs, followed by monocytes and finally lymphocytes. The process for both the microphages and macrophages are similar and involve following four steps:

- Recognition and attachment stage
- Engulfment stage
- Secretion stage
- Digestion stages

1.1.2. CHRONIC INFLAMMATION

It is defined as prolonged process in which tissue destruction and inflammation occurs at the same time. Chronic inflammation can be caused by one of the following three ways.
Chronic inflammation followed by acute inflammation like pneumonia terminating in lung abscess.

Recurrent attacks of acute inflammation.

Chronic inflammation due to infection.

Chronic inflammation associated with the systemic features like fever, anemia, lueukocytosis, amyloidosis.

Chronic inflammation can be divided into 2 types

1. **Non-specific**, When irritant substance produces non-specific chronic inflammatory reaction with formation of granuloma tissue and healing by fibrosis e.g. osteomyilits, chronic ulcer.

2. **Specific**, when injurious agent causes characteristics histologic tissues response e.g. tuberculosis, leprosy, syphilis.

### 1.1.3. MEDIATORS OF INFLAMMATION (Table 1.1.)

These are also called as permeability factor or endogeneous mediator. These causes vasodilation, chemotaxis, fever, pain and cause tissue damage. The substance act as chemical mediator released from cells, plasma or damaged tissues and classified as shown in Table 1.1.

**Histamine**- Histamine (β-Imidazolylethylamine) is a vasodilator, a constrictor of smooth muscle and a potent stimulant of vascular permeability, respiratory mucus and gastric acid secretion⁷.

**Leukotriene** - Leukotriene (LT) C4 and its products, LTD4 and LTE4, make up the biologic mixture previously known as the slow-reacting substance of anaphylaxis. Leukotrienes are generated by most cell types that participate in inflammatory reactions including mast cells, basophils, eosinophils, neutrophils and monocytes⁸.

**Cytokins** - Cytokines are polypeptide substances produced by activated lymphocytes and activated monocytes. Main cytokines acting as mediators of inflammation are: Interleukin-1 (IL-1), tumor necrosis factor (TNF) α and β, Interferon (IF)-γ, Chemokines (IL-8, PF-4).

An imbalance of IL-4 and IFN-g production is present in atopic asthma. A deficient release of the type 1 cytokine IFN-g seems to play an important role in the pathogenesis
of allergic inflammation. Regardless of whether the defective IFN-γ secretion is primary or a consequence of suppression by other cytokines, it will in atopic subjects enhance the release of type-2 cytokines, which in turn will facilitate the development of allergic inflammation.

**Prostaglandins** - Prostaglandins are the naturally occurring 20 carbon cyclopentano fatty acid derivatives produced in mammalian tissues from polyunsaturated fatty acid. The most abundant cyclooxygenase product generated by the immunologic activation of human lung mast cells is PGD_2_. Intermediates like PGG_2_ may then be converted nonenzymatically or enzymatically by specific isomerase/peroxidase or synthase enzymes to yield the primary prostaglandins PGD_2_, PGE_2_, and PGFα_7_.

In the cardiovascular system, PGD_2_ and PGE_2_ as well as PGI_2_ are potent vasodilators whereas TXA_2_ displays vasoconstrictor properties. TXA_2_ also plays a major role in the induction of platelet aggregation while PGI_2_ presents anticoagulant properties.

In the airways, PGF_2α_ and TXA_2_ are bronchoconstrictor whereas PGI_2_ and PGE_2_ act as bronchodilators.

In the GI tract, PGE_2_ and PGF_2α_ as well as PGI_2_ ensure the protection of the gastric mucosa by lowering acid secretions, enhancing mucosal blood flow and stimulating

<table>
<thead>
<tr>
<th>SOURCE</th>
<th>MEDIATOR</th>
<th>ACTION</th>
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<tbody>
<tr>
<td><strong>Mast cells, basophils</strong></td>
<td>Histamine</td>
<td>↑Permeability</td>
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<tr>
<td><strong>Platelets</strong></td>
<td>Serotonin</td>
<td>↑Permeability</td>
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<td><strong>Inflammatory cell</strong></td>
<td>Lysosomal enzymes</td>
<td>Tissue damage</td>
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<tr>
<td><strong>Prostaglandins</strong></td>
<td>Vasodilation</td>
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<td><strong>Leukotrienes</strong></td>
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<td><strong>Cytokines</strong></td>
<td>Fever</td>
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<tr>
<td><strong>Nitric oxide</strong></td>
<td>Tissue damage</td>
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<td>Fibrin split product</td>
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<td><strong>Kinin system</strong></td>
<td>Kinin/bradykinin</td>
<td>↑Permeability</td>
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<tr>
<td><strong>Complement system</strong></td>
<td>Anaphylatoxins</td>
<td>↑Permeability</td>
</tr>
</tbody>
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Table 1.1. Inflammatory mediators and their actions

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mucus formation and bicarbonate secretion. TXA₂ induces increased vascular permeability, leading to edema.

In the kidney, PGE₂ and PGI₂, unlike TXA₂, stimulate renal blood flow and diuresis. PGE₂ and PGF₂α, in contrast to PGI₂, strongly contract the uterine smooth muscle.

**Kininns**—These are potent peptide hormones formed de novo in body fluids and tissues during inflammation. They are derived from α-2 globulins (high and low molecular weight kininogens) through proteolytic cleavage by a variety of enzymes, the most important of which are plasma and tissue kallikreins.

Generation of Kinins in the inflammatory response occurs through a plasma pathways, a tissue pathways, and a plasma/tissue-independent pathways. Kinin production by the plasma pathway is initiated by the interaction of activated factor XII (Hageman factor) with prekallikrein and high molecular weight kininogen. Activated Hageman factor (factor XIIa) initiates the conversion of prekallikrein to kallikrein, which furthers the conversion of factor XII to XIIa. The action of kallikrein to further conversion of factor XII to XIIa is augmented by high molecular weight kininogen. The active kallikrein released by this sequence of events cleaves high molecular weight kininogen to release bradykinin.

**Anaphylatoxins**

The actions of anaphylatoxins in inflammation are release of histamine from mast cells and basophils, increased vascular permeability causing edema in tissue.

**1.1.4. CYCLOOXYGENASE (Figure 1.4.)**

Cyclooxygenases (COXs) catalyze the committed step in the conversion of arachidonic acid to prostaglandins and thromboxane. Arachidonic acid derived from dietary linoleic acid which esterified to phospholipid in cell membranes which hydrolyses by phospholipase A₂.

They oxygenate arachidonic acid to the hydroperoxy endoperoxide PGG₂ (prostaglandin G₂), followed by reduction of PGG₂ to the alcohol PGH₂. PGH₂ is converted by isomerases to prostaglandins and thromboxane, which exert numerous physiological and pathophysiological effects. Thus, COX enzymes play a key role in the biosynthesis of a family of important bioactive lipids.
Cyclooxygenase active sites of COX-1 and COX-2 are very similar but there are subtle structural differences that give rise to functional differences between the two proteins. Both COX-1 and COX-2 have a molecular weight of 71kDa and the amino acid sequence of COX-2 shows a 60% homology with the sequence of the non-inducible enzyme.

**Figure 1.4.** Mediators from arachidonic acid and sites of drug action.

### 1.1.4.1. Cyclooxygenase-1

In 1976, COX-1 was isolated from sheep seminal vesicles. Binding sites for arachidonic acid and NSAID, tyrosine 385 and serine 530, are positioned at the apex of the long active site whereas arginine 120 lies close to the opening of the COX channel. There may be other sub-sites for binding of the precursor in this narrow channel. COX-1 is the constitutive isoform of COX which has clear physiological functions. **(Figure 1.5.)** It performs a “housekeeping” function to synthesize prostaglandins which regulate normal cell activity. Its activation leads, for instance, to the production of prostacyclin which, when released by the endothelium, is anti-thrombogenic and when released by the
gastric mucosa, it is cytoprotective. It is also COX-1 in platelet that leads to TXA₂ production, causing aggregation of the platelets to prevent inappropriate bleeding\textsuperscript{17,18}.

![Diagram of COX-1 and COX-2](image)

**Figure 1.5.** Hatched areas in COX-1 are those that are more accessible in COX-2 due to amino acid substitutions. Tyr 385 & Arg 120 function as binding sites for arachidonic acid & NSAIDs. SP - side pocket; ES - extra space\textsuperscript{19}.

### 1.1.4.2. Cyclooxygenase-2

It closely resembles the structure of COX-1, except that the COX-2 active site is slightly larger and can accommodate bigger structures than those which are able to fit into the active site of COX-1. A secondary internal side pocket of COX-2 contributes significantly to the larger volume of the active site in this enzyme, although the central channel is also wider by approximately 17\%\textsuperscript{20,21,22}.

The COX-2 selectivity of diarylheterocycles appears because of the insertion of the sulfonamide or sulfone moiety into a side pocket of the main arachidonic acid binding channel. This side pocket is bordered by Val523 and the corresponding region of COX-1 is inaccessible because of the presence of an Ile residue instead of Val at position 523, which sterically hinders binding of inhibitors. Other changes between COX-2 and COX-1 that contribute to increasing the rigidity of this side pocket include the substitutions Arg513His and Val434Ile. The site-directed COX-2 mutant Val523 to Ile is resistant to
inhibition by diarylheterocycles and acidic sulfonamides but not to alkanoic-acid-type NSAIDs\textsuperscript{21,23}.

1.1.5. CYCLOOXYGENASE PATHWAYS\textsuperscript{24}

Once proper binding of Arachidonic acid occurs, Tyr-385 abstracts the hydrogen from carbon 13 and molecular oxygen forms the endoperoxide bridge connecting carbons 9 and 11. Ring closure then occurs between carbons 8 and 12, resulting the bicyclic cyclopentydioxygen prostaglandin structure. The addition of a second molecule of oxygen at carbon 15 forms the product PGG\textsubscript{2}. PGG\textsubscript{2} must then migrate to the peroxidase (POX) active site, located on the luminal side of the enzyme to be reduced to PGH\textsubscript{2}\textsuperscript{25}.

The endoperoxide, PGH\textsubscript{2}, is also metabolised into two unstable and highly biologically active compounds with structures that differ from those of the primary prostaglandins. One of these is thromboxane A\textsubscript{2} (TXA\textsubscript{2}), formed by an enzyme, thromboxane synthase, first isolated from platelets. TXA\textsubscript{2} has a very short chemical half-life of about 30s; it breaks down nonenzymatically into the stable and relatively inactive thromboxane B\textsubscript{2} (TXB\textsubscript{2}). The other route of metabolism of PGH\textsubscript{2} is to prostacyclin or prostaglandin I\textsubscript{2} (PGI\textsubscript{2}), yet another unstable compound with a half-life of around 3 min, formed by the enzyme, prostacyclin synthase. PGI\textsubscript{2} has a double-ring structure, closed by an oxygen bridge between carbons 6 and 9. It is hydrolysed non-enzymatically to a much less active, stable compound, 6-keto-PGF\textsubscript{1α}. The presence of the different prostaglandin synthases varies from tissue to tissue. For example, lung and spleen tissues are able to synthesize the whole range of products but other tissues cannot; hence, platelets synthesize mainly TXA\textsubscript{2}, whereas the blood vessel walls primarily produce PGI\textsubscript{2}\textsuperscript{26}. (\textbf{Figure 1.6.})
Figure 1.6. Cyclooxygenase Pathway showing conversion of arachidonic acid to various prostaglandins acts as mediator for inflammation.

1.1.6. LIPOOXYGENASE PATHWAY

Lipoxygenase are group of enzyme which oxidizes polysaturated fatty acid possessing two cis double bonds separated by methylene group to produce lipid hydroperoxide. Arachidonic acid metabolized to number of hydroperoxy eicosatetraenoic acid derivatives (HPETEs). HPETE derivative is not stable, being rapidly converted to a number of metabolite. (Figure 1.7)
Leukotrienes are the products of the Lipooxygenase pathway which are divided into two classes: hydroxylated eicosatetraenoic acid (LTs) represented by LTB₄ and peptidoleukotrienes (PLTs) such as LTC₄, LTD₄, and LTE₄. These compounds (LTC₄, D₄, and E₄) are collectively referred to as cysteinyl- or peptido-LTs. Unlike prostanoids, LTs are almost exclusively produced by inflammatory cells. However, while 5-LOX is specifically expressed in cells of myeloid lineage, LTA₄ hydrolase and LTC₄ synthase are more widely distributed throughout the body. LTA₄

Figure 1.7. Lipooxygenase Pathway, showing conversion of arachidonic acid to various leukotrienes, a product responsible for inflammatory response.

LOX; Lipooxygenase, LT; Leukotrienes, HPETE; Hydroperoxy Eicosatetraenoic Acid, a; LTA hydrolase, b; LTA synthase.
hydrolase is particularly abundant in the intestine, spleen, lung, kidney and erythrocytes; LTC₄ synthase is expressed in mast cells, basophiles, eosinophils, endothelial cells and platelets. The broad distribution of these two enzymes allows transcellular metabolisms to occur. Indeed, once available in the extracellular space, LTA₄ can be exported to another cell, which contains enzymes able to metabolize it further. For instance, LTA₄ produced by polymorphonuclear leukocytes can be converted either into LTB₄ in erythrocytes, or to LTC₄ in endothelial cells²⁸.

LTB₄ is potent chemotactic agent for leukocytes causes accumulation of leukocyte at inflammation sites and lead to symptoms characteristics of inflammatory disorder. LTC₄ and LTD₄ are potent hypotensive and bronchoconstrictor.

1.1.7. NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS)

1.1.7.1. HISTORY OF NSAIDs

Salicylic acid was introduced by Carl Buss in Switzerland to cure typhoid fever and it was found to be effective antipyretic. Phenazone was synthesized by Luswing Knorr in 1884 with mistaken assumption that he was preparing portion of quinine molecule; later it was found that it is pyrazolone. Phenazone was most widely used drug of its time and it is known to cure agranulocytosis. These were recommended for ability to ease joint pain and were used for rheumatic condition. Then attempt to reduce toxicity phenylbutazone was introduced but it was found to have side effect²⁹.

In 1971 Vane and co-workers made the landmark observation that aspirin and some NSAID blocked Prostaglandin G generation. These are considered major mode of action of NSAID and are used for the treatment of pain and inflammation, especially arthritis. From a historical viewpoint, the first NSAID with therapeutic benefits was aspirin, which has now been used for more than 100 years as NSAID. The overall worldwide production of about 50 000 tons a year reflects the importance of this substance even today³⁰. The anti-inflammatory potency of different compounds roughly corresponds with their potency to inhibit COX³¹. NSAID are the mostly used and abused drug in the world today³².
1.1.7.2. CLASSIFICATION OF NSAIDs$^{1, 23, 27, 33}$

*Non-selective COX inhibitor*

1. Salicylic acid derivatives  
   e.g. Aspirin, phenyl salicylate

   ![Aspirin](image1)

2. N-aryl anthranilic acid derivatives  
   e.g. Mefenamic acid

   ![Mefenamic acid](image2)

3. Aryl acetic acid derivative  
   e.g. Diclofenac, aceclofenac

   ![Diclofenac](image3)

4. Propanoic acid derivative  
   e.g. Ibuprofen, Ketoprofen

   ![Ibuprofen](image4)

5. Oxicam Derivatives  
   e.g. Piroxicam, Tenoxicam

   ![Meloxicam](image5)

6. Pyrrolo-pyrrole derivative  
   e.g. Keterolac

   ![Keterolac](image6)

7. Indole derivative  
   e.g. Indomethacin

   ![Indomethacin](image7)

8. Pyrazolone derivative  
   e.g. Phenylbutazone

   ![Phenylbutazone](image8)
Selective COX inhibitors.

9. Methane Sulfonamides e.g. Nimesulide,

10. Alkanones e.g. Nabumeton

e.g. Celecoxib.

\[ \text{Nimesulide} \]

\[ \text{Nabumeton} \]

\[ \text{Celecoxib} \]
1.1.7.3. MECHANISM OF ACTION OF NSAIDs\textsuperscript{34,35} 

NSAID block cyclooxygenase enzyme. Both COX-1 and COX-2 associated with membrane and each consist of long channel with bend at the end, the channel being widen in COX-2 the operating channel is largely hydrophobic. (Figure 1.8.)

\textbf{Figure 1.8.} Comparing the action of traditional NSAID and that are selective for COX-1 or COX-2, A; Arachidonic acid enters COX enzyme and converted PGG2 and then to PGE2, B; Nonselective drug block COX-1 and COX-2 Channel; C Specificity for COX-2 enzyme\textsuperscript{36}.

Arachidonic acid enters twisted round form in COX channel and has two oxygen inserted and free radical extracted, resulting in 5-carban ring characteristic of prostaglandins. Most traditional NSAID block the COX by hydrogen bonding to a polar arginine at 120. The difference between COX-1 and COX-2 responsible for selective COX-2 enzyme inhibition, which contain small side pocket in which agent get fit. these agent may be too bulky to fit in COX-1 channel\textsuperscript{36}. Figure 7 shows above consequences during the inhibition.
Many of the side effects that limit the role of nonselective NSAIDs in the peri- or immediately preoperative setting are related to their nonselective inhibition of the COX isoenzymes. As shown in figure 8, COX-1, a constitutively expressed enzyme, plays a role in platelet aggregation, hemostasis, and the protection of gastric mucosa. COX-2, an inducible enzyme, is a crucial mediator of pain, inflammation, and fever. Coxibs selectively inhibit COX-2 without compromising the constitutive role of COX-1. They have been shown to have a minimal effect on platelet aggregation and gastric mucosa, while exerting a major effect on pain and inflammation37,38 (Figure 1.9).

**Figure 1.9.** NSAID mechanism of action. COX-1, a constitutive enzyme, plays a major role in the release of PG to protect gastric mucosa and regulate hemostasis. COX-2, an inducible enzyme, releases PG that mediates pain, inflammation, and fever. Nonselective NSAIDs inhibit both forms of COX.

1.1.7.4. SELECTIVE COX-2 INHIBITION39,40

Prior to the identification of the COX-2 enzyme, researchers identified a potent anti-inflammatory compound, DuP-697, which was a relatively weak inhibitor of bovine seminal vesicle PG synthesis, but potent in a variety of anti-inflammatory assays. At first these results could not be explained, but after identification of COX-2 it became evident that this compound possessed a selective inhibitory activity against COX-2. This was the beginning of the search for new anti-inflammatory compounds focusing on COX-2 as the target enzyme. Contrary to the classic NSAIDs, this new class of enzyme inhibitors is lacking a carboxylic group, thus effecting COX-2 affinity by a different orientation.
within the enzyme without formation of a salt bridge in the hydrophobic channel of the enzyme. Pharmacophore requirement for selective COX-2 inhibition shown in figure 1.10. Selective COX-2 inhibitors belong to different structural classes,

**Diaryl-or aryl-heteroaryl-ethers (sulfonanilide inhibitors): Nimesulide, Flosulide**

There are two compounds, nimesulide and flosulide, diaryl ether and thioether structure, respectively, which bear a methansulfonanilide moiety. The sulfonamide structure with its NH-acidity in all these compounds seems to be obligatory. It appears that nimesulide was the first member of this class of drugs. Its mechanism of action, pharmacology and clinical results in rheumatic diseases, osteoarthritis and acute inflammation demonstrated that nimesulide possesses novel anti-inflammatory qualities. 

**Vicinal diaryl heterocycles: celecoxib, Rofecoxib**

The compounds are characterized by a central carbocyclic or heterocyclic ring system bearing two vicinal aryl moieties. These compounds represent the most important group of COX-2 inhibitors. It can be assumed that the heterocycle is responsible for the appropriate orientation to the aromatic rings in space and finally for the binding to the enzyme. A wide variety of heterocycles can serve as a template for COX-2 inhibitors, i.e. pyrrole, thiazole, oxazole, furane, imidazole, isoxazole, pyrimidine and thiophene, but at the moment pyrazole and cyclopentenone seem to be the most appropriate tools for COX-
2 specificity. For optimal activity, one aromatic ring must be substituted with a methylsulfonyl or a sulfonamide substituent in para position. Substitution at position 4 of one of the aromatic systems with a sulfonamide or a methylsulfonyl group is essential for COX inhibition. Replacement of the methylsulfonyl group by a sulfonamide group reduces COX-2 selectivity but improves oral bioavailability\textsuperscript{42,43}.

Modified, known NSAIDs to improve COX-2 selectivity:
Modifying well known NSAIDs into selective COX-2 inhibitors represents an interesting strategy. Indomethacin, zomepirac, aspirin and flurbiprofen have been successfully elaborated into selective COX-2 inhibitors. However, the methodology utilized in NSAID modification does not follow a general scheme. Classic NSAIDs such as indomethacin possess both COX-1 and COX-2 inhibiting activity. Various attempts have been made to shift the enzyme selectivity of indomethacin from COX-1 to COX-2 while keeping the potency on the same level and reducing the unwanted side-effects at the same time. In principle, the strategy consisted of introducing larger substituents to fit into the active site volume of COX-2. Transformation of the aryl acetic acid moiety of indomethacin to esters or amides furnishes molecules capable of binding tightly to COX-2 but not COX-1.

Modern medicinal chemistry offers a wide range of different strategies for finding selective COX-2 inhibitors with a novel structure. Computational and combinatorial chemistry methodology helped to create a highly selective phenothiazine derivative which can serve as a novel lead compound for further developments in the field of COX-2 inhibitors\textsuperscript{44,45}.

Antioxidative compounds:
These compounds, which are under investigation, develop their mode of action by an antioxidative mechanism. Since COX enzyme catalysis involves radical intermediates, a radical scavenging moiety such as a di-tert-butylphenol interferes with the cyclooxygenase reaction. Linkage of phenolic substructure with a thiazolone, oxazolone, thiadiazole or oxadiazole derivative produces non-ulcerogenic, orally active anti-inflammatory agents as a novel class of COX-2 inhibitors\textsuperscript{46}.

1, 2-Diarylethylene derivatives (cis-stilbenes):
Reduction of the furanone ring led to active inhibitors with a ring open diol structure. Ring opening and elimination of the heteroatom led to cis-stilbene derivatives which still
contain the pre-requisites for COX-2 inhibition: vicinal orientation of two aromatic rings, substitution pattern at the aryl moiety as seen in potent COX-2 inhibitors, i.e. methylsulfonyl moiety in combination with a halogen. This group of compounds is presently undergoing biological testing\textsuperscript{47}.

1.1.7.5. ADVERSE EFFECTS OF NSAID

As COX-1 responsible for normal physiological function in platelets, GI tract and kidney but COX-2 induced in inflammatory sites and have cause no protective Prostaglandins. Platelets contain only the COX-1 form of COX and are therefore inhibited by all currently available nonselective NSAIDs with the exception of nonacetylated salicylates and moderate doses of nabumetone. The platelet inhibiting effects of aspirin, an irreversible inhibitor of COX, have been used to clinical advantage in the prophylaxis of coronary artery disease and cerebrovascular thrombotic disease. Clinical problems that may arise from the use of platelet altering NSAIDs include gastrointestinal bleeding from a preexisting lesion (erosion, ulcer, polyp, etc.)\textsuperscript{48}. The NSAID causes gastrointestinal bleeding with anemia, gastritis, epigastric pain and hemorrhage and ulcerative oesophagitis. NSAID cause water retention resulting in cerebral oedema\textsuperscript{49}.

Besides gastrointestinal effect of NSAID, the other adverse effect is observed in renal system like renal arteriolar constriction, renal tubular necrosis, congestive cardiac failure, cirrhosis of liver, renal failure hyperkalemia, renal papillary necrosis. The nephritic syndrome and renal lesions associated with use of NSAID\textsuperscript{31, 50}.

NSAID related gastrointestinal death put in to perspective, the rate is higher than that found from cervical cancer, asthmas. In 1991 more than 99 million prescription NSAID and billion OTC NSAID were sold annually in US. USFDA reported in 1988 that every year approximately 20,000 bleeding or perforated NSAID induced ulcer result in at least 10,000 deaths in US alone\textsuperscript{51}.

1.1.7.6. CHOICE OF NSAID

At present NSAID are bewildering array of strongly promoted drug. No single drug is superior to all other for every patient. Choice of drug is especially empirical. The societal burden of arthritis and its treatment with nonselective nonsteroidal antiinflammatory drugs (NSAIDs) is substantial because of the numbers of people
affected. Both the prevalence and incidence of osteoarthritis (OA) and rheumatoid arthritis (RA) increase dramatically as people age, with more than 33 million people affected, of whom 28 million are older than 45 years of age\textsuperscript{52}.

The cause and nature of pain like mild, moderate acute, chronic along with consideration of risk factor in the given patient governs selection of drug . also consider past experience of patient, acceptability and preference. The choice of drug for children is considerably restricted and only drugs that have been extensively tested in children should be used.

The use of any NSAID in pregnant women generally not recommended, to avoid complication such as prolongation of labor, increased rise of post partum haemorrage and intrauterine closure of ductus arterioles.

For seriously delibilitated patients who do cannot tolerate these drugs or in whom they are not adequately effective, other forms of therapy should considered.

**MARKETED NSAIDs**

<table>
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<tr>
<th>Sr.No.</th>
<th>Drug</th>
<th>Major therapeutic use</th>
<th>Major side effect</th>
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<td>Ketalac.</td>
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<td>3</td>
<td>Mefenamic acid</td>
<td>RA, Dysmenorrhoea, Dental pain</td>
<td>Visual disturbances, CHF, Agranulocytosis</td>
<td>100,250, 500</td>
<td>Ponstan, Mefdol</td>
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<td>forty, Lenagesic</td>
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<td>4</td>
<td>Paracetamol</td>
<td>Pain and fever</td>
<td>Liver damage, Blood dyscrasias</td>
<td>100,250, 500, 650</td>
<td>Crocin, Dolopar,</td>
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<td>5</td>
<td>Aceclofenac</td>
<td>Ankylosing spondylitis, osteoarthritis.</td>
<td>Nephrotoxicity , GI bleeding</td>
<td>100,200</td>
<td>Aclofen, Aceclo,</td>
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<td>Celecoxib</td>
<td>Osteoarthritis, RA, edematous polyposis</td>
<td>Stevan jonsons syndrome, Epidermal necrosis</td>
<td>100,200, 250</td>
<td>Celicb, Coxib, Zecoxib</td>
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<td>Diclofenac</td>
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<td>Kidney failure</td>
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<td>Dicloact, Adiflam,</td>
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<td>Dosages</td>
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<td>Ibuprofen</td>
<td>Juvenile arthritis, closure of patent ductus arteriosus</td>
<td>Ulceration, Hypoglycemia.</td>
<td>200, 400, 300</td>
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<td>Indomethacin</td>
<td>Musculoskeletal and joint disorder, Gout</td>
<td>Psychiatric disturbances, renal failure.</td>
<td>25, 50, 75</td>
<td>Artesid, Indocid</td>
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<td>Acute intestinal nephritis</td>
<td>30, 50, 100</td>
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<td>Gastritis, Nephritis</td>
<td>500, 750</td>
<td>Nabuflam, Niltis</td>
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<td>12</td>
<td>Napropane</td>
<td>Gout, Migrane,</td>
<td>Visual and GI disturbances</td>
<td>25, 250, 500</td>
<td>Artagen, Movibon</td>
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<td>13</td>
<td>Nimesulide</td>
<td>Acute pain, arthritis</td>
<td>Rhinitis, thrombocytopenia</td>
<td>50, 100</td>
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<td>14</td>
<td>Piroxicam</td>
<td>Juvenile idiopathic arthritis</td>
<td>Pruritus, photosensitivity</td>
<td>10, 20, 25</td>
<td>Brexic, Dolokam</td>
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<td>15</td>
<td>Valdecoxib</td>
<td>Osteoarthritis</td>
<td>Hypertention</td>
<td>10, 20, 40</td>
<td>Vabra, Valbc, Valdez</td>
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1.2. p38 MAP Kinase inhibitors

TNFα is one of several cytokines that plays a significant role in the development of an acute or chronic inflammatory response. The success of the soluble TNFα receptor fusion protein Enbrel (etanercept)\textsuperscript{53} and the monoclonal TNFα antibody Remicade (infliximab)\textsuperscript{54} in the treatment of rheumatoid arthritis and Crohn’s disease has provided a proof of concept for the treatment of these autoimmune diseases. These biologics are generally well tolerated to date but have drawbacks related to patient cost, efficiency of production, and administration by injection. Therefore, an orally active small-molecule drug that blocks or modulates circulating TNFα remains an attractive therapy. Of the different approaches toward this end, inhibition of p38 MAP kinase results in the suppression of not only TNFα but also IL-1β, another significant proinflammatory cytokine.\textsuperscript{55} The biosynthesis of TNFα and IL-1β occurs predominately in activated monocytes and macrophages via an intracellular signaling cascade that involves the dual phosphorylation (via MKK3 and MKK6) of Thr180 and Tyr182 within a TGY motif of p38 kinase. Phosphorylated p38 subsequently phosphorylates a variety of substrates, including kinases and transcription factors.\textsuperscript{56} Two of these substrates that are activated by p38 are MAPKAP kinases 2 and 3, which in turn phosphorylate heat shock protein HSP 27, ultimately leading to gene transcription and protein translation. This cascade of events can be initiated by a wide variety of extracellular stimuli or stresses such as endotoxin bacterial lipopolysaccharide and cytokines, or osmotic shock, heat shock, UV light, ionizing radiation, and oxidative stress.

Four isozymes of p38 have been cloned and characterized to include the ubiquitously expressed p38α and p38 β; p38γ is primarily expressed in skeletal muscle, and p38δ is highly distributed within lung, kidney, endocrine glandular, and small intestinal tissues.\textsuperscript{57} Historically, SmithKline Beecham demonstrated early on that an orally active small molecule, SB203580 (1), could reduce TNF α levels in vivo, (Figure 1.11,) validating the pyridinylimidazole structural class.\textsuperscript{58} Significant improvements to the kinase selectivity and whole blood potency of 1 were subsequently accomplished at Merck with the discovery of the (S)-sec-phenethylamine moiety represented in the second-generation
pyridinylimidazole 2.\textsuperscript{59} Within a few years, Vertex demonstrated that even greater kinase selectivity could be obtained with VX-745 (3).\textsuperscript{60} Human clinical trials with 3 have been reported,\textsuperscript{61} reviewed,\textsuperscript{62} and claimed to have achieved proof of concept, although this press release lacks scientifically reviewed data.\textsuperscript{63} In an effort to increase the whole blood potency of 3, chemists at Merck designed inhibitors such as 4 that retained the structural attributes of 3 in addition to the basic saturated heterocycle present in 2.\textsuperscript{64}

Figure 1.11. Selected p38 inhibitors
1.3. References


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