INTRODUCTION

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Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used drugs all over the world. Reverend Edmund stone in the mid eighteenth century successfully used bark of willow to relive fever, its active ingredient was salicilin, NSAIDs act mainly thruogh inhibition of cycloxygenase enzynes, the finding in 1991 of a second form of cyclooxygenase (COX) has opened a new era of non-steroidal anti-inflammatory drugs (NSAID) research. *(Xie et al., 1991)* Comparing COX-1 and COX-2 expression has led to the generalization that COX-1 which is found in most tissuesof mammals is involved in physiological regulation of homeostasis, COX-2, on the other hand, is involved in the inflammatory response, that explains the low level of COX-2 in case of absence of external stimuli and the increase in its level in the presence of inflammation. *(Sirois et al., 1992).*

Inflammation:

Inflammation is the response of the living tissue to noxious and injurious external and internal stimuli, such as toxic chemical agents, physical factors, microorganisms and their metabolic by-products, immune responses (hypersensitivity, immune complex, autoimmune reactions). It involves a complex of enzyme activation, mediator release, extravasation of fluid, cell migration, tissue breakdown and repair. *(Sporn and Roberts, 1997)*

Multiple cells are involved in inflammation process such as, *Neutrophils,* that are the predominant cells in acute inflammation, their

intracellular granules (lysosomes) contain everal active enzymes, together with extra cellular release of the highly irritating lysosomal enzymes into tissues, contributes to the local inflammation. ***Basophils*** contain granules that contain histamine, heparin, and slow-reacting substance of anaphylaxis (SRS-A). ***Eosinophils*** the eosinophilic granulocytes contain hydrolytic enzymes (eg., histaminase, which inactivates histamine, arylsulfatase B, which inactivates SRS-A). ***Mast cells*** contain numerous granules that release histamine, heparin, and SRS-A. *Macrophages* the mononuclear phagocyte systemis an extensive network of macrophages that exists throughout the body. ***Lymphocytes*** and their derivatives are found in the tissues in all types of inflammation. *(Nathan, 1987)*

A variety of chemical substances are synthesized in response to inflammation and emitted. They are known to have vascular effects vasoactive **amines** (e.g. histamine, and serotonin) they are responsible for haemodynamic and vascular changes. *Histamine*, most of the body's histamine is stored in the granules of mast cells, it is also found in basophils and platelets. Histamine is released by degranulation in response to various stimuli, once released, histamine causes direct vascular effects and some degree of vasoconstriction followed by vasodilatation, histamine also causes an increase in the vascular permeability of small veins and venules. *Serotonin*, most of the body's serotonin is stored in the gastrointestinal tract and the central nervous system (CNS) serotonin is known to have opsonizing actions, immune functions, and secretory function. *(Clark and Henson, 1988)*

Plasma factors have an important role in inflammation that can not be ignored, ***Kinin system*** when activated, leads to the formation of bradykinin,

its actions are much like those of histamine, it causes increased vascular permeability, vasoconstriction, smooth muscle contraction, and pain .*The Complement system* is an important mediator of the inflammatory process, its components play an important role in the increased vascular permeability. *Arachidonic acid* metabolites (derivatives), these comprise prostaglandin's (PGs) and leukotrienes (LT); ***Prostaglandin's*** and leukotrienes have functional roles in coagulation and haemostaisis, and in cardiovascular, respiratory, renal, endocrine and osteo-articular systems. They can be synthesized and released by many cell types and are not stored. Thus, in effect they resemble hormones, exerting local paracrine and autocrine effects following by which they are inactivated ***(Morrison, 1986)***

Arachidonic acid cascade: ***(Figure 1)***

Arachidonic acid is a 20-carbon polyunsaturated fatty acid that is derived from the diet or from linoleic acid. It is present in the cell membrane phospholipids and following an appropriate stimulus it is released by one of two phospholipases, of which phospholipase A_2 is the major one in inflammatory cells. ***(Sraer et al., 1983)***

Intracellular cyclo-oxygenase and lipo-oxygenase produce the biologically active prostaglandins and leukotrienes respectively. The exact prostaglandin product depends upon the cell or tissue involved. Thromboxan-A_2 and prostaglandin I_2 (prostacyclin) are the major prostaglandins of platelets and endothelial cells respectively and have an important role in haemostaisis. PGD_2, PGE_2, $PGF2\alpha$ are synthesized by macrophages and neutrophils and cause vasodilatation. PGE_2 produces pain if injected intradermally and is involved in producing fever. ***(Needleman, 1994).***

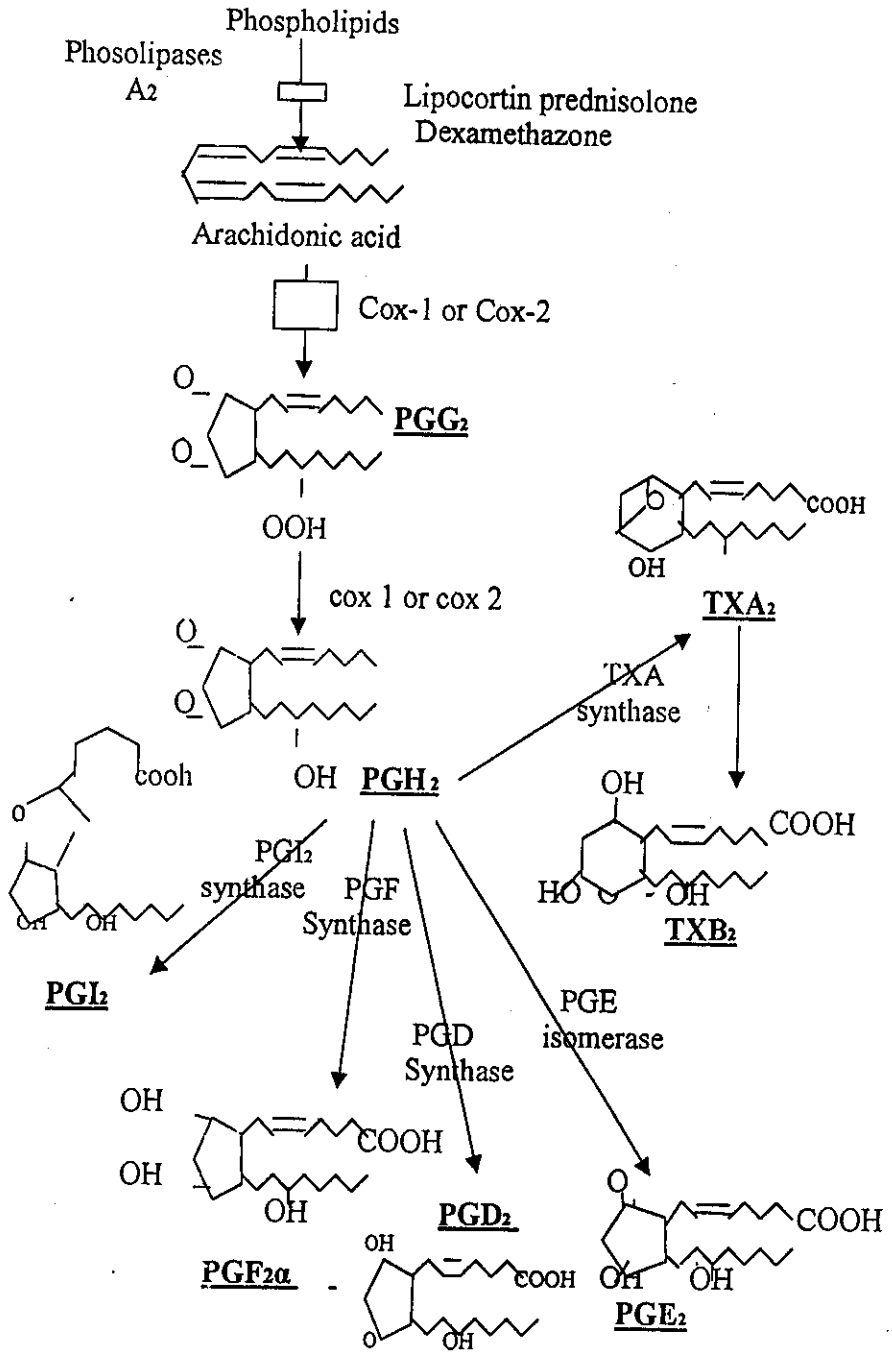

FIG. (1) **ARACHIDONIC ACID CASCADE**

Some physiological effects of prostaglandins:

Inflammation

Prostaglandins appear to be one of the natural mediators of inflammation. Administration of prostaglandins PGE_2 and PGE_2 induce the signs of inflammation that include redness, hotness (due to arteriolar vasodilatation), and oedema resulting from increased capillary permeability. ***(Premack and Schall, 1996)***

Cardiovascular system:

In most vascular beds, the PGs are potent vasodilators. The dilatation appears to involve arterioles, precapillaries and post-capillary venules. Large veins are not affected by PGs. However, PGs are not universally vasodilatory; constrictor effects have been noted in selected sites. In vascular beds including the mesenteric, coronary and renal, vasodilatation occurs at lower concentrations than does vasoconstriction Systemic blood pressure generally fall in response to PGs, and blood flow to most organs, including mesentery and kidney is increased. Blood pressure is increased by $PGF_{2\alpha}$ in some experimental animals due to vasoconstriction. ***(copland et al., 1994)***.

Cardiac output is generally increased by PGE and PGF, weak direct inotropic effects have been noted In various isolated preparations. In the intact animals, however increased force of contraction as well as increased force of contraction as well as increased heart rate is in large measures a reflex consequence of fall in total peripheral resistance. ***(Lee, 1973)***

Blood.

Ecosanoids modify the function of the formed elements of the blood, in some instances these actions reflects their physiological role. The PGs and related products exert a powerful action on platelets. PGI_2 inhibits the aggregation of platelets and contributes to the non thrombogenic properties of the vascular wall as they are synthesized by the vascular endothelium *(Moncada and Vane, 1979)*

Gastric and intestinal secretions:

PGE and PGI2 inhibit gastric acid secretion probably by an action exerted directly on the secretory cells. In addition, these prostaglandins are vasodilators in the gastric mucosa and PGI_2 may be involved in the local regulation of blood flow. Mucous secretion in the stomach and small intestine is increased by prostaglandins. These effects help to maintain the integrity of the gastric mucosa. *(Patoia et al., 1995)*

Central nervous system:

Although a large number of observations have been made on the effects of prostaglandins on the central nervous system (CNS), evidence for a particular physiological role has yet to emerge. Both stimulant and depressor effect of prostaglandins on the CNS have been reported following injection into the cerebral ventricles. The release of PGE_2 in the hypothalamus has been proposed to explain the genesis of pyrogen induced fever *(Barbour et al., 1989)*

Afferent nerve and pain:

PGE causes pain when injected intradermally these are generally not as immediate or intense as those caused by bradykinin or histamine. Prostaglandin E and prostacycline ~~synthetize~~ sensitize the afferent nerve endings to the effect of chemical or mechanical stimuli by lowering the threshold of the nociceptors. The release of these prostaglandins during the inflammatory process thus serves as an amplification system for the pain mechanism *(Moncada and Vane, 1979)*

Kidney and urine formation:

Prostaglandins influence renal salt and water excretion by alteration renal blood flow and by direct effects on renal tubules. PGE_2 and PGI_2 infused directly into the renal arteries of dogs increase renal blood flow and provoke diuresis, natriuresis, and kaliuresis; there is little change in the rate of glomerular filtration. TXA_2 decreases renal blood flow and glomerular filtration rate. *(Walker and Frolich, 1987)*

Vascular and pulmonary smooth muscle:

Local generation of PGE_2 and PGI_2 modulate vascular tone. They appear to counteract the effects of circulating vasoconstrictor autacoids and to maintain blood flow to vital organs *(Aiken and Vane, 1973)*.

Platelets:

An area in which there has been considerable interest is the elucidation of the role played by PG endoperoxides and TXA_2 in platelet aggregation and by PGI_2 in the prevention of such aggregation. It is

generally accepted that stimulation of platelets to aggregate leads to activation of membrane phospholipases with the consequent release of arachidonate and its transformation into prostaglandin endoperoxides and TXA_2, these substances induce platelet aggregation *(De Gaetano et al., 1985)*.

PGI_2 that is generated in the vessel wall inhibits platelet aggregation and contributs to the non thrombogenic properties of the endothelium according to this concept, PGI_2 and TXA_2 represent biologically opposite pools of a mechanism for regulating platelet vessel wall interaction *(Moncada and Vane, 1979)*

Inflammatory and immune responses:

Prostaglandin are released by a host of mechanical, thermal, bacterial and other insults and they contributes importantly to the genesis of the signs and symptoms of inflammation, although PGs do not appear to have direct effect on vascular permeability, both PGE_2 and PGI_2 markedly enhance edema formation and leukocyte infiltration by promoting blood flow in the inflamed area. More over, they potentiate the pain producing activity of bradykinin and other autacoids, however PGE_2 inhibit the participation of lymphocytes in delayed hypersensitivity reactions *(Vane et al., 1994)*.

<u>Lipo-oxygenase pathway</u>:

Is responsible for the formation of leukotrienes which are a family of conjugated trienes formed form eicosanoic acid in the leukocytes and

macrophages this pathway in response to both immunologic and non immunologic stimuli, The products of the lipoxygenase reaction which arises by addition of hydroperoxy group to arachidonic acid, are designated as hydro-peroxy- eicosatetraenoic acids (HPETEs). ***(Bennett et al., 1988)***

Hydroperoxy substitution of arachidonic acid by lipoxygenases occur at positions 5, 12, or 15. 5-HPETE is the major lipoxygenase product in basophils, polymorphnuclear leukocytes, macrophages, and mast cells .12-HPETE predominates in platelets, pancreatic endocrine islet cells, vascular smooth muscle, and glomerular cells.15-HPETE is the principal lipoxygenase product in reticulocytes, eosinophils and T-lymphocytes. ***(Marcus et al., 1982)***

The HPETE-hydroperoxides themselves are not hormones, but instead are highly reactive, unstable intermediates that are converted either to the analogous alcohol or to leukotrienes. Leukotrienes are derived from the unstable precursor 5-HPETE by a reaction catalyzed by LTA_4 synthase that generates LTA_4, which is then converted to LTB_4, LTC_4 and LTD_4. ***(Serhan, 1994)***

The biological actions of the LTC_4, LTD_4, and LTE_4 comprise what has been refferd to for decades as the slow-reacting substance of anaphylaxis (SRS-A). The LTC_4 is rapidly converted to LTD_4 and then slowly converted to LTE_4.

In general the HPETEs and LTB_4 are involved mainly in regulating neutrophil and eosinophil function, they mediate chemotaxis and induce

polymorphneuclear leukocyte degranulation. In contrast, the leukotrienes LTC4 and LTD4 are humoral agents that promote smooth muscle contraction, constriction of pulmonary airways, trachea and intestine and capillary permeability. The mono-hydroxy-eicostetraenoic acids that compromise the lipoxygenase pathway are potent mediators of processes involved in allergy and inflammation as they stimulate migration of eosinophils and neutrophils, making them the principal mediators of PMN-leukocyte infiltration in inflammatory reactions. *(Sraer et al., 1983)*

COX ACTIVITY AND EXPRESSION:

COX activity was determined by measuring the capacity of air pouch tissues to produce prostanoids from exogenous arachidonic acid that progressively rose during the first 24h of acute inflammation, accompanied by an increase in COX-2. COX activity does not discriminate between the contributions from either of the COX isoforms to prostanoid formation; however a western blot, using antibodies specific to COX-1 and COX-2, could give us an idea on the levels of each isoform of the protein over time. **(Davies et al., 1984)**

In the chronic phase of the inflammatory response COX activity was 2-3 times greater than in the acute phase, with prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) as the predominant metabolites. The increase in COX activity was mirrored by an increase in COX-2 protein levels. Levels of COX-1 protein appeared unchanged throughout the time course. *(Vane et al., 1994)*.

COX-1, first isolated from ram seminal vesicles, is constitutily expressed and responsible for the production of homeostatic prostanoids in tissues such as stomach mucosa and kidney. ***(Glerse et al., 1995).***

COX-2 is the inducible isoform of the enzyme, the expression of which is glucocorticoid sensitive and can be stimulated by mitogens, cytokines, inflammation, and tissue injury. The unwanted side effects of NSAIDs, including gastrointestinal distress, are thought to be due the inhibition of *COX-1*, while their primary anti-inflammatory effects are due to inhibition of COX-2. ***(Herschman, 1994)***COX is an integral membrane protein located primarily in the endoplasmic reticulum nuclear membrane network of the cell. The X-ray crystal structure of COX-1 has been determined that the enzyme is a monotopic dimer, that is, the binding surface of the protein extents through only one leaflet of the membrane with the bulk of the protein facing the lumen ***(Smith and Marnet, 1994).***The active site of the enzyme at which NSAIDs are thought to bind exists as a long narrow hydrophobic channel extending from the outer surface to the center of the protein. Interestingly the active site structure of COX is believed to be located in the hydrocarbon domain of the lipid bilayer, endogenous arachidonic acid may gain access to the active site of the COX enzyme via the lipid bilayer structure of the membrane, as with other ligand/membrane receptor systems. ***(Picot et al., 1994)***

COX gene expression:

In the COX gene and protein structure have been reviewed ***(Goppelt and Strube, 1995)*** COX-1 and COX-2 have been found on distinct

chromosomes: chromosome 2 and 9 for murine and human COX-1 respectively, and chromosome 1 for both murine and human COX-2. The COX-2 gene is smaller than the COX-1 gene, however the size of the mRNA for COX-2 is larger than that for COX-1. The COX enzyme exists in two isoforms encoded by separate genes, and has 60% homology in their amino acid sequences. *(Otto et al., 1993)*

Recently it has been found that some NSAIDs appear to show significant selectivity towards the inhibition of COX-2 rather than COX-1. In vivo these compounds demonstrate good anti-inflammatory effects with little unwanted ulcerogenicity. *(Copland et al., 1994)* suggested that this selective inhibition is due to a slow structural transition induced in the COX-2 enzyme upon binding of the drug. Although these compounds initially inhibit both iso enzymes with equal potency, the COX-1 enzyme fails to undergo such a time dependent transition and is only moderately inhibited.

Herschman et al, (1994) inhibited COX-2 with antisense oligonucleotide in macrophages, COX-1 was unable to convert the released arachidonic acid to prostaglandins. This suggested that there may be two distinct endogenous pools of arachidonic acid, one constantly available to COX-1 and its house keeping production of prostaglandins, and a second pool which may be released on induction and available only to COX2.This may be related to the recent finding of two different intracellular locations for COX-1 and COX-2, COX-2 is much more concentrated in the nuclear envelope rather than in the endoplasmic reticulum where COX-1 predominantly resides.

Cyclooxygenase-1: (COX-1)

It is a membrane bound haemo and glycoprotein with a molecular weight of 71kDa is found in greatest amounts in the endoplasmic reticulum of prostanoid forming cells. It both cyclizes arachidonic acid and adds the 15-hydroperoxy group to form prostaglandin G_2 *(Heuler et al., 1976).*

Picot and his colleagues (1994) gave a strong boost to the field of COX research by determining the three dimensional structure of COX-1.The enzyme is a monotopic arrangement that is, it integrates into only a single leaflet of the lipid bilayer .the bifunctional enzyme comprises three independent folding units: an epidermal growth factor-like domain, a membrane – binding motif and an enzymatic domain. The sites for peroxidase and cyclooxygenase activity are adjacent but distinct. Three of the helices of the structure from an entrance channel to the active site and their insertion into the membrane could allow arachidonic acid to gain access from the interior of the bilayer.

The COX activity site is a long, hydrophobic channel and ***Picot et al.(1994)*** presented arguments that some NSAIDs such as flubiprofen inhibit COX-1 by excluding arachidonate from the upper portion of the channel. Tyrosine and serine are at the apex of the long active site. Aspirin irreversibly inhibits COX-1 by acetylation of serine, thereby excluding excess for arachidonic acid to Tyrosine By steric hindrance. The acetyle serine side chain can rotate in the slightly larger channel of COX-2; thus allowing limited access of substrate to the active site.

COX-1 is constitutivly expressed in most tissues, and performs a "housekeeping" function to synthesis prostaglandins, which regulate normal cell activity. The concentration of the enzyme remains largely stable, but small 2 to 4 fold increases can occur in response to stimulation with hormones or growth factors *(DeWitt, 1991).*

Cyclooxygenase-2: **(COX-2)**

COX-2 seems to be catalytically indistinguishable from COX-1 with respect to its general structure, biochemistry and mechanism of prostaglandin biosynthesis. COX-2 is induced in certain cells and tissues after exposure to a variety of cytokines. The DNA sequences of this 'inducible' COX gene product suggest that subtle, but significant amino acid differences occur between COX-1 and COX-2 (around 60% identities); COX-2 sequences for several species have greater degrees of amino acid identities *(Simmons et al., 1991).*

This isoform effect is rather pronounced with aspirin: aspirin acetylation completely blocks the activity of COX-1, but aspirin acetylated COX-2 is still capable of oxygenating arachidonic acid, moreover the serine acetylation in COX-2 must alter the substrate position for catalysis instead of blocking binding as in COX-1, suggesting that the active site topographies of COX-1 and COX-2 are subtly, but distinctly different. *(Rosen et al., 1989)*

Non-Steroidal Anti-inflammatory Drugs (NSAIDs)

NSAIDs are an important group that are used to relief pain and inflammation acting by inhibiting the synthesis of endogenous compounds that are responsible for the inflammatory process, that are often associated with eryithema and oedema formation at the site of inflammation. NSAIDs display an unusually large degree of structural diversity and include classes of compounds such as:

Salicylates.

Despite the introduction of many new drugs, aspirin (acetyl salicylic acid) is still the most widely prescribed analgesic- antipyretic and anti-inflammatory agent, and it is the standard for the comparison and evaluation for the others. ***(Brenner and Simons, 1982).***

Members of this class of NSAIDs include aspirin, sodium salicylate, salicylic acid, methylesalicylate, and diflunisal. ***Pharmacological properties:*** *analgesia*, the types of pain usually relieved by salicylates are those of low intensity especially headache, myalgia, and arthralgia long term use does not lead to tolerance or addiction. *Antipyresis*, salicylates usually lower elevated body temperatures rapidly and effectively, however, in toxic doses these compounds have a pyretic effect. ***(Meade et al., 1993)***

Pharmacokinetics and metabolism: ***absorption,*** salicylate absorption occurs by passive diffusion across the gastrointestinal membrane,

its peak value is reached in about two hours after oral intake and then gradually declines. After absorption salicylate is distributed throughout most body tissues and most transcellular fluids, primarily by pH dependent passive process, ***Biotransformation and execration***: Biotransformation of salicylates occurs in the hepatic endoplasmic reticulum and mitochondria. ***(Jaffe and Weksler, 1997)*** The three chief metabolic products are salicyluric acid, phenolic-glucuronide, acyl- glucuronide. Salicylates are execrated mainly in urine and gastric acid the plasma half-life is 8 to 12 hours with greater than 99% bound to plasma protein.

Pyrazolone derivatives,

This group of drugs include Apasone, Phenylbutazone, sulfinpyrazone, oxyphenbutazone, Antipyrine **Phenylbutazone:** ***Pharmacokinetic and metabolism***: It is rapidly and completely absorbed after oral administration, it is extensively protein bound and has a very long half life of 50 to 100 hours. ***(Shen, 1979)*** Phenylbutazone is slowly metabolized into hydroxyphenylbutazone and oxyphenbutazone, both of which have activities similar to Phenylbutazone these metabolites contribute to the prolonged pharmacologoic effect of its administration.

Pharmacological properties: The anti-inflammatory effects of phenylebutazone are similar to those of salicylates, but its toxicity differs significantly, for it can cause agranulocytosis. ***(Meade et al., 1993)*** In accordance to its analgesic and anti-pyretic effects it's efficacy is inferior to that of salicylates, it also has uricosuric effect in doses of 600 mg / day, this is probably attributable to one of its metabolites, however low

concentrations of the drug inhibit tubular re-absorption of uric acid and cause retention of urate, another effect that cannot be neglected is it's effect on water and electrolytes, for it causes significant retention of Na and Cl accompanied with a reduction in urine volume. ***(Hart and Huskissor, 1988)***

Apazone

Is a pyrazolon, aspirin like agent with a spectrum of activity very similar to phenylebutazone, although it is much less toxic. Thus, it is anti-inflammatory, analgesic, and antipyretic. In addition, ***Apazone*** is a potent uricosuric agent and is particularly useful in the treatment of acute gout. *(Fries et al., 1993).*

It is rapidly and almost completely absorbed from the gastrointestinal tract after oral administration to man: peak concentrations in plasma are achieved 4 hours later. The compound is extensively bound to plasma proteins (>95%), and the biological half-life is about 20 to 24 hours. Most of the drug (65%) is execrated in the urine unchanged; approximately 20% are present, as the 6-hydroxy derivative. There may be significant enterohepatic cycling ***(shen, 1979)***

Para-aminophenol derivatives:

Phenacetin and its active metabolite acetaminophen, are effective alternatives for aspirin as anti-pyretic and analgesic, however, unlike aspirin their anti-inflammatory activity is weak. The antipyretic effect of these compounds resides in the aminobezene structure, introduction of other

radicals into the hydroxyl group of paraaminophenol and into the free amino group of aniline reduces toxicity without loss of the antipyretic effect. *(Smilkstein et al., 1988)*

Parmacokinetics and metabolism: Acetaminophen and phenacetin are metabolized primarily by hepatic microsomal enzymes. Acetaminophen is rapidly and almost completely absorbed from the GIT, the concentration in plasma reaches a peak in 30 to 60 minutes, and the half-life in plasma is about 2 hours after therapeutic doses. Acetaminophen is relatively uniformly distributed throughout most body fluids. Binding of the drug to the plasma protein is variable: only 20 to 50%may be bound at the concentrations encountered during acute intoxication. After therapeutic doses, 90 to 100% of the drug may be recovered in the urine within the first day. It must be kept in mind that children have less capacity for glucuronation of the drug than do adults. ***(Hart and Huskisson, 1988).***

A small portion of acetaminophen undergoes cytochrome P_{450} mediated N-hydroxylation to form N-acetyle-benzo-quinoneimine, a highly reactive intermediate. However after large doses of acetaminophen the metabolite is formed in amounts sufficient to deplete hepatic glutathion; under these circumstances reaction with sulfhydryl groups in hepatic proteins increased and hepatic necrosis may result. ***(Ferreira, 1972)***

Phenylproprionic acid,

Members of this class of NSAIDs –ibuprofen, fenoprofen, flurbiprofen, ketoprofen, naproxen, are all derivatives of phenylepropionic

acid however their individual chemical structures are quite diverse. e.g. **Ibuprofen,** at lower doses it is more effective as an analgesic rather than an anti-inflammatory. Its peak plasma levels occur 1 to 2 hours after oral administration. **Ketoprofen,** it is absorbed rapidly and completely after oral administration, it inhibits both cyclooxygenase and lipooxygenase enzyme activities *(Shen, 1979).*

Phenyleacetic acid derivatives:

Diclofenac, is the first of a series of phenyleacetic acid derivatives it is a very potent inhibitor of cyclo-oxygenase, it posses analgesic, antipyretic, and anti inflammatory activities. It is rapidly and completely absorbed after oral administration, reaching peak plasma concentration within 2 to 3 hours. Diclofenac is extensively bound to plasma protein 99% and has plasma half life of 1 to 2 hours, Diclofenac is metabolized in the liver to 4-hydroxydiclofenac, the principal metabolite, and other hydroxylated forms; after glucuronidation and sulfation, the metabolites are excreted in the urine (65%) and bile (35%). **(Meade et al., 1993)**

Pyrole acetic acid derivatives:

Members of this group include indomethacin, sulindac, tolmetin, ketorolac, and etodolac. **Indomethacin** is a very potent NSAID absorbed well after oral administration, it is metabolized in the liver and has a relatively short serum half-life of 4 hours, and a component of its anti-inflammatory activity is its ability to inhibit polymorphnuclear leukocyte chemotaxis. *(Ferreira , 1972)*

Napthylacetic acid:

Nabumetone, it is a pro drug that is rapidly biotranformed by the liver to the active compound 6-methoxy-2-naphthylacetic acid, it is well absorbed by the GIT, more than 99% of the active compound is bound to plasma proteins. *(Brooks and Day, 1991)*.

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on water and electrolytes, for it causes significant retention of Na and CL accompanied with a reduction in urine volume.*(Brooks and Day, 1991)*

Anthranilic acid derivatives:

Are a family of aspirin like drugs that are derivatives of N-phenylanthranilic acid they include, Meclofenamate, Mefenamic acid e.g. Meclofenamate, is completely absorbed after oral administration reaching peak plasma concentration in half to one hour, its plasma half life is two hours it is highly bound to plasma proteins and displaces warfarin. In man approximately 50% of a dose of mefenamic acid is execrated in the urine, while 20% of the drug is recovered in the feces. *(Hart and Huskisson, 1988)*

Oxicams:

This group contains piroxicam, Meloxicam, oxicams are structurally different from other NSAIDs in that they are not carboxylic acid. Like other NSAIDs they pocess anti-inflammatory, antipyretic, and analgesic activity by virtue of its ability to inhibit cyclooxygenase, piroxicam also inhibits chemotaxis, release of lysosomal enzymes, and neutrophil aggregation*(Shen, 1979)*

Piroxicam: ***pharmacokinetics and metabolism,*** it is completely absorbed after oral administration, the peak concentration in plasma occur within 2-4 hours neither food nor antacids ulter the rate or extent of absorption. There is enterohepatic cycling of piroxicam, and estimates of the

half-life of plasma have been variable, a mean value appears to be about 50 hours. After absorption piroxicam is extensively (99%) bound to plasma proteins. The drug is mainly execrated via the urine and feces *(Hart and Huskisson, 1988).*

Some selective inhibitors of COX-2

NSAIDs exert their anti-inflammatory activity by the inhibition of cyclooxygenase and thus the inhibition of the formation of the prostaglandins. The discovery of two different isoforms of cyclooxygenase enzyme, a constitutive COX-1, and a form that is expressed in inflammation COX-2, has stimulated several laboratories to search for selective inhibitors of this enzyme *(Mitchell et al., 1994).*

A number of compounds have been described which preferentially inhibit COX-2 rather than COX-1 these compounds can be classified into two groups: substances which have been initially selected for development by drug companies because of an improved pharmacological profile in animal models, and were only later shown to preferentially inhibit COX-2 relative to COX-1; and newly designed COX-2 inhibitors, i.e. substances screened in vitro for their selectivity for COX-2, such as SC 58125, and L-745,337. The former group of compounds includes meloxicam, CGP 28238, NS 398, and DuP 697 *(Battistini et al., 1994).*

Nimesulide:

Nimesulide was patented in 1974 in Belgium and USA and is currently sold in several European countries for the relief of pain associated with inflammatory conditions. It has an unusual pharmacological profile compared with other NSAIDs in that it barely inhibited the bovine seminal vesicle microsomal cyclooxygenase with 1/1000 of the activity of indomethacin, while reducing the inflammation of rat adjuvant arthritis and carrageenin paw oedema at similar doses to indomethacin, diclophenac or piroxicame *(Bottchcher et al., 1987).*

1,2-Diarylcyclopentene derivatives:

(Needleman, 1994) have made inhibitors, which are some 1000-fold, more potent against COX-2 than against COX-1. Their prototype SC 58125, emerged as an effective anti-inflammatory agent in various models of chronic inflammation such as carrageenin-induced oedema of the rate paw, the carrageenin-injected air-pouch and rat adjuvant-induced arthritis.

Its in vitro selectivity was tested against COX-1 in platelets and against COX-2 in IL-1 stimulated fibroblasts .The IC50 was 0.09*u*M for COX-2 and >100*u*M for COX-1, making it 1400-fold more selective for COX-2.). *(Reitz et al.,1994).*

Even at greater than anti-inflammatory doses, SC 58125 did not inhibit PGE2 synthesis in the stomach or cause gastric Ulceration. Under similar experimental conditions, aspirin caused a 55% incidence of gastric

ulcers and fenprofen caused a 15% incidence. No changes in generation of renal PGs were observed at high doses of SC58125. ***(Gans et al., 1990)*** DuP 697 was reported some years ago to be a potent anti-inflammatory drug in the rat adjuvant-induced arthritis model, with an ED50 of 0.18 mg/kg .It did not cause stomach ulcers or alter renal blood flow at doses of up to 400mg/kg.

L-475, 337:

Recent reports have described the selective and orally active COX-2 inhibitor L-475, 337. In whole cell assay, this compound inhibited COX-2 with an IC50 of 20nM, but it was inactive on COX-1 even at doses of 10umol (COX-2/COX-1 ratio of 1/500). ***(Chan et al., 1995)*** It reduced carrageenin-induced rat paw oedema with an ED50 of 2mg/kg.

Oral doses of L-475, 337 reduced carrageenin hyperalgesia in the rat paw with ED50 values similar to those of piroxicame and indomethacin (0.37,0.51 and 1047 mg/kg respectively). It did not cause stomach lesions at doses of up to 30mg/kg, whereas the ED50 for the ulcerogenic actions of piroxicam and indomethacin was 14mg/kg. No gastrointestinal bleeding was detected in a Cr excretion assay in monkeys at doses of 10mg/kg given twice daily for 5 days indomethacin and flurbiprofen had a significant effect in this test when given at 5mg/kg for one day. Further more, no obvious stomach lesions where found in rats at doses, which reduced PGE2, levels in the inflammatory exudate from the pleural cavity ***(Reitz et al., 1994).***

CGP 28238:

CGP 28238, also known as flosulide (but not on the market), has been described as a highly potent NASID which dose not inhibit COX prepared from sheep seminal vesicles.

It is now clear that flosulide is a highly potent, selective inhibitor of COX-2 with an IC50 of 15 nM in IL-1 stimulated mesangial cells. At least 1000- fold higher concentrations where required for 50% inhibition of COX–1 in human washed platelets. Assessment of gastric damage by measuring Cr excretion in faeces showed that the two COX-2selective NSAIDs, flosulide and DuP 697, were considerably less toxic to the stomach than other standard NSAIDs *(Tagari et al., 1994)*.

NS-398:

An impressive selectivity for COX-2 was also shown for NS-398, made by Taisho in Japan. This compound inhibited the COX-2 of sheep placenta with potency equal to that of indomethacin, but even at high concentrations it had no effect on the ram seminal vesicles. In analgesic and anti-inflammatory tests in rats it was almost as potent as indomethacin, where as no significant gastric lesions were seen even when 1000mg/kg was given as a single oral dose. *(Futaki et al., 1993)*

MELOXICAM A SELECTIVE COX-2 INHIBITOR NSAID

Meloxicam is a new non-steroidal anti-inflammatory drug (NSAID) that selectively inhibits the inducible isoform of the cyclooxygenase (COX)-2 enzyme. This enzyme has a major role in mediating the inflammatory response while synthesis of prostaglandins required for normal physiological functioning of the stomach and kidneys is under the control of the constitutive isoform, COX-1. ***(Herbette et al., 1996)***

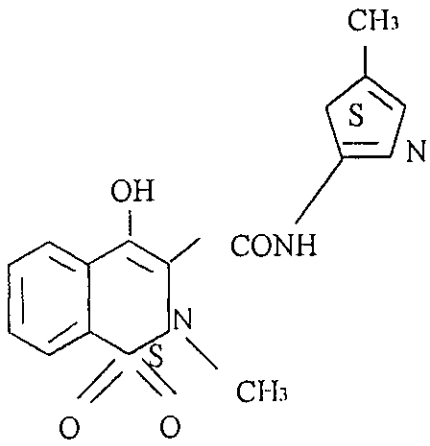
Other NSAIDs in clinical use show varying degrees of selectivity towards COX-1. Only meloxicam and (to a lesser extent) nimesulide could be described as selective for COX-2. In comparative trials of patients with osteo- and rheumatoid arthritis, Meloxicam has been found to be as effective as other NSAIDs, but with a greatly reduced incidence of gastrointestinal side effects. Meloxicam Safety and tolerability make it a significant advance in the treatment of rheumatic diseases. ***(Pairet and Englhardt, 1996)***

Chemical name:

4-hydroxy-2-methyl-N- (5methyl-2-thiazolyl) -2H-1, 2 benzotha- zine-3-carboxamide--dioxide. ***(Engelhardt et al., 1995)***

Empirical formula: C14H13N3O4S2

Chemical structure: ***(Figure 2)***



MELOXICAM (Figure 2)

Key pharmacokinetic features:

(Luger et al., 1996) Stated that the pharmacokinetic profile of meloxicam in man has been extensively investigated in over than 150 male and female volunteers and a number of special patient groups (patients with impaired renal function and patients with hepatic insufficiency and the elderly). Meloxicam is suitable for once daily administration and changing of formulation does not compromise efficacy or safety, thus allowing patients to switch between formulation at their convenience. ***(Engelhardt et al.,1995)***

The elimination half-life of meloxicam is approximately 20h, making it ideal for once daily dosing. Meloxicam is metabolized to four biologically inactive main metabolites. The individual metabolites are excreted in equal proportions via urine and faeces. Meloxicam is almost completely absorbed, and is more than 99% bound to plasma proteins. *(Vane, 1996)*

Meloxicam does not relevantly interact with food, antacid, aspirin, cimetidine, B acetyl-digoxin, methotrexate, warfarin or fursemide. The pharmacokinetics of meloxicam are not affected by hepatic insufficiency or mild to moderate renal dysfunction. End stage renal failure decreases total drug concentrations. Plasma concentrations of meloxicam do not alter in elderly males, although an increase is seen in elderly female patients. These pharmacokinetic parameters are linear over the dose range 7.5-30 mg and bioequivalence has been shown in a number of different formulations. *(Turck and Busche, 1996)*

Absorption:

The absorption of meloxicam by IM, oral, and rectal routes have been reviewed by ***(Turck and Busch, 1996)*** Meloxicam is almost completely absorbed after oral administration, with a bioavailability of 89% after a single 30-mg dose. Peak plasma concentrations (Cmax) are reached 5-6 h (t max) after a light meal. Cmax occur later when meloxicam was administrated in a fasted state. However, in clinical trials the time to onset of analgesic action was quite different when compared to t max. In a double-blind trial in sciatica, the time onset of action was 8-9 min for the oral formulation 30 min and 45 min for I.V. and i.m. Injections respectively *(Englelhardt et al., 1996)*.

As interaction with food may influence may influence the pharmacouinetic behavior of a drug; the effect on the pharmacokinetics of meloxicam has been studied in design studies involving 22 healthy male volunteers. Results indicate that the amount of drug absorption is not affected by a continental breakfast, and a high fat breakfast resulted in slightly increased C max. Absorption after rectal administration is similar to that following oral route and absorption after i.m. Injection is faster than oral administration, with C max occurring after 1-1.5 h. absorption is independent of dose over the range 7.5 to 30mg, leading to dose linear increase in plasma concentrations. This allows for easy dose titration for patients switching to higher doses *(Narjes et al., 1996)*.

Distribution:

Meloxicam is highly protein bound, being more than 99% bound to albumin in accordance with a restricted volume of distribution. This characteristic of most other NSAIDs and also enolcarboxamide derivatives (piroxicam, tenoxicam) in which the unbound fraction of these drugs amounts to 1-3. *(Schmid et al., 1995).*

The pharmacokinetics of meloxicam in rats and mini-pigs suggest that the highest tissue concentrations of meloxicam can be found in blood (albumin) rich compartments such as the liver, kidney, and lungs, with low levels in the CNS. As the pharmacokinetics of the rate most closely resemble that of man, we should also expect this to be the case in human tissue. The volume of distribution is around 10-151, which equates approximately with the extracellular space, although meloxicam has also been shown to penetrate other tissues *(Turck and Busch, 1996).*

Studies by *(Degner et al., 1995)* Show that the concentration in the synovial fluid is approximately half that in plasma (40-57%), with slightly lower concentrations found in adjacent tissues. These findings are similar to results reported for piroxicam and tenoxicam, with synovial fluid: plasma ratios of 0.6 and 0.43 respectively. Meloxicam, therefore, shows consistently similar steady state trans-synovial pharmacokinetics to that of other NSAIDs. Studies on animals also show meloxicam to be highly concentrated in inflamed tissue.

As inflamed tissue are characterized by extravasation and probably a decrease in PH values, these factors may bring about suitable conditions in which to trap meloxicam from the circulation. *(Lehmann et al., 1996)*

Metabolism:

Meloxicam is extensively metabolized, with only traces of the parent drug appearing in urine (0.2%) and faeces (1.6%). Four main metabolites have been identified in both rat and man. These are formed by oxidation of the methyl group of the thiazolyl moiety, followed by oxidation cleavage of the benzothiazine ring *(Schmid et al., 1995)*

Elimination:

Meloxicam has a relatively short elimination half-life in comparison with other oxicams (e.g. piroxicam and tenoxicam). The elimination half-life for meloxicam is approximately 20h in comparison with 53h for piroxicam

and 65-70 h for tenoxicam. Total clearance of the oral form is in the range of 0.42-0.48 l/h, (7-8 ml/min) and steady state plasma concentrations are achieved within 3-4 days. ***(Boulton et al., 1996)***

In patients with renal failure:

Studies in-patients with mild to moderate renal impairment show this condition to have no relevant effect on the parmacokinetics of meloxicam. On this basis, no dosage adjustments are required when administrating meloxicam in these patients. In a further study in patients with end stage renal failure meloxicam showed decreased total drug concentrations. Free Cmax was doubled and thus a 7.5mg dose is recommended in these patients as a safety precaution. ***(Busch et al., 1996).***

In patients with hepatic insufficiency:

The pharmacokinetics of oral meloxicam also showed no relevant effect in patients with impaired hepatic function. The comparative study on patients with clinically stable liver cirrhosis and healthy volunteers showed that the pharmacokinetic parameters for both groups are similar, and there is no evidence of impaired elimination and drug retention. No special dosage adjustments are therefor necessary in this group of patients ***(Busch et al., 1997).***

In elderly patients:

Increased age may alter excretory functions, resulting in drug accumulation. This effect has already been reported for some NSAIDs .The pharmacokinetics of meloxicam in elderly patients suffering from RA have been compared with younger male and female patients, interestingly the results show there to be no difference in the pharmacokinetics between younger male patients and elderly male patients, but there is a small and not clinically relevant reduction in meloxicam clearance in elderly female patients when compared to younger female patient. However as adverse event profiles and efficacy data were similar between young and elderly females no dosage adjustment was necessary. ***(Sander et al., 1995)***

Drug interactions:

Many patients taking NSAIDs are elderly and may be taking other medications. However, no drug interactions of clinical significance have been demonstrated with digoxin ***(Degner et al., 1995)***, methotrexate ***(Hubner et al., 1994)***, cimetidin ***(Busch et al., 1996)***, antacids, frusimide ***(Muller et al., 1995)***, or warfarin. ***(Turck et al., 1998)***

Side effects:

Meloxicam had no significant adverse effects, other than the usual adverse effects of NSAIDs, such as gastric disturbance (the most serious gastrointestinal adverse effects are grouped under the acronym PUB, perforation, ulceration, and bleeding) and the unwanted renal effects that researches have found to be significantly fewer in meloxicam than with other NSAIDs (**Table 1**). ***(Sandler et al., 1990).***

Table(1): Comparison between COX-1 and COX-2 effect on the kidney

	COX-1inhibition	COX-2 inhibition
Reduction of Na elimination	+	nc
Hyperkalaemia	+	nc
Reduction of H2O clearance	+	nc

n.c : No change

Safety and tolerability of Meloxicam

Meloxicam in a doses of 7.5 mg was significantly better tolerated in respect of all upper gastrointestinal events (duodenal ulcer, dyspepsia, eructation, nausea, vomiting, gastric ulcer, haematemesis, and melena) ***(Lemmel et al., 1996).*** The well documented concern over NSAIDs have lead to extensive efforts to develop new agents with similar analgesic and anti-inflammatory properties, but better safety profiles ***(Prouse et al., 1996).***

The discovery that the COX enzyme exists in two distinct isoforms with different functions has made it possible to develop new compounds, which selectively inhibit COX-2, The isoform responsible for mediating the inflammatory response. Meloxicam is the first of these to become available for clinical use. ***(Langman et al., 1994).***

When compared with existing NSAIDs, Meloxicam is as effective as its compurgators, but causes significantly fewer adverse reactions. The most likely explanation for these findings is the COX-2 selectivity of Meloxicam, enabling it to inhibit the inflammatory response while sparing PG syntheses in those sites where it is needed for normal physiological functions, particularly the stomach and the kidneys. ***(Linden et al., 1996).***

The most selective COX-2 inhibitors are still in the experimental stage, but Meloxicam has been registered in many countries. Clearly, anti-inflammatory agents will be far less toxic in the near future.