ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Cyclooxygenase-2: Pathway form anti-inflammatory to Anti-cancer drugs

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Abstract

Cyclooxygenase, an enzyme involved in the conversion of C-20 acids to prostaglandins, exists in two isoforms. COX-1 is constitutively expressed and has a gastroprotective function. COX-2, induced at the site of injury, is responsible for the expression of pro-inflammatory prostaglandins. Despite overall similarities, COX-1 and COX-2 show subtle difference in amino acid composition at the active sites. COX-2 has valine at positions 89 and 523, while COX-1 has isoleucine, resulting in larger space availability in the former. Further, the presence of valine at position 434 in COX-2 as against isoleucine in COX-1 allows a gate mechanism to operate in favour of the former Numerous experimental, epidemiologic, and clinical studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs), particularly the highly selective cyclooxygenase (COX)-2 inhibitors, have promising anticancer as well as anti-inflammatory activity. NSAIDs restore normal apoptosis in human adenomatous colorectal polyps and in various cancer cell lines that have lost adenomatous polyposis coli gene function. NSAIDs also inhibit angiogenesis in cell culture and rodent models of angiogenesis. Many epidemiologic studies have found that long-term use of NSAIDs is associated with a lower risk of colorectal cancer, adenomatous polyps, and, to some extent, other carcinogens. Non steroidal anti-inflammatory drugs (NSAIDs) produce their therapeutic effects through inhibition of COX, the enzyme that makes prostaglandins. Nonselective inhibition of COX isoenzyme leads to not only beneficial therapeutic effects but also a number of damaging effects. Beneficial effects are due to inhibition of COX-2 and damaging effects are due to inhibition of physiological COX-1. The present review discusses the biology as well as the role of these COX isoenzymes in various prevalent pathophysiological conditions

Key-Words: Cyclooxygenase-2, NSAID'S, Prostaglandins, Anti-cancer, Anti-inflammatory agents.

Introduction

The revolution in biology over the past two decades has resulted in radically new approaches and opportunities for drug discovery. There has been an incredibly rapid increase in the rate of determination of three-dimensional structures of biomolecules. Many of these macromolecules are important drug targets and it is now possible to use the knowledge of the three-dimensional structures as a good basis for drug design. We propose to illustrate this in the case of cyclooxygenase-2, an enzyme responsible for inflammation[1]. This area has attracted immense attention in the last few years and a large number of original research articles and a good number of scientific and popular review articles have been published[1-6].

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Aspirin or acetylsalicyclic acid, the prototype of non steroidal anti-inflammatory agents (NSAIDs) was first produced and marketed by Bayer in March 1899. NSAIDs are even today among the most widely used therapeutic agents with a total annual sale in excess of US $ 10 billion. They are used for the treatment of a broad spectrum of pathophysiological conditions such as headaches, discomfort associated with minor injuries and alleviation of severe pain caused by inflammatory, cancer and degenerative joint diseases such as osteo and rheumatoid arthritis[1].

COX: Biological function and regulation

In the 1980s, Bailey and Needleman proposed the concept of the cyclooxygenase (COX) enzyme as a major regulatory step in prostaglandin (PG) synthesis[7]. COX enzymes catalyze the formation of the prostaglandin endoperoxide from arachidonic acid (AA) to prostaglandin H2 (PGH2). It has both cyclooxygenase activity, which catalyzes the

conversion of AA to prostaglandin G2 (PGG2), and a peroxidase activity, which catalyzes the conversion of PGG2 to PGH2. After the unstable PGH2 is produced, it is rapidly converted by tissue specific isomerases into more stable PGs, such as PGE2, PGF2, PGI2, and thromboxanes as well as other metabolites[8-10].

COX molecules are comprised of an epidermal growth factor-like domain, a membrane binding moiety and an enzymatic domain[11]. In the early 1990s, it was discovered that COX exists in two forms, COX-1 and COX-2. Both enzymes have a molecular weight of approximately 70 kDa, and have approximately the same capacity to convert AA to PGE2. Within the cell, both isoforms are located on the endoplasmic reticulum and the nuclear envelope. COX-1 is found in equivalent concentrations in both intracellular locations, while the concentration of COX-2 within the nuclear envelope is about twice that noted in the endoplasmic reticulum[12].

The two isoforms are encoded by genes located on different chromosomes. COX-1 gene is located on human chromosome[13], while the COX-2 gene is located on chromosome1[14]. COX-1 and COX-2 share 60% homology in their coding regions. Sizes of mRNA for COX-1 and COX-2 are 2.8 kb and 4.6 kb, respectively[15]. 3'-mapping studies have indicated that alternative polyadenylation of the gene occurs at the 3'-UTR (untranslated region) and results in the formation of two distinct mRNA isoforms; COX-24.6 and COX-12.8 [16]. The 5'-flanking region of the COX-2 gene contains important regulatory elements such as a TATA box, cyclic AMP-responsive element (CRE) motifs, CCAAT/enhancer-binding protein (C/EBP) and transcription regulatory sequences for the activator binding protein-2 (AP-2), NF-kappa B (NF-kB) and the ubiquitous DNA binding transcription factor Sp1[17-18]. The COX-2 gene contains a large 3'-UTR. Multiple elements in the 3'-UTR cooperate to destabilize the mRNA[19].

Quiescent cells express COX-1, and it is constitutively expressed in most tissues [20]. COX-1 is the only COX isoform expressed in platelets and gastric mucosa of normal humans. COX-1 produces PGs that regulate essential physiologic functions such as gastric mucosal protection, maintenance of normal kidney function, and platelet aggregation. COX-1 expression can be increased only two- to four- fold under most circumstances. In contrast, COX-2 is usually barely detectable during normal physiologic conditions. It is an immediate-early gene induced upon cell activation and stimulation by pathophysiological stimuli, and it can be rapidly induced to increase PG production ten- to eighty-fold[21].

Deregulated COX-2 expression is associated with a variety of pathological conditions, including colorectal cancer [22, 23], rheumatoid arthritis[20, 21], gastric cancer[24], breast cancer[25-27], prostate cancer[28], and non-small cell lung cancer[29, 30]. COX-2 has also been found to be expressed constitutively but only in a few tissues such as the rat kidney and brain, and human prostate and lung[31, 32].

COX-1 and COX-2: Two isoforms of cyclooxygenase

An early clue to the existence of COX-2 came from a study of cell-growth signaling pathways, which pointed to a unique inducible gene product related to the known COX (i.e. COX-1)[33]. Meanwhile investigators looking at PG production in response to cytokines and other inflammatory factors observed increase in COX activity that could only arise by increased expression of another cyclooxygenase[34]. Immunoprecipitation techniques allowed the isolation of the COX-2 protein and the identification of the two distinct isoforms. Subsequent research established that the COX-1 and COX-2 proteins are derived from distinct genes that diverged well before birds and mammals[35].COX isoforms are bifunctional hemoproteins that catalyze both the bioxygenation of arachidonic acid to form PGG_2 and the peroxidative reduction of PGG_2 to form PGH_2. For a given COX isoform there is approximately 90% (81-98%) identity between species. The tissue distribution of COX-1 and COX-2 differs notably between species and can be heterogenous within the same tissue. For example, COX-2 is the major isoform in rat and mouse brain, whereas similar levels of both isoforms have been detected in human brain. Both isoforms were detected in the stomach with COX-1 being the most abundant in mouse and rat, whereas COX-2 was found to be expressed to a similar extent as COX-1 in human tissue. In the rat stomach, COX-2 was found in the surface mucous cell while COX-1 was found in mucous neck cells (Table 1)[36, 37].

In addition, COX isoenzymes also partition at the cellular level since COX-1 function primarily in the endoplasmic reticulum, whereas COX-2 activity is located both in the endoplasmic reticulum and in the nuclear envelope[12]. This compartmentalization suggests that COX isoenzymes may represent two temporally and spatially separated prostanoid bisynthetic systems with COX-2 producing prostanoids for intracellular differential or replicative events[38, 39]. The compartmentalization of COX isoenzymes is an attempt to explain their respective role at the cellular level when gene distribution techniques may allow an understanding of their biological relevance.

Cyclooxygenase-1 (COX-1)

Picot *et al.*[11] reported the three dimensional structure of COX-1, providing a new therapeutic understanding for the actions of COX inhibitors. This bifunctional enzyme is composed of three independent folding units-an epidermal growth factor-like domain, a membrane-binding motive and an enzymatic domain. The sites for cyclooxygenase and peroxidase are adjacent but spatially distinct. The COX active site is a long, hydrophobic channel. Aspirin-like drugs, such as flurbiprofen, inhibit COX-1 by excluding arachidonate from the upper portion of the channel. Tyrosine 385 and serine 530 are at the apex of the long active site. Aspirin irreversibly inhibits COX-1 by acetylation of the serine 530, thereby excluding access for arachidonic acid[40].

Cyclooxygenase-2 (COX-2)

The COX-2 enzyme is dimeric and each monomer consists of a catalytic domain and a membrane-binding domain, connected by the N-terminal EGF domain. The membrane-binding domain forms a channel, which leads to the active site[41]. The roentgenogram crystal structure of COX-2 closely resembles COX-1 and the binding sites for arachidonic acid for these enzymes are also very similar. The active site of COX-2 is slightly larger and can accommodate bigger structures than those which are able to reach the active site of COX-1. Selectivity for COX-2 inhibitors can be conferred by replacing the His513 and Ile523 of COX-1 with Arg and Val, respectively. This replacement removes the constriction at the mouth of the secondary side channel and allows the more bulky selective COX-2 inhibitors[42].

Structure of COX-1 and COX-2

X-ray crystallography of the 3-D structures of COX-1 and COX-2 as well as complexes with NSAIDs has thrown light on the mechanism of action[11, 43]. COX-1 and COX-2 are very similar enzymes consisting of a long narrow channel with a hairpin bend at the end. Both isoforms are membrane associated. Arachidonic acid released from damaged membranes adjacent to the opening of the enzyme channel, mostly hydrophobic, is sucked in, twisted around the hairpin bend and subjected to chemical reactions, resulting in the formation of the cyclopenta ring of PGs. Experiments have revealed the site of catalysis at about half-way down the channel and mechanism of action of NSAIDs at that site[44]. Subtle differences existing at the active site in COX-1 and COX-2 can be expected to regulate specificity as has been convincingly shown by the elegant study of complexes of the classical, nonspecific NSAIDs, flurbiprofen and indometacin with selectivity for COX-2[45]. It was postulated that L-valine at 523 in the active site of COX-2 as against the bulkier isoleucine in COX-1 gave better access to the inhibitor in the case of former (Fig. 1 a & b).

COX-2 Selective inhibition: Newer therapeutic targets

The potential improvement in the therapeutic ratio of NSAIDs which inhibit inducible COX-2 at the inflammed site but have no effect on constitutive COX-1 is likely to change the use of the classical NSAIDs. Beside their therapeutic indication, these selective COX-2 inhibitors might have potential use in various diseases such as colorectal cancer and neurodegenerative disease of the alzheimer type.

COX-2 inhibitors in colon cancer

Various laboratory studies suggested that NSAIDs reduce the risk of colon cancer and that inhibition of colon carcinogenesis is mediated through modulation of prostaglandin by COX isoenzyme. Over expression of COX-2 has been observed in colon tumors therefore specific inhibitors of COX-2 could potentially serve as chemopreventive agents[46, 47].

A recent study with celecoxib and nimesulide in intestinal polyphs in mice and colonic aberrant crypt foci (ACF) formation in rats induced by azoxymethane indicated that both agents possess strong chemopreventive activity against colon carcinogenesis[48]. This finding is further supported by Reddy *et al.*[47,49] who suggested that SC-58635 (a COX-2 inhibitor) significantly suppressed colonic ACF formation and crypt multiplicity, and strengthens the hypothesis that selective COX-2 inhibitors have promising therapeutic potential against colon carcinogenesis.

COX-2 inhibitors in Alzheimer's disease

Recent studies suggest that inflammatory events are associated with plaque formation in the brains of patients with Alzheimer's disease (AD). Treatment of these patients with NSAIDs slows the progression of disease. Pepeu reviewed the evidence of inflammatory mechanisms in the pathogenesis of AD. It was shown that the intra-cerebral injection of b-amyloid produces extensive glial reaction in the brain and induces COX-2 expression in neuronal culture[56]. Moreover, experimental neurodegenerative lesions cause up-regulation of neuronal COX-2, and COX-2 inhibition can be neuroprotective in animal and cell culture models[50]. Ho *et al.*[51] also reported an elevated expression of neuronal COX-2 in sub-regions of the hippocampal formation in AD and that such elevation may potentiate b-amyloid-mediated oxidative stress. Thus, the efficacy of NSAIDs in slowing AD may be explained by inhibition of neuronal COX-2, the activity of which promotes neurodegeneration. On this hypothesis, the selective COX-2 inhibitor which

penetrates the blood-brain barrier may be a good therapeutic candidate for alzheimer's disease.

COX-2 inhibitors in delaying premature labor

Eicosanoids are important for inducing uterine contractions during labor. A significant increase in COX-2 occurs in amnion and placenta immediately before and after the start of labor. It is thus likely that COX-2 produces the oxytocic PGs that are responsible for preterm labor[60]. One cause of preterm labor could be an intrauterine infection, resulting in the release of endogenous factors that increase PG production by up-regulating COX-2. These data indicate that selective COX-2 inhibition may be of use in preventing contractions in premature labor, being preferable to b-sympathomimetics (which produce maternal cardiovascular, respiratory and metabolic side effects) and indomethacin (which produces oligohydramnios and closure of the ductus arteriosus due to reduced synthesis of vasodilator PGs)[51, 52].

COX-2 inhibitors in bone resorption

Prostaglandins and IL-1 have long been known to be major mediators of osteoclast activation and bone resorption. Recent studies indicate that IL-1 causes initial PGE_2-independent bone resorption followed by induction of COX-2. A variety of other chemokines are also involved in the process (IL-6, IL-11 and TGF-b (which is stored in the bone matrix and is released during bone damage), all induce COX-2 and bone resorption. In the study by Macial *et al*.[53], it was reported that PTH induces COX-2 expression in human osteoblast that is significantly altered by NS-398 (a specific COX-2 inhibitor). These studies suggest that the COX-2 inhibitors may be therapeutic agents for bone-related disorders.

NSAIDs and COX-2 specific inhibitors classic NSAIDs

Nonselective NSAIDs inhibit both COX-1 and COX-2 enzymes. They are commonly used in the therapy of inflammatory diseases. The observation of decreased risk of colorectal cancer among aspirin and other NSAID users suggests promise that NSAIDs may play a role in future chemoprevention strategies. Over the years the therapeutic usage of NSAIDs has grown rapidly. Kurumbail classified inhibitors according to their interaction with the enzyme protein as four classes[45].

Irreversible inhibitors of COX-1 or COX-2

Aspirin is the most ancient NSAIDs used as an anti-inflammatory agent, whose history traces back to more than 100 years ago. Aspirin, and a more recently designed aspirin- like molecule o-(acetoxyphenyl) hept-2-inyl-sulfide (APHS), acetylates the serine residues of COX-1 and COX-2 thus preventing AA from reaching the catalytic center. This covalent modification irreversibly inactivates COX [54].

Reversible, competitive inhibitors of COX-1 and COX-2

Inhibitors such as ibuprofen, compete with AA to bind to the catalytic center of COX.

Slow, time-dependent, reversible inhibitors of COX-1 and COX-2

Acting through ionic interactions between a carboxylic moiety on the inhibitor and an arginine residue of COX, this group of NSAIDs, such as indomethacin and flurbiprofen, seem to influence the helix D region of COX protein rendering it less flexible and thus less active.

Slow, time-dependent inhibitors of COX-2

Representatives of this group such as celecoxib, and rofecoxib are selective COX-2 inhibitors. They inhibit COX-2 in a slow time-dependent process, and are weak competitive inhibitors of COX-1.

All currently marketed classic NSAIDs, such as indomethacin, ibuprofen, and sulindac sulfide are inhibitors of both COX-1 and COX-2. NSAID toxicity is the result of inhibition of COX-1 activity leading to ulceration, bleeding, and perforation in the gastrointestinal mucosa [1]. Long-term aspirin use results in an increased risk of gastrointestinal bleeding, even at relatively low doses of drug. Therefore, the classic non-selective NSAIDs are being pushed gradually into the background, whereas selective COX-2 inhibitors are being favored with their reduced side effects and attractive pharmacological profile.

COX-2 selective inhibitors

Given the evidence linking COX-2 expression to tumor development, COX-2 selective inhibitors are not only preferable as anti-inflammatory agents, but also may represent novel chemopreventive drugs. In the past few years, COX- 2 selective inhibitors have come to the forefront of cancer research and their effects in inhibiting tumor growth have been shown in both in vitro and in vivo studies. A large number of COX-2 inhibitors have been developed. 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. They were grouped into different structural classes as summarized by G. Dannhardt and W. Kiefer[55].

1. Diaryl- or aryl- heteroaryl-ethers (sulfonanilide inhibitors): nimesulide, NS398, flosulide, L-745337
2. Vicinal diaryl heterocycles: celecoxib, rofecoxib, SC-57666, DuP-697

3. Modified, known NSAIDs to improve COX-2 selectivity: L-748780, L-761066, meloxicam, etodolac
4. Antioxidative compounds
5. 1,2-Diarylethylene derivatives (*cis*-stilbenes)

COX and anti-inflammatory activity

Mode of action: Corticosteroids inhibit the activity of phospholipase A_2 and hence reduce the release of arachidonic acid and ultimately inhibit the formation of proinflammatory prostaglandins. Vane[56] made the seminal proposal in 1971 that in contrast to steroids, NSAIDs exerted their activity by inhibiting cyclooxygenase (COX), a dual function enzyme. Prostaglandins are formed by the oxidative cyclization of the central 5 carbons within 20 carbon polyunsaturated fatty acids. The key regulatory enzyme of this pathway is COX, also known as PGH synthase, which catalyses the conversion of C-20 acids with varying degrees of unsaturation to prostaglandins PGG_2 and PGH_2. The latter is subsequently transformed to a variety of eicosanoids such as PGE_2 and thrombaxane (TXA_2). Apart from the activity to bring about cyclization, COX has also peroxidase activity which leads to the hydroxylation of cyclopentenes through endo-peroxidation. All NSAIDs in clinical use have been shown to inhibit COX, leading to a marked reduction in PG synthesis[57]. The inhibition by aspirin is due to irreversible acetylation of the cyclooxygenase component of COX, leaving the peroxidase activity unaffected[58]. In contrast, NSAIDs like indomethacin or ibuprofen inhibit COX reversibly by competing with the substrate, arachidonic acid, for the active site of the enzyme[59]. All the activities of NSAIDs such as prevention of pathological overproduction of pro-inflammatory prostaglandins and the physiological formation of prostanoids are explained well by the postulate of inhibition of prostaglandin synthesis. The unwelcome ulcerogenic and renal side effects of NSAIDs such as aspirin and ibuprofen have been related to the inhibition of production of prostacyclin, which has a cytoprotective effect on the gastric mucosa and regulation of kidney function. It thus appeared that the ulcerative effect of classical NSAIDs was an inevitable price to be paid for the desired anti-inflammatory activity, until the discovery that COX existed in two isoforms, COX-1 and COX-2. The protective effects of NSAIDs are based on the following mechanisms

COX and anti-cancer activity

Inflammatory mediators such as cytokines, eicosanoids, and growth factors are thought to play a critical role in the initiation and maintenance of cancer cell survival and growth[60]. One of these mediators, PGE_2[61], is produced in large amounts by tumors. PGE_2 is produced from arachidonic acid by either of two enzymes: COX-1 or COX-2. Both COX isozymes can be inhibited by traditional NSAIDs, such as aspirin and indomethacin. Several studies show that regularly taking aspirin or other conventional NSAIDs provides a 40–50% reduction in relative risk of death by colon cancer, indicating that inhibition of COX in humans has a chemopreventive effect[1]. In rodent models of FAP, a genetic disease leading to colon carcinoma, blockade of COX-2, either by gene deletion or by pharmacological inhibition of enzyme activity, suppresses intestinal polyp formation. COX-2 inhibition also demonstrates chemopreventive activity against colon carcinogenesis. Taken together, these data provide strong evidence for the importance of COX-2 enzyme activity in oncogenesis.

Several recent reviews[62-64] have summarized the intriguing and accumulating evidence that non steroidal anti-inflammatory drugs (NSAIDs) have a promise as anticancer drugs. NSAIDs have been shown experimentally to stimulate apoptosis and to inhibit angiogenesis, two mechanisms that help to suppress malignant transformation and tumor growth. Randomized clinical trials have confirmed that two NSAIDs, the prodrug sulindac[65] and the selective cyclooxygenase (COX-2) inhibitor celecoxib[66], effectively inhibit the growth of adenomatous polyps and cause regression of existing polyps in patients with the unusual hereditary condition familial adenomatous polyposis (FAP).

Evidence for cancer prevention properties of NSAIDs

The hypothesis that NSAIDs might inhibit the occurrence or growth of colorectal cancer arose in the mid-70s, when Bennett and Del Tacca[67] and Jaffe[68] reported that the concentration of prostaglandin E2 was higher in human colorectal tumor tissue than in the surrounding normal mucosa. Conventional NSAIDs (such as piroxicam, indomethacin, sulindac, ibuprofen, and ketoprofen), and selective COX-2 inhibitors [e.g., celecoxib] inhibit chemically induced carcinogenesis in rats and mice. Nonselective NSAIDs suppress tumor growth to a greater extent and at lower doses when treatment is begun before or coincident with exposure to the carcinogen than when it is delayed until the tumor promotion/progression phase. For example, low-dose piroxicam (25 ppm in food) caused a 30% reduction in tumors when treatment was begun soon after exposure to the carcinogen but only a 12% reduction when treatment was begun 23 weeks after exposure[69]. Early initiation of treatment also improves tumor suppression by sulindac sulfone and celecoxib. Both nonselective and selective NSAIDs effectively inhibit the early stages of tumor development, whereas

only selective COX-2 inhibitors are effective when treatment is delayed. For example, celecoxib (1500 ppm in food) reduced tumor incidence and multiplicity by approximately half, even when treatment was delayed until the tumor Promotion/progression stage.

Mechanism of inhibition of apoptosis

Despite continuing uncertainty about the molecular pathways by which NSAIDs may inhibit colorectal neoplasia, there is mounting evidence that tumor inhibition may be mediated by at least two distinct cellular processes. These involve the ability of NSAIDs to restore apoptosis in APC-deficient cells[70] and their capacity, particularly in the case of coxibs, to inhibit angiogenesis. Apoptosis, or programmed cell death, is needed to maintain homeostasis in continuously replicating tissues such as the intestine. Partial suppression of apoptosis occurs early in tumorigenesis in approximately 85% of human colorectal cancers due to the inactivation of both alleles of the APC gene. The suppression of apoptosis allows APC-deficient cells to accumulate in adenomatous polyps. Further suppression of apoptosis occurs as these cells develop additional genetic mutations and phenotypic changes[71]. In vitro, both nonselective NSAIDs and selective COX-2 inhibitors stimulate apoptosis in APC-deficient cells that have not yet undergone malignant transformation. Nonselective NSAIDs lose their ability to inhibit chemically induced tumors when polyps undergo malignant transformation. In contrast, selective COX-2 inhibitors stimulate apoptosis and suppress growth in many carcinomas, including human cancers of the stomach[72], esophagus[73-74], tongue[75], brain[76], lung[77], and pancreas[78]. In human HT-29 colon cancer cells, apoptosis can be restored by treatment with selective[79] or nonselective COX inhibitors. Apoptosis becomes progressively more inhibited during the development of colorectal cancer[71], coincident with the increasing expression of COX-2

Mechanism of inhibition of angiogenesis

A second cellular process by which COX-2 inhibitors may inhibit tumor growth is through inhibition of angiogenesis and neovascularization. Solid tumors must stimulate the formation of new capillary blood vessels to grow larger than approximately 2 mm in diameter [80]. COX-2 expression is widely induced in the angiogenic vasculature of colorectal adenomatous polyps and in carcinomas of the colon, lung, breast, oesophagus, and prostate[81]. Selective COX-2 inhibitors suppress the growth of corneal capillary blood vessels in rats exposed to basic fibroblast growth factor and inhibit the growth of several human tumors transplanted into mice[81]. Therapeutic (low micromolar) concentrations of coxibs also suppress the release of angiogenic growth factors by human or rodent colorectal cancer cells that are co-cultured with vascular endothelial cells[80] and block migration and tube formation by the endothelial cells.

Conclusions and future trends

The discovery of inducible cyclooxygenase enzyme has given a new impetus in the development of safer anti-inflammatory and anti-cancer drugs. The results of animal experiments and early clinical studies with selective COX-2 inhibitors are quite impressive and support that these selective COX-2 inhibitors will represent an effective gastrointestinal-sparing alternative to classical NSAIDs and will be beneficial in other clinical situations in which COX-2 is over expressed, besides their therapeutic indication. Recently, various challenges posed to the COX theory that prompt a reevaluation of the original theory and a reexamination of whether the selective inhibition of COX-2 might not be as effective or as safe as anticipated. Ferreri *et al*.[83] reported that COX-2-deficient genetically engineered mice develop a severe nephropathy. Moreover, an important consideration is the potential consequences of inhibition of COX-2 in tissues where this enzyme has been constitutively expressed (i.e., brain and kidney). Whether or not selective inhibition of COX-2 fulfills the therapeutic potential will depend on long-term safety of selective COX-2 inhibitors. Owing to gastrointestinal safety concerns with traditional nonselective COX inhibitors, derivatives that selectively target COX-2 have been developed for applications in arthritis, analgesia, and the treatment of neoplasia. COX-2 selective inhibitors serve as a paradigm of molecularly targeted, cytostatic, anti-neoplastic agents[84]. COX-2 is consistently over expressed in a large percentage and variety of human and rodent tumors[85]. At the cellular level, COX inhibitors have been shown to inhibit proliferation, induce apoptosis, inhibit angiogenesis, reduce carcinogen activation, and stimulate the immune system[85]. Currently, most publicly sponsored chemoprevention trials in this area are testing celecoxib, a circumstance that commends the foresight of its discoverers. If celecoxib proves to be the most active among a growing field of COX-2 inhibitors, the fact that it is already being tested in advanced clinical trials against a variety of epithelial malignancies (e.g., colon, esophagus, skin, and bladder cancers) may have enormous impact on the rate at which the true potential of this class of agents will be definitively assessed.

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Table-1: Difference between COX-1 and COX-2 Isoenzymes

COX-1	COX-2
Physiology	
[1]Constitute form of COX.	[1]Inducible form of COX.
[2] "Housekeeping gene" produces PG that regulates normal kidney and stomach function and vascular homeostasis.	[2] "Inflammation response gene" induced during inflammation, produces PG involved in inflammation.
	"Immediate early gene" thought to control mitogenesis, may, produces PG involved in cell growth.
Localization	
[1]Present in platelets, endothelial cells, stomach, kidney, smooth muscle, most tissues.	[1]Present in brain- Control & limbic neurons, activated monocytes of fibroblasts and synovlocytes during inflammation and in follicles proceeding ovulation.
[2] Lumen of ER.	[2] ER and nuclear envelope.
Amino acids	
[1] 599 amino acids	[1] 604 amino acids
[2] a cassette of 17 a.a. sequence near the N-terminal that is absent in COX-2	[2] a cassette of 18 a.a. sequence near the C-terminal that is absent in COX-1
[3] N-terminal sequence begins with ADPGA	[3] N-terminal sequence begins with ANPCC
Molecular Weight	
73000	74000
Regulation of expression	
1 gene is 22 Kb with 11 exons	1 gene is 83 with 11 exons
2 gene located on chromosome 9	2 gene located on chromosome 10
3 mRNA transcript is 2.8 to 3.0 Kb	3 mRNA transcript is 3.0 to 4.5 Kb
4 mRNA transcript is not degraded fast	4 mRNA transcript is degraded quickly
5 promoter region of gene has poor inducibility.	5 promoter region contains many transcriptional factors which can be upregulated by proinflammatory cytokines.
6 post-transcriptional additions of 3 high mannose oligosaccharides.	6 post-transcriptional additions of 5 high mannose oligosaccharides.
7 not inhibited by glucocorticosides .	7 inhibited by glucocorticosides .
Active Site	
Smaller active size	larger active size
Substrate	
Only C20 carboxylic acid	both C18 and C20 carboxylic acids.
Phase of inflammation	
Main source of PG in chronic inflammation phase	Main source of PG in acute inflammation phase
Acetylation by aspirin	
1 acetylation of ser 530	1 acetylation of ser 516
2 Complete inhibition of COX activity	2 modification of enzyme to produce 15-hydroxyeicosutetraenoic acid(15 HETE)

ISSN: 0976-7126

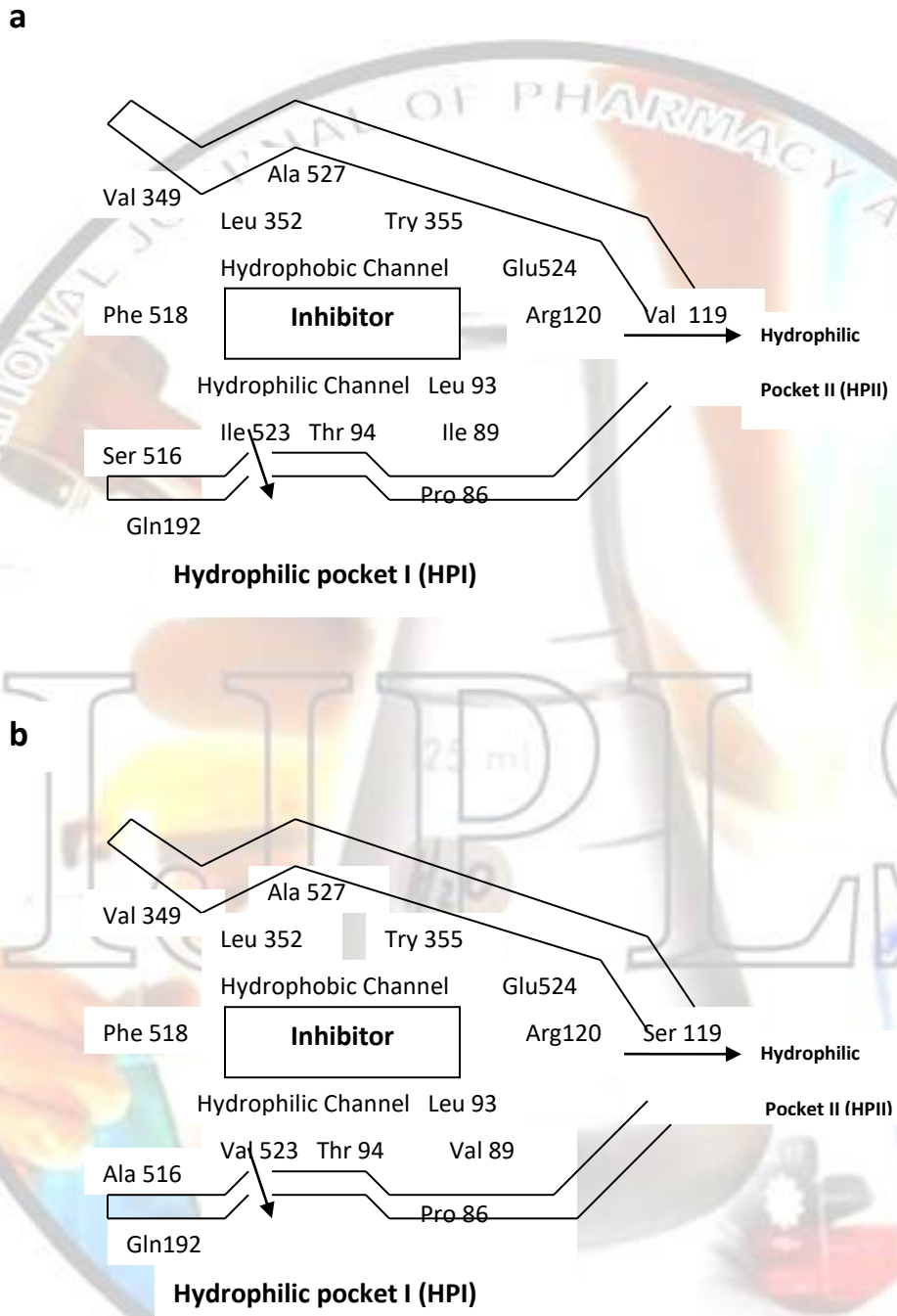

Fig. 1. Schematic representation of active sites of (a) COX-1 and (b) COX-2