Therapeutic and Toxic Effects of New NSAIDs and Related Compounds: A Review and Prospective Study

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Abstract: The discovery of the existence of two cyclooxygenases greatly refines the understanding of how the therapeutic and toxic effect of NSAIDs relate to inhibition of PG synthesis. As was reported, NSAIDs inhibit both COX-1 and COX-2 to different extents. This accounts for their anti-inflammatory and analgesic activities and also their unwanted GI side effect. Current evidence indicates that selective COX-2 inhibitors have important adverse cardiovascular effects that include increased risk for myocardial infarction, stroke, heart failure and hypertension. Thus, the development of selective COX-2 inhibitors could be a big step ahead in the therapeutic treatment of anti-inflammatory diseases with fewer risks and side effects. The selective COX-2 diaryl heterocyclic, Coxib group, is characterized by having different 1,2-diaryl five-membered or six-membered heterocycles that are considered as pharmacophore templates, such as, Celecoxib, Rofecoxib, Valdecoxib and Etoricoxib. Moreover, the SAR studies have shown that the substituted sulfonfyl group present in the structure of Coxibs, is considered one of the pharmacophoric moieties responsible for the selective recognition with the key amino acid residues at COX-2 active site pocket. Also, it has been reported that compounds having aryl methylsulfone or aryl sulfonamide moieties display a propensity for COX-2 selectivity. Furthermore, taking in consideration the main difference between the two COX active sites which is the replacement of Ile523 in COX-1 by the less bulky Val523 in COX-2 active site, results in opening a polar side pocket that enlarges the volume of COX-2 active site and is considered a prerequisite for COX-2 drug selectivity.

Key words: Cyclooxygenases, NSAIDs, Coxib, anti-inflammatory, COX-2

INTRODUCTION

Inflammation is a normal and essential response to any noxious stimulus. The typical symptoms of inflammation are redness, swelling, local heat and the patient may be febrile. At the microscopic level, dilatation of the small blood vessels lead to increase in vascular permeability that leads to the leak of fluid and elements from blood into tissue spaces, leukocytes and other phagocytic cells migrate into the area and rupture of cell lysosomes releases lytic enzymes into the tissues. This process is accompanied by the local liberation of chemical mediators that include histamine, bradykinin and prostaglandins (Meyerson and Linderoth, 2006).

Prostaglandins (PGs) comprise a group of naturally occurring 20-carbon cyclopentano-fatty acid derivatives. They belong to a class of autacoids called eicosanoids derived from membrane phospholipids. Upon tissue exposure to any of the inflammation-precipitating factors, cell membranes release Arachidonic Acid (AA) by partial hydrolysis of lipids mediated by the membrane-bound enzyme phospholipase (Kalgutkar et al., 2000a). AA is subjected to one of two biochemical transformation routes. One route involves hydroxylation of the fatty acid by the enzyme lipooxygenase, resulting in the formation of a group of autacoids Called Leukotrienes (LT). The second route involves oxygenation and a process of cyclization by cyclooxygenase enzyme (COX) to produce different types of PGs (Al-Turki, 2010; Vane and Botting, 1992; Flower and Vane, 1972; Vane, 1971; Wu et al., 2003) (Fig. 1).

PGs are produced by most cells and also present in tissues, this explain their broad spectrum of biological responses. The outstanding effects of the PGs include their cycloprotective properties in the gastrointestinal (GI) tract and control renal functions in the kidney (Meyerson and Linderoth, 2006). The general structure of PGs is shown in Fig. 2.
The major effect of prostacycline (PGI₂) is inhibition of the platelet aggregation process, while thromboxane (TXA₂) has the opposite effect on platelets (Wu et al., 2003). Due to the apparent role of PGs in the process of inflammation, inhibiting PG biosynthesis has become an attractive approach to fighting inflammation.

**ANTI-INFLAMMATORY DRUGS**

Anti-inflammatory drugs are type of drugs that influence the inflammatory process or its manifestations and they do so by a variety of actions. They include corticosteroids, immunosuppressive agents, colchicines, chloroquine, penicillamine, gold salt and non-steroidal anti-inflammatory drugs (NSAIDs) of which Aspirin is the prototype. Corticosteroids diminish inflammation of all types by preventing prostaglandin synthesis through inhibition of phospholipase A₂ that releases the AA required. Long term use of corticosteroids poses many problems and in general this group of drugs should not be stopped immediately; gradual withdrawing under physician supervision is required (Kalguarkar et al., 2000b). NSAIDs will be the topic of concern in the following part.
NONSTEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs)

Traditional NSAIDs are one of the most commonly used class of medication worldwide, primarily because of their effectiveness as anti-inflammatory, analgesic and antipyretic agents (Montvale, 2000). The first NSAID, sodium salicylate, was discovered in 1763, however, the side effects associated with the use of salicylates, particularly the GI toxicity, led to the introduction of non-salicylate NSAIDs (Houston and Tech, 2004) (Fig. 3).

All drugs in this class cause adverse effects in a significant number of patients who use them and these are frequently dose limiting. None of the currently available NSAIDs is free of GI complications (Rodriguez et al., 1998).

Among the most widely prescribed drugs for treatment of rheumatic disorders and other degenerative inflammatory joint diseases are: Diclofenac (1), Indomethacin (2), Sulindac (3), Ketoprofen (4), Flurbiprofen (5), Ibuprofen (6), Naproxen (7), piroxicam (8), Tenoxicam (9), Tolmetin (10), Ketorolac (11) (Fig. 4).

**Cyclooxygenase (COX Enzyme):** The common mechanism of action for NSAIDs is inhibition of the synthesis of PGs by inhibiting the key regulatory COX enzyme (Fig. 5).

Fig. 3: NSAIDs. The 9 chemical groupings of NSAIDs are shown, along with key compounds in each class.

Fig. 4: Classic NSAIDs
In 1989, it was determined that there were at least 2 isoforms of cyclooxygenase: COX-1, or prostaglandin H₂ synthase and COX-2, prostaglandin H₂ synthase. COX-1 is expressed in most tissues, regulates physiologic processes such as gastric cytoprotection, kidney function and platelet aggregation and is stimulated by growth factors and hormones. It has been called the housekeeping enzyme (Sperling, 1995; Paloucek and Ryan, 2001; Lee, 2003; Singh, 1998; Graumlich, 2001; Simon, 2001; Noble et al., 2000).

Many toxic effects of NSAIDs, such as prolonged bleeding times and gastrointestinal side effects are attributed to the inhibition of COX-1 (Griffin et al., 1991; Hollander, 1994; Laine, 1996; Scheiman, 1996). COX-2 is found at low or undetectable levels in most tissues. It is an inducible enzyme whose expression is increased in response to inflammation or experimentally in response to mitogenic stimuli (Crostford et al., 2000; Dubois et al., 1998; Smith, 1992). COX-2 is constitutively expressed in the brain, specifically in the cortex, in the female reproductive system where it is associated with ovulation and implantation, in the male reproductive system, in bones where it is associated with osteoblast activity and in the kidney (Crostford et al., 2000; Dubois et al., 1998). In persons with normal kidney function, COX-2 facilitates the regulation of water and electrolyte balance (Chrischilles and Wallace, 1993; Handel and Nielsen, 1997; Johnson et al., 1993; Pope et al., 1993; Whelton, 1999).

Recently, COX-2 over expression has been demonstrated in several types of cancer, in angiogenesis and in neurodegenerative diseases such as Alzheimer's or Parkinson's (Dannenberg et al., 2001; Shiff et al., 2003; Leahy et al., 2000; Masferrer et al., 2000; Hoozemans et al., 2003; Teismann et al., 2003).

Prostaglandins produced by COX-2 are responsible for pain and inflammation where as those from COX-1 have a protective effect on the stomach lining, for this reason NSAIDs which blocks both COX-1 and COX-2 may cause peptic ulcer, while this unwanted effect is eliminated when COX-2 specific inhibitors are used.

Selective COX-2 inhibitors: Cyclooxygenase-2-inhibitors are a relatively new group of NSAIDs which at recommended doses block prostaglandin production by COX-2. Typical COX-2 inhibitors are drugs that show in vitro a minimum of 10-100 times stronger inhibition of COX-2 than COX-1 (Dewitt and Smith, 1988).

However, emerging evidence suggests that adverse reactions such as GI irritation or ulceration and renal liabilities are associated with prolonged use of COX-2 selective inhibitors (Kawaguchi et al., 1995). COX-2 selective inhibitors are also known to suppress synthesis of prostacyclin, a potent vasodilator, gastro-protectant and platelet inhibitors, via inhibition of COX-2. However COX-2 inhibitors do not inhibit production of thromboxane, a vasoconstrictor and promotor of platelet aggregation, which is synthesized in platelets by COX-1 (Silverstein et al., 1995; MacDonald et al., 1997) (Fig. 6a-c). Therefore, COX-2 inhibitors intrinsically lack antithrombotic activity and some cardiovascular liabilities have been associated preclinically with them (Fries, 1991).

It has been reported that, COX-2 inhibitors have not been approved for use in children younger than 18 years old. In adults, candidate criteria for the use of COX-2 inhibitors over other NSAIDs have been suggested by
Simon (2001): Age >60 years. History of GI bleeding, History of NSAID-induced GI toxicity. History of cardiovascular disease, requiring high-dose NSAIDs, concomitant use of glucocorticoids and requiring a combination of NSAIDs.

More than 500 COX-2 inhibitors have been described over the past few years. The large number of newly developed COX-2 inhibitors demonstrates how promising this group of anti-inflammatory agents is expected (Talley, 1999).

**Classes of COX-2 inhibitors:** From a structural point of view selective COX-2 inhibitors are divided into five classes (Gauthier et al., 2006): diaryl or aryl-heteroarylethers, structurally modified NSAIDs, compounds with antioxidative moieties, diethylene derivatives and vicinal diacylcyclohexene or heterocycles (Coxibs).

**Diaryl or aryl-heteroarylethers:** The first selective COX-2 inhibitor discovered in this class was compound NS-398 (12). It showed inhibition of PG synthesis in inflammatory cells and was free of unwanted GI effects in animal models.

Nimesulide (13) and Flosulide (14) are two more closely related members of this group of compounds that is characterized by having a methanesulfonyl moiety (Patrignani et al., 1994; Huff et al., 1995; Davis and Brogden, 1994; Li et al., 1995).

The thioether analogue of Flosulide L-745337 (15) was reported to have higher COX-2 specificity, better bioavailability, improved in vivo potency and greater GI safety than Flosulide.

Furthermore a series of isobenzofuran derivatives was synthesized and compound 16 was reported to be the most potent member of this series in cell culture with COX-1: IC_{50} >100 μmol and COX-2: IC_{50} = 0.005 μmol.

**Structurally modified NSAIDs:** Modifying well known NSAIDs into selective COX-2 inhibitors represents an interesting strategy (Darnhardt and Lauer, 2000). Classic NSAIDs such as indomethacin (2) possess both COX-1 and COX-2 inhibitory activity. Introduction of larger substituents as trichlorobenzoyl moiety and altering the side chain by a beta-branched butyric acid afforded compounds L-748780 (17) and L-761066 (18), respectively with high potency and remarkable activity (Black et al., 1996; Thersen et al., 1996). However, it was reported that esterification or amide formation of the arylacetic acid moiety of indomethacin gave compound 19 capable of binding tightly to COX-2 but not to COX-1 (Kalugutkar et al., 2000b) (Fig. 7).

A similar strategy was used for modification of Zompirac (20) (Darnhardt and Kief, 2001; Saari and King, 1973), Flubiprofen (5) and Aspirin (21) (Kalugutkar et al., 1998) to obtain selective COX-2 inhibitors as shown in Fig. 8.

**Compounds with antioxidative moieties:** Song et al. (1999) reported that since COX enzyme catalysis involves radical intermediates, a radical scavenging moiety such as a di-tert-butylphenol interferes with COX reaction. Accordingly a series of compounds carrying this functional group was prepared and it was found that the thiazole derivative (24) was the most potent and COX-2 selective compound of this class with COX-1: IC_{50} >100 μmol and COX-2: IC_{50} = 0.14 μmol on purified enzymes (Song et al., 1999).
1, 2-Diarylethane derivatives: Compounds 25 and 26 are examples of this group of compounds that is still undergoing biological testing (Black et al., 1996).

Vicinal diarylcarbocycles or heterocycles (Coxibs): These compounds represent the most important group of COX-2 inhibitors. DuP-697 (27) is the prototype of this class of compounds that is called Coxibs (Bertenshaw et al., 1995).

Clinical data of 27 showed selective inhibitory activity against COX-2 (Gaus et al., 1990), but showed very long plasma half-life of 242 h in human and because of its enterohepatic recirculation, it was unacceptable for further evaluation.

All Coxibs characterized by having a central carbocyclic or heterocyclic five membered ring system bearing two vicinal aryl moieties, such as, cyclobuteneone (SC-57666) (28), pyrazole (Penning et al., 1997) (Celecoxib (celebrex®) (29), 2 (SH) furanone (Li et al., 1999) (Rofecoxib (vioxx®)) (30) and isoxazole (Talley et al., 2000a, b, Li et al., 2003) (Valdecoxib®) (31). Some Coxibs have a six-membered ring as the central heterocycle such as the pyridine derivative Etoricoxib (32) (Fig. 9).

A novel class of 6-alkylthio-substituted six membered lactone (pyranone-2-one) ring (33) has been reported to exhibit very good in vitro COX-2 inhibitory potency and selectivity (Joo et al., 2004; Kuel et al., 1984) (Fig. 8).

Structure activity relationship studies (SAR) of Coxibs showed that, substitution at position 4 of one of the aromatic ring system with a sulfonamide or a methylsulfonyl group is essential for optimum COX-2 selectivity and inhibitory potency and the presence of a p-F substituent on a non-sulfonyl vicinal phenyl ring improve in vivo activity (Venturini et al., 1998).
In addition to the methylthioether derivatives 36 and the 2,6-dichloroanilinobenzyl derivatives 37 which produced good anti-inflammatory activity (Gosowami et al., 1984; Amir and Shikla, 2004).

Recently Navidapour et al. (2006) designed and synthesized a new type of 4,5-diaryl-4H-1,2,4-triazole, possessing C-3 thio and alkylthio substituents of the structure 38 for evaluation as selective COX-2 inhibitors. It was reported that compound 3-ethylthio-5-(4-fluorophenyl)-4-(4-methylsulfonylphenyl)-4H-1,2,4-triazole exhibited a high in vitro selectivity and showed good antiinflammatory activity compared to celecoxib in a carrageenan-induced rat paw edema assay (Navidapour et al., 2006).

**MOLECULAR MODELLING STUDIES**

The molecular modeling studies linked between the pharmacological effect and chemical structure which shed more light on structure activity relationship (Al-Rashood et al., 2006, Abou-Zeid, 2002, Abou-Zeid...
and El-Mowafy, 2002; Abou-Zeid et al., 2007; Goda et al., 2008).

Inhibition of COX enzyme at the molecular level is mediated through the blockade of AA to access the COX active site. Accordingly, comparative molecular modelling studies of several selective and non-selective NSAIDs in complex with COX-1 and COX-2 have been done to delineate features that differentiate their mode of interaction with COX-1 and COX-2 (Juni et al., 2002). The molecular modelling will be discussed in section 3.

Structure of COX binding sites: The reported molecular modelling studies based on the x-ray crystallography of the 3-D structures of COX-1 and COX-2 indicated that COX-1 and COX-2 are 63% identical and 77% similar at the amino acid level (Al-Turki, 2010; Fabiola et al., 2001) (Fig. 10).

COX binding site can be considered as a hydrophobic channel extending from the membrane binding domain. In the upper part of the channel, both isozyme possess a Ser 530 which is the amino acid acetylated by aspirin, whereas Tyr 385 located at top of the channel.

The main difference between the two COX active sites is the replacement of the relatively bulky isoleucin (Ile) residue in COX-1 by Valine (Val) at position 523 of the active site of the enzyme. This substitution opens a polar side pocket, enlarging the volume of COX-2 active site and giving access to Arg 513 replaced in COX-1 by a histidine at the same position (Fig. 11). This will cause a
structural modification in COX-2 enzyme that allows access to an additional side pocket which is a prerequisite for COX-2 drug selectivity.

Second, in the apex of the COX-2 binding site, substitution of Phe 503 in COX-1 by Leu 503 generates a small acove which is hydrophobic due to the presence of Leu 384, Tyr 385 and Tryp 387 (Fig. 12).

Thus, there is still a need for novel, selective and potent COX-2 inhibitors with an improved profile compared to current COX-2 inhibitors.

CONCLUSIONS

The discovery of the existence of two cyclooxygenases greatly refines the understanding of how the therapeutic and toxic effect of NSAIDs relate to inhibition of PG synthesis.

As was reported, NSAIDs inhibit both COX-1 and COX-2 to different extents. This accounts for their anti-inflammatory and analgesic activities and also their unwanted GI side effect (Carabaza et al., 1996).

Current evidence indicates that selective COX-2 inhibitors have important adverse cardiovascular effects that include increased risk for myocardial infarction, stroke, heart failure and hypertension (Antman et al., 2007).

Thus, the development of selective COX-2 inhibitors could be a big step ahead in the therapeutic treatment of anti-inflammatory diseases with fewer risks and side effects (Al-Turki, 2010). As shown in this study, the selective COX-2 diaryl heterocyclic, Coxib group; is characterized by having different 1,2-diaryl five-membered or six-membered heterocycles that are considered as pharmacophore templates such as, Celecoxib (29), Refecoxib (30), Valdecoxib (31) and Etoricoxib (32) (Rieudeau et al., 2002; Friesen et al., 1996; Prasit et al., 1999).

Moreover, the SAR studies have shown that the substituted sulfonyl group present in the structure of Coxibs, is considered one of the pharmacophoric moieties responsible for the selective recognition with the key amino acid residues at COX-2 active site pocket.
Also, it has been reported that compounds having aryl methyl sulfone or aryl sulfonamide moieties display a propensity for COX-2 selectivity (Smith et al., 2000).

Furthermore, taking into consideration the main difference between the two COX active sites which is the replacement of Ile523 in COX-1 by the less bulky Val523 in COX-2 active site, results in opening a polar side pocket that enlarges the volume of COX-2 active site and is considered a prerequisite for COX-2 drug selectivity (Mengle-Gaw and Schwartz, 2002) (Fig. 13).

REFERENCES


