

## A Pharmacological Review on Cyclooxygenase Enzyme

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**Abstract:** *This review provides brief summary of structure, physiology and pharmacological role of Cyclooxygenase. Cyclooxygenase, an enzyme involved in the production of prostaglandins, exists in two isoforms, third isoform has been recently encountered. 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 differences 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. The activity of cyclo-oxygenase-1 and 2 results in the production of a variety of potent biological mediators (the prostaglandins) that regulate homeostatic and disease processes.*

**Keywords:** *Cyclooxygenase, COX-1, COX-2, Inflammation, Alzheimer's disease*

### Introduction

Cyclooxygenase is a rate-limiting enzyme that catalyzes the biosynthesis of prostaglandins and thromboxanes from arachidonic acid. These bioactive metabolites play an important role in the regulation of physiological process, such as mucosa secretion and smooth muscle contraction, and in the regulation of pathological condition, such as allergic diseases and rheumatoid arthritis [1, 2]. Two isoforms of cyclooxygenase, COX-1 and COX-2, have been identified [3, 4, 5]. COX-1 is constitutively expressed in most human tissues and functions as a housekeeping gene, whereas COX-2 is an immediate-early gene and is induced by oncogenes, growth factors, cytokines, endotoxins, and phorbol esters [6, 7, 8]. Overexpression of COX-2 has been related to chronic inflammation, angiogenesis, and carcinogenesis [9]. COX-2 expression is tightly regulated at both the transcriptional and the post transcriptional levels. The *cis*-acting elements in the 5-flanking promoter region of COX-2 contains a canonical TATAbox and multiple regulatory elements of NF-B, NF-IL6 (CCAAT/enhancer-binding protein; C/EBP), and cAMP response element (CRE) [10, 11]. The activation of intracellular signaling pathway induces the recruitment of specific transcription factors to these elements and triggers COX-2 expression.

### Physiology

COX converts arachidonic acid (AA, an  $\omega$ -6 PUFA) to prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), the precursor of the series-2 prostanoids. The enzyme contains two active sites: a heme with peroxidase activity, responsible for the reduction of prostaglandin G<sub>2</sub> to prostaglandin H<sub>2</sub>, and a cyclooxygenase site, where arachidonic acid is converted into the hydroperoxy endoperoxide prostaglandin G<sub>2</sub> (PGG<sub>2</sub>). The reaction proceeds through H atom abstraction from arachidonic acid by a tyrosine radical generated by the peroxidase active site. Two O<sub>2</sub> molecules then react with the arachidonic acid radical, yielding prostaglandin G<sub>2</sub>.

Currently three COX isoenzymes are known—COX-1, COX-2 and COX-3. COX-3 is a splice variant of COX-1 which retains intron one and has a frameshift mutation, thus some prefer the name COX-1b or COX-1 variant (COX-1v). [12]

Different tissues express varying levels of COX-1 and COX-2. Although both enzymes act basically in the same fashion, selective inhibition can make a difference in terms of side-

effects. COX-1 is considered a constitutive enzyme, being found in most mammalian cells. More recently it has been shown to be upregulated in various carcinomas and to have a central role in tumor genesis. COX-2, on the other hand, is undetectable in most normal tissues. It is an inducible enzyme, becoming abundant in activated macrophages and other cells at sites of inflammation.

Both COX-1 and -2 also oxygenate two other essential fatty acids – DGLA ( $\omega$ -6) and EPA ( $\omega$ -3) – to give the series-1 and series-3 prostanoids, which are less inflammatory than those of series-2. DGLA and EPA are competitive inhibitors with AA for the COX pathways. This inhibition is a major mode of action in the way that dietary sources of DGLA and EPA (e.g. borage, fish oil) reduce inflammation.

### **Properties of COX-1 and COX-2**

COX-1 is constitutively expressed in most tissues and performs a housekeeping function to synthesize PGs with normal cell regulatory activity. It is a membrane-bound haemo and glycoprotein with a molecular weight of 71 kDa. It cyclizes arachidonic acid and adds the 15- hydroperoxy group to PGG<sub>2</sub> and then reduces the molecule to PGH<sub>2</sub>, both through its own peroxidase activity. COX-2 with a molecular weight of 70 kDa has similar functions. However, it is not found to any appreciable extent in resting cells. On the other hand, it is expressed considerably after exposure to fibroblasts, cytokines, etc.

Levels of COX-2 protein increase in parallel with overproduction of prostaglandins in many cells and tissues in chronic inflammation. Both COX-1 and COX-2 are homodimeric proteins. With the availability of purified COX-1 and COX-2 enzymes of different origins and by various methods, including recombinant technology, the classical non-steroidal anti-inflammatory drugs could be readily tested and were shown to inhibit both COX-1 and COX-2, some of them in fact inhibiting the first more than the second. A logical outcome of the recognition of the role of the two COX forms appeared to be that a selective COX-2 inhibiting NSAID should not have the principal side effects associated with use of the earlier drugs, such as gastrointestinal inhibition and ulcers as well as renal perturbations.<sup>[13,14]</sup> Additionally, since COX-2 overexpression is observed in diseases like Alzheimer's and colorectal cancer, selective COX-2 inhibitors may have useful therapeutic benefits in such conditions<sup>[15]</sup> COX-2 selective NSAID treatment may be an important advance in the prevention of preterm delivery.<sup>[16]</sup> The design of a selective COX-2 inhibitor would be desirable and possible if structural differences could be identified between the two isoforms and exploited, although doubts have been raised to temper this optimism, since among other reasons, COX-2 has also useful physiological functions which may suffer in the process of inhibition<sup>[17]</sup> The situation is confounded further by the preliminary data on COX-3 which is expressed in the 'resolution' phase of the inflammatory process. Since this has anti-inflammatory activity, the administration of selective COX-2 inhibitors at this stage may actually retard the healing phase<sup>[18]</sup>.

### **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 [19,20,21] 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. 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 indomethacin and the recently developed SC-558 with selectivity for COX-2

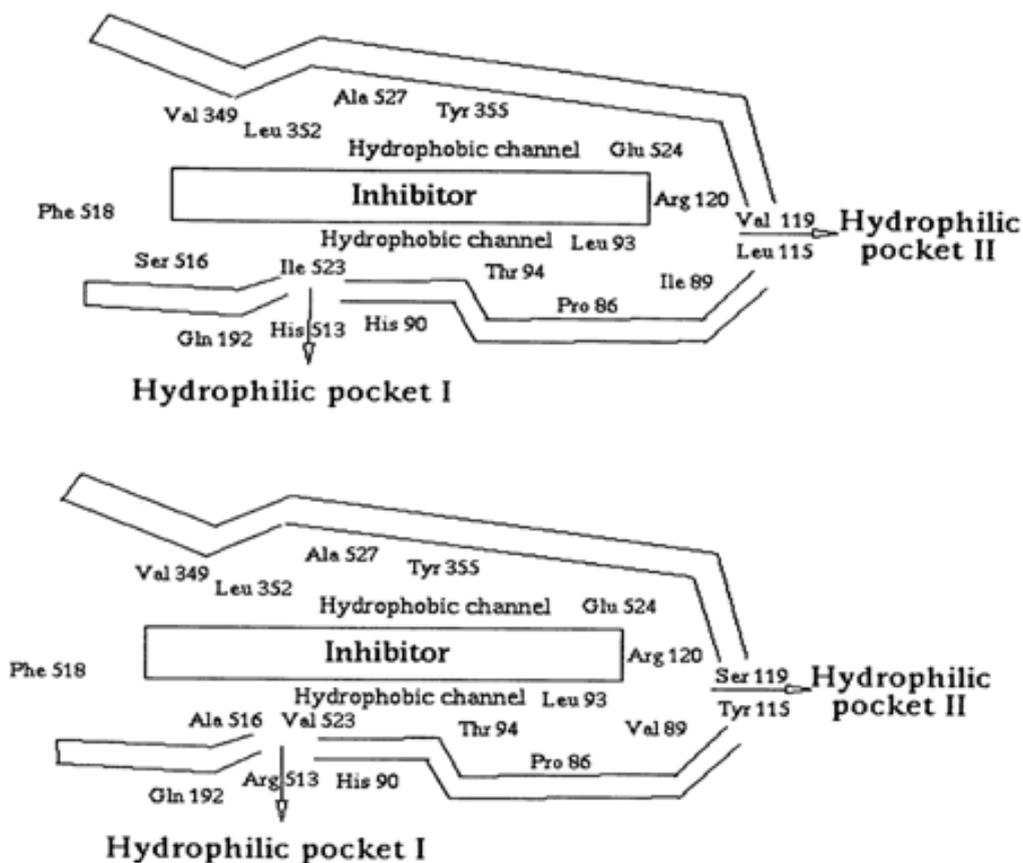
[21]. It was postulated that L-valine at 523 in the active site of COX-2 as against the bulkier isoleucine in COX-1 gave better access to the inhibitor in the case of former. This has been convincingly demonstrated with SC-58125, an analogue of SC-558, which selectively inhibited COX-2. Targetted single amino acid substitution of valine in COX-2 at position 509 gave a mutant with a COX-1 profile, which was poorly inhibited by SC-58125. [22] We now proceed to discuss in somewhat greater detail the differences between the binding sites of COX-1 and COX-2.

### ***Differences between binding sites of COX-1 and COX-2***

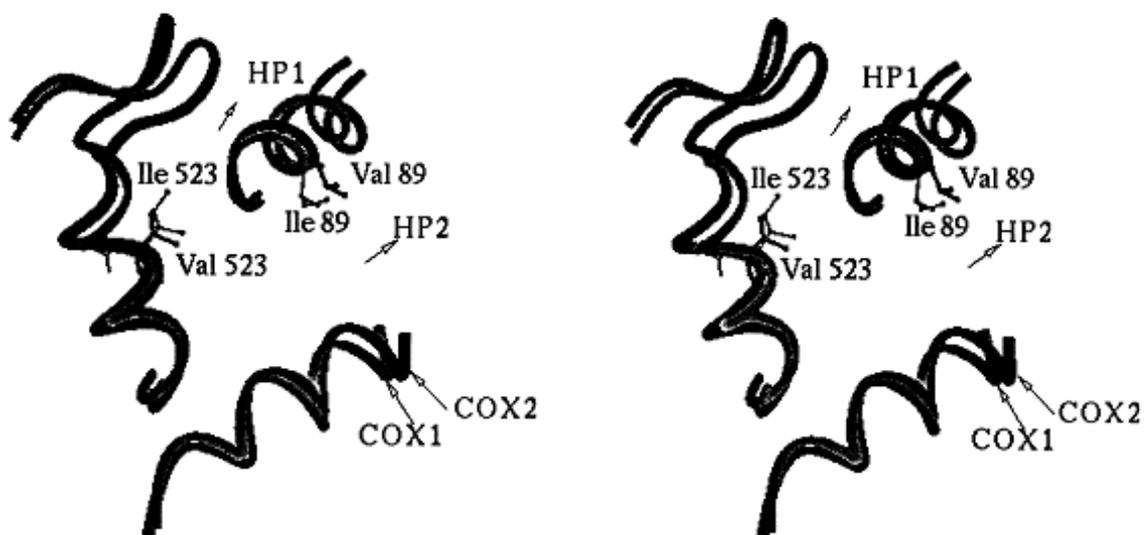
COX-1 and COX-2 are 63% identical and 77% similar at the amino acid level. The catalytic domain is highly conserved, with the major residues known to be involved in catalysis, Arg 120, His 206, Tyr 385, His 386 and His 388, all conserved along with the residue Ser 530. Differences that could be responsible for the selectivity are most likely to be found in the cyclooxygenase active site, due to the fact that the known selective inhibitors inhibit the cyclooxygenase activity. The active site is preponderantly hydrophobic in nature with two internal hydrophilic pockets I and II (Figure 1 *a* and *b*), both of which have a valine in COX-2 and an isoleucine in COX-1 (positions 523 and 89) at the opening of the pocket, leading to the constriction of this pocket in COX-1.

Figure 2 gives a stereoview of the environments at the binding centres. The accessibility of these pockets is reported to be controlled by a valine in COX-2 as against isoleucine in COX-1, at position 434. The side chain of Ile residue at 434 packs against Phe 518 which forms a molecular gate that extends to the hydrophilic pocket I. In COX-1, this gate is closed because of the bulkier Ile side-chain, whereas in COX-2, with the less voluminous Val at 434, the gate has room to swing open, allowing the entry of the inhibitor<sup>19</sup>. The substitution of Val 523 in COX-2 by Ile in COX-1 has consequences for the size and shape of this hydrophobic region. This difference has been implicated in the selectivity of some inhibitors<sup>19</sup>. Access to the hydrophilic pocket II situated at one end of the hydrophobic channel is facilitated in COX-2 because of Val at 89 instead of Ile in COX-1 at that position. The combined effect of the amino acid differences at 89 and 523 contributes to the larger space of this site in COX-2. NSAIDs, occupying both the pockets in COX-2, may be expected to have greater specificity compared to drugs which may bind at only one of them.

In addition to the above-mentioned differences between COX-1 and COX-2, significant changes occur at position 115, where a nonpolar leucine is replaced by an uncharged polar tyrosine; at position 119, where a nonpolar valine is replaced by an uncharged polar serine, and at position 357, where a nonpolar leucine is replaced by the much larger nonpolar phenylalanine. The substrate channel is oriented from top to bottom with heme at the top and amino acid residues 112, 115 and 119 at the bottom. Leucine 357 is situated slightly below the active site/NSAID binding pocket.



**Figure 1:** Schematic representation of active sites of (a) COX-1 and (b) COX-2.



**Figure 2:** Stereo view of the environment of binding centres of COX-1 and COX-2.

## Genetics and Structure: Evidence for Two Separate Enzyme Systems

### *Molecular Biology*

The genes for COX-1 and COX-2 are located on separate chromosomes, with COX-1 on chromosome 9 and COX-2 on chromosome 1. The COX-2 gene is smaller than COX-1. Exons 1 and 2 of COX-1 are condensed into a single exon in COX-2. The introns of COX-2 are smaller than COX-1. COX-2 has a TATA box promoter and COX-1 lacks a TATA box. Lastly, the mRNA of COX-2 contains long 3' untranslated regions containing several different polyadenylation signals and multiple "AUUUA" instability sequences that act to mediate rapid degradation of the transcript. These features differentiate the gene for COX-1 into a gene consistent with rapid transcription and mRNA processing for processing a continuously transcribed stable message. The provides for a constant level of enzyme in most cell types to synthesize prostaglandins responsible for homeostatic functions. In contrast, the features of the COX-2 gene are those of an "immediate-early" gene that is not always present but is highly regulated and upregulated during inflammation or pathological processes.

### *Protein Structure*

Although there are clear differences in DNA/mRNA structure and function between COX-1 and COX-2, there is less difference between the protein structure and function of these enzymes. The core sequences of both enzymes, as well as their crystal structures, are 60% identical. Both enzymes have similar kinetics for arachidonic acid. Despite these similarities, evidence indicates that COX-1 and COX-2 function as separate enzyme systems. COX-1 is localized to the endoplasmic reticulum, whereas COX-2 is localized to the endoplasmic reticulum and the nuclear membrane. In addition, COX-1 and COX-2 use different pools of arachidonate that are mobilized in response to different cellular stimuli for prostaglandin synthesis.

### *Tissue Expression*

COX-1 is constitutively present in virtually all tissues under basal conditions. COX-2 is constitutively expressed under basal conditions in many areas of the central nervous system (CNS). Most information on localization is based on animal studies. The highest level in the central nervous system has been found in the hippocampus associated with granule and pyramidal cell layers.<sup>[23]</sup> Moderate levels have been found in pyramidal cell, piriform cortex, neocortex, and amygdala. Lower levels have also been found in caudate-putamen, thalamus, hypothalamus, striatum, and preoptic levels.<sup>[24]</sup> Increased levels have also been found in cortical neurons in response to natural N-methyl D-aspartic acid (NMDA) receptor-mediated neuronal activity.<sup>[23]</sup> Expression here may be involved in modulation of pain. This is important because it suggests that blocking COX-2 centrally may be important in control of pain.

Data relating to the kidney is not clear, and there are differences between human and animal data. Animal studies demonstrate that COX-1 is constitutively located in medullary collecting ducts and medullary interstitial cells with less expression in ascending collecting tubules. There is none in the macula densa or cortical thick ascending limbs. COX-2 is constitutively expressed in the cortex and medullary interstitial cells, particularly at the renal papillae. COX-2 is also expressed in the macula densa of the juxtaglomerular (JG) apparatus and in the epithelial cells of the cortical ascending limb. In animal models, salt restriction leads to increased COX-2 levels in the JG apparatus with increased renin and decreased salt excretion. In the medulla, the opposite occurs, with increased COX-1 expression seen with increased salt intake in accord with the physiologic action of medullary prostaglandins to promote salt excretion. Medullary COX-1 produces prostaglandins that are important for renal adaptation to states of high salt intake.<sup>[25,26]</sup> Localization of COX-1 and COX-2 is somewhat

different in humans (Table no.1). This may result in different actions on renal function depending on which species is examined. In rats, COX-2 in the macula densa increases with salt depletion and medullary COX-1 increases with salt repletion. In humans, COX-2 is seen in podocytes and arterioles. Low salt intake stimulates prostaglandin E synthesis in the JG apparatus with secretion of renin. COX-2 stimulates thromboxane and with resultant vasoconstriction could cause podocyte contraction with a possible decreased single nephron glomerular filtration rate.

### **Cyclo-oxygenase isoforms**

**Cyclo-oxygenase-1;** the constitutive isoform Cyclo-oxygenase-1 is expressed in most mammalian cells under physiological conditions. [27] However endothelial cells, platelets and kidney tubule cells are notable in that they express particularly large amounts of cyclo-oxygenase-1. Cyclo-oxygenase-1 was initially identified and purified in the 1970s, using classical biochemical techniques, from bovine [28] and sheep [29] vesicular glands and found to be a membrane bound homo-dimer of 70 kDa. The protein contained both the cyclo-oxygenase and peroxidase activities required to form, respectively, prostaglandin PGG<sub>2</sub> and PGH<sub>2</sub>. Either free or protein-bound heme was required for activity. The primary structure of cyclo-oxygenase-1 was later determined from the complementary DNA sequence of 2.7 kilobases. [30]

**Cyclo-oxygenase-2;** the inducible isoform Cyclo-oxygenase metabolites are released in high amounts locally at the site of inflammation or systemically after infection. Initially it was believed that this was due to an increase in supply of arachidonic acid. However, in 1990 it was demonstrated that the increase in prostaglandin formation following exposure of isolated cells in culture to inflammatory stimuli [31] was due to an increase in cyclo-oxygenase enzyme expression [32,33] We now know that this increased cyclo-oxygenase is not cyclo-oxygenase-1 but an inducible isoform, cyclo-oxygenase-2. The identification of cyclo-oxygenase-2 was, in many respects, a triumph of molecular biology. Early experiments demonstrated that cultured epithelial cells contained two distinct mRNA species, recognized under low stringency conditions by cDNA probes designed for the known cyclo-oxygenase (cyclo-oxygenase-1). [34] These probes hybridized to the predicted 2.8 kilobase mRNA but also to a novel 4.0 kilobase product. Importantly, these researchers also demonstrated that increases in the 4.0 kilobase product paralleled increases in enzymatic activity supporting the conclusion that this mRNA encoded for active protein. Later studies from the same group confirmed that these epithelial cells did indeed express two distinct forms of cyclo-oxygenase. [35] In separate studies, Xie and co-workers [36] showed that mitogen stimulated chicken fibroblasts expressed a 4.1 kilobase mRNA which encoded a protein with 59% homology to the well characterized cyclo-oxygenase identified in sheep seminal vesicle (cyclo-oxygenase-1). Furthermore, phorbol esters were also shown to induce mouse fibroblasts to express an inducible cyclo-oxygenase (TIS10) [37] with striking similarities to the protein identified in chicken cells. [36] Finally, the inducible cyclo-oxygenase gene was directly demonstrated to encode a protein which had cyclo-oxygenase activities. [2, 38] Differential induction of cyclo-oxygenase-1 and cyclo-oxygenase-2 transcription. The human cyclo-oxygenase-2 gene is 8.3 kilobases whereas the cyclo-oxygenase-1 gene is much larger at 22 kilobases. [39, 40] As discussed above, the gene products also differ in size. Cyclo-oxygenase-1 mRNA is approximately 2.8 kilobases whilst cyclo-oxygenase-2 mRNA is approximately 4.0 kilobases. Analysis of the 5'- Flanking untranslated regions of cyclo-oxygenase-1 and cyclo-oxygenase-2 show that the cyclo-oxygenase-1 gene exhibits the features of a housekeeping gene whereas the gene for cyclo-oxygenase-2 appears to be a primary response gene. For example, the 5'-Flanking region of the cyclo-oxygenase-2 gene has a canonic TATA box 30 base pairs upstream from the transcription start site [11] whereas the same region in the cyclo-oxygenase-1 gene has no canonic TATA box. [39] The cyclo-oxygenase-2 gene also contains a number of putative regulatory sites, including cyclic AMP response element, IL-6 response element, CCAAT/enhancer binding proteins, AP-2, nuclear factor-kB (NF-kB), Sp-1, PEA-3, GATA-1, and glucocorticoid response element. [39] Interestingly, the cyclo-oxygenase-1 gene also has some putative regulatory sites, including Sp-1, PEA-3, AP2, NF-IL6, GATA-1 and shear stress response element. [39] Characterization of the cyclo-oxygenase-2 gene

as being a primary response gene has led to a larger number of studies investigating potential regulatory factors. These include in various cell types: cytokines, e.g. interleukin-1 $\alpha$ <sup>[41]</sup>, tumour necrosis factor- $\alpha$ , inter- leukin-6, bacterial endotoxin and PMA <sup>[42]</sup>; growth factors, e.g. epidermal growth factor, platelet derived growth factor, serum <sup>[43]</sup> and chorionic gonadotrophin <sup>[44]</sup>; locally acting mediators, e.g. 5-hydroxytryptamine <sup>[45]</sup> and endothelin <sup>[46]</sup>; fatty acid related mediators, e.g. arachidonic acid <sup>[47]</sup>, thromboxane A2 <sup>[45]</sup> and platelet activating factor mechanical forces, e.g. pulsatile flow<sup>[48]</sup> and cyclic stretch <sup>[49]</sup>; and other stimuli such as circulating hormones, e.g. parathyroid hormone. <sup>[50]</sup> In vivo local increases in cyclo-oxygenase-2 expression have been associated with inflammation <sup>[51]</sup>, rheuma-toid arthritis <sup>[52]</sup>, seizures <sup>[53]</sup> and ischaemia. <sup>[54]</sup> Cyclo- oxygenase-2 expression in the spinal cord is also elevated following peripheral inflammation. <sup>[55]</sup> The intracellular pathways regulating these events appear numerous and complicated, varying between cell types and cellular stimulus. These pathways include, but are not limited to, reactive oxygen intermediates <sup>[56]</sup>, NF-kB and NF-IL6 <sup>[57]</sup>, Ras/Rac1/MEKK-/JNK kinase/JNK signal transduction leading to phosphorylation of c-Jun, ceramide <sup>[58]</sup>, mitogen-activated protein kinase <sup>[59]</sup>, AP-1 and CRE nuclear binding proteins <sup>[60]</sup>, CCAAT/enhancer-binding proteins <sup>[61]</sup> and protein kinase C/p42/p44 MAPK/p38 kinase<sup>[47]</sup>. Despite the presence of many pathways regulating the expression of cyclo-oxygenase-2 it is widely found that cyclo-oxygenase-2 is down-regulated by glucocorticosteroids <sup>[62]</sup>, and also by related agents such as 17 $\beta$ -estradiol. <sup>[63]</sup>

### **Enzyme homology**

Interestingly, although the genes for cyclo-oxygenase-1 and cyclo-oxygenase-2 are clearly different, the proteins share approximately 60% homology at the amino acid level. <sup>[36]</sup> Cyclo-oxygenase-1 and cyclo-oxygenase-2 also catalyse the formation of prostaglandin (PG) G2 followed by PGH2 from arachidonic acid, have a molecular weight of 70 kDa, and are identical in length. Studies of the tertiary structures of cyclo-oxygenase-1 and cyclo-oxygenase-2 have demonstrated that the amino acid conformation for the substrate binding sites and catalytic regions are almost identical. <sup>[19, 21]</sup> cyclo-oxygenase-2 has larger and more flexible substrate channel than in cyclo-oxygenase-1 and also the inhibitor binding site in cyclo-oxygenase-2 being 25% larger than that in cyclo-oxygenase-1. There are also differences in the amino acid sequences in the N and C terminus of these enzymes. <sup>[19, 21]</sup> Cyclo-oxygenase-2 for instance, is lacking in a 17 amino acid sequence at the N terminus but has an extra 18 amino acid sequence at the C terminus. The functionality of these differences are yet not known. Cyclo-oxygenase-1 and cyclo-oxygenase-2 are membrane bound proteins that reside, after synthesis and transport, primarily in the endoplasmic reticulum. <sup>[64]</sup> After the crystal structures of both isoforms of cyclo-oxygenase has been elucidated, it appeared likely that four amphipathic helices near the amino termini of these proteins would act as membrane binding domains a hypothesis that has been confirmed by experiments using chimeric proteins. <sup>[65]</sup>

## **Pharmacological Role of Cyclo-Oxygenase Enzyme**

### **Inflammation**

Cyclo-oxygenase products mainly PGE2 modulate the classical signs of inflammation. The two best studied inflammatory role of cyclo-oxygenase products are induction of swelling and pain. <sup>[66]</sup> One of most important inflammatory disease targets associated with cyclo-oxygenase 2 is arthritis. <sup>[52]</sup> In addition, cyclo-oxygenase products are through act in spinal cord to facilitate the transmission of pain response. However, despite a clear role for cyclo-oxygenase -1 as time for induction must elapse for cyclo-oxygenase-2.<sup>[55,57]</sup> The relative contribution of cyclo-oxygenase -1 and cyclo-oxygenase -2 in chronic pains (i.e. associated with rheumatoid ar osteoarthritis) remain to be established.

## **Cancer**

Cyclo-oxygenase 2 an inducible prostaglandin synthase, participates in inflammatory and neoplastic process, it is expressed by various tumors and contributes to carcinogenesis.<sup>[67]</sup> One of exciting observations associated with use of NSAID's is association with a reduction in the incidence of colon cancer indeed, a retrospective study revealed the startling findings that patients taking relative low doses of aspirin, a maximum effect being seen at four to six tablets per week for long period of time had substantially reduce risk of developing colon cancer.<sup>[68]</sup>

The process underlying these effects is through to be ability of prostaglandins produced by cyclo-oxygenase 2 to slow down rate of apoptosis in cancerous cells. This response is reduced by exposure to NSAID's of colon cancer cells. The effect of selective COX-2 inhibitor in colon cancer in man is currently being investigated.<sup>[69]</sup>

## **Alzheimer's disease**

In addition to colon cancer, epidemiological evidence of patients taking NSAID's there is reduced risk of developing Alzheimer's disease indeed in 1997 and also inverse correlation was drawn between onset and severity of Alzheimer's and the intake of NSAID's, usually ibuprofen. It has been reported that after kainic acid induced seizures, cyclo-oxygenase-2 is induced in neurons that are susceptible to apoptosis.<sup>[70]</sup> Nevertheless, epidemiology data shows that NSAID's reduce Alzheimer's may have nothing to do with cyclo-oxygenase -2 inhibition.

## **Cyclooxygenase-2 and Renal Renin-Angiotensin System**

In the kidney, cyclooxygenase-2 is expressed in the macula densa/cTALH and medullary interstitial cells. The macula densa is involved in regulating afferent arteriolar tone and renin release by sensing alterations in luminal chloride via changes in the rate of Na<sup>+</sup>/K<sup>+</sup>/2Cl<sup>-</sup> cotransport, and administration of non-specific cyclooxygenase inhibitors will blunt increases in renin release mediated by macula densa sensing of decreases in luminal NaCl. There is increasing evidence of COX-2 expression in the macula densa and surrounding cortical thick ascending limb cells which is regulated by angiotensin II and is a modulator of renal renin production. These interactions of COX-2 derived prostaglandins and the renin-angiotensin system may underlie physiological and pathophysiological regulation of renal function.<sup>[71]</sup>

## **Gastrulation**

Gastrulation is a fundamental process during embryogenesis that shapes body architecture properly and establishes three germ layers through coordinated cellular actions of proliferation, fate specification, and movement. Although many molecular pathways involved in the specification of cell fate and polarity during vertebrate gastrulation have been identified, little is known of the signaling that imparts cell motility. Prostaglandin E<sub>2</sub> production by microsomal PGE<sub>2</sub> synthase is essential for gastrulation movements. Furthermore, PGE<sub>2</sub> signaling regulates morphogenetic movements of convergence and extension as well as epiboly through the G-protein-coupled PGE<sub>2</sub> receptor (EP<sub>4</sub>) via phosphatidylinositol 3-kinase/Akt.<sup>[72]</sup>

## **Cyclooxygenase-2, Decidual Growth and Embryo Development**

Deficient cyclooxygenase-2 leads to largely infertility because of failure to ovulate, poor fertilization, and defective implantation and decidualization. COX2 has a role in mediating the initial uterine decidual response but is not essential to sustaining decidual growth and embryo development throughout the remainder of pregnancy. COX2 is required at the start of decidualization, because within 24–36 h, decidual growth is restored to a normal rate.<sup>[73]</sup>

### ***Inflammatory Lung Diseases***

There is involvement of cyclooxygenase- 2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases. Inducible cyclo-oxygenase and its metabolites have diverse and potent biological actions that are important for both physiological and disease states of lung. The wide variety of prostaglandin products are influenced by the level of cellular activation, the exact nature of the stimulus, and the specific cell type involved in their production. In turn, the anti and proinflammatory response of PG is mediated by a blend of specific surface and intracellular receptors that mediate diverse cellular events. COX-2 and its metabolites have diverse actions as both pro and anti-inflammatory mediators in lung injury and inflammation.<sup>[74]</sup>

### ***pH Control At The Gastric Surface***

Endogenous cyclooxygenase activity is required to maintain a relatively alkaline surface pH at the gastric luminal surface. COX isoform, COX-1 or COX-2, is responsible for regulating the protective surface pH gradient. When the gastric mucosal surface was exposed and superfused with a weakly buffered saline solution containing the pH indicator Cl-NERF, the pH directly at the gastric surface and thickness of the pH gradient COX-1 is the dominant isoform regulating the normal thickness of the protective surface pH gradient in stomach. <sup>[75]</sup>

### ***Regulation of the Brain Upstream Nf-Kb Pathway and Downstream Enzymes***

Cyclooxygenase -2-deficiency affects brain upstream and downstream enzymes in the arachidonic acid metabolic pathway to prostaglandin E<sub>2</sub>, as well as enzyme activity, protein and mRNA levels of the reciprocal isozyme, COX-1. There was a compensatory up-regulation of COX-2, accompanied by the activation of the Nuclear factor kappa light chain enhancer B cell (NF-κB) pathway, and also an increase in the upstream Cytosolic Phospholipase A<sub>2</sub> (cPLA<sub>2</sub>) and secretory Phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) enzymes. The mechanism of NF-κB activation with COX-1 involved the upregulation of protein expression of the p50 and p65 subunits of NF-κB, as well as the increase in protein levels of phosphorylated IκBα and of phosphorylated IKKα/β. This suggest that COX-1 and COX-2 play a distinct role in brain PG biosynthesis, with basal PGE<sub>2</sub> production being metabolically coupled with COX-2 and TXB<sub>2</sub> production being preferentially linked to COX-1.<sup>[76]</sup>

### ***Fracture healing***

The physiological mechanisms of fracture healing have been the topic of active investigation for many years. At the present time ample data exist on the histological, mechanical and biochemical aspects of this process. Most investigators now believe that many of the steps in fracture healing are a recapitulation of what happens in endochondral bone formation. The class of pharmacologic agents that requires investigation are COX-1 and COX-2 inhibitors. These agents are one of the most commonly used medications for minor musculoskeletal injuries and pain throughout the world. Unlike other tissues that heal through the generation of scar tissue, bone heals by regenerating new bone. This capacity may be due to similarities in the molecular programs of foetal skeletogenesis and adult fracture repair. The process of fracture healing can be subdivided into 3 phases: inflammatory, reparative, and remodeling. The first phase begins immediately following fracture and is characterized by the formation of a hematoma, migration of mesenchymal cells to the fracture site, and the release of cytokines and growth factors from leukocytes and fibroblasts. Following the initial inflammation, new bone is formed by intramembranous ossification as well as endochondral ossification; these processes are predominately mediated by osteoblasts. This phase is followed by an extended period of remodeling involving osteoblasts that resorb the new woven bone and osteoblasts that replace this matrix with lamellar bone. As with homeostatic remodeling, the important functional outcome of the remodeling phase of fracture healing is the restoration of

mechanical strength and stability Prostaglandins comprise a group of short fatty acid derivatives that are the most abundant eicosanoids in bone. Prostaglandin synthesis is controlled by three different enzymes: phospholipase A2s which release arachidonic acid from the cell membrane; the cyclooxygenases which catalyze the oxygenation and further reduction of AA to form PGH<sub>2</sub>; and isomerases which convert PGH<sub>2</sub> to individual prostaglandins. Among these, the cyclooxygenases are the most important rate-limiting enzymes in the pathway.<sup>[77]</sup>

### ***Cyclooxygenase-2 in Signaling Pathway***

The gap junction communication and the presence of soluble mediator are both known to play important roles in the bystander response; the precise signaling molecules have yet to be identified. By using the particle beam in conjunction with a strip dish design, it was reported that the cyclooxygenase-2 signaling cascade plays an essential role in the bystander process. Because the critical event of the COX-2 signaling is the activation of the mitogen-activated protein kinase pathways, that inhibition of the extracellular signal-related kinase phosphorylation suppressed bystander response further confirmed the important role of mitogen-activated protein kinase signaling cascade in the bystander process.<sup>[78]</sup>

### ***As a Cardioprotective Protein***

The function of cyclooxygenase-2 in the cardiovascular system remains largely an enigma. It has been demonstrated that ischemic preconditioning upregulates the expression and activity of COX-2 in the heart, and this increase in COX-2 activity mediates the protective effects against myocardial stunning and myocardial infarction. The possibility that inhibition of COX-2 activity may augment myocardial cell death by obliterating the innate defensive response of the heart against ischemia/reperfusion injury.<sup>[79]</sup>

### ***Cyclo-Oxygenase-2 in Reproduction***

There are several lines of evidence which suggest that cyclooxygenase-2 is important in positive feed-back mechanisms associated with reproduction. Both cyclo-oxygenase-1 and cyclo-oxygenase-2 are expressed in the uterine epithelium at different times in early pregnancy. The expression of cyclo-oxygenase-2 may be important for localized increased uterine permeability and the attachment reaction.<sup>[80]</sup>

### **Conclusion**

There are clearly identified areas where cyclo-oxygenase induction may be beneficial or harmful to the body. These are in major airways, gastro-intestinal, urogenital tract etc. Thus, we could anticipate potential role of cyclo-oxygenase in prevention and production of certain diseases.

### **References**

1. Smith, W. L., Prostanoid biosynthesis and mechanisms of action. *Am. J. Physiology* 1992, 263, F181–F191.
2. Goetzl, E. J., An, S., and Smith, W. L., Specificity of expression and effects of eicosanoid mediators in normal physiology and human diseases. *FASEB J.* 1995, 9, 1051–1058.
3. Fletcher, B. S., Kujubu, D. A., Perrin, D. M., and Herschman, H. R. Structure of the mitogen inducible TIS10 gene and demonstration that the TIS10-encoded protein is a functional prostaglandin G/H synthase. *J. Biol. Chem.* 1992, 267, 4338–4344.
4. Hla, T. and Neilson, K., Human cyclooxygenase-2 cDNA. *Proc. Natl. Acad. Sci. USA* 1992, 89, 7384–7388.
5. Kraemer, S. A., Meade, E. A., and DeWitt, D. L., Prostaglandin endoperoxide synthase gene structure: identification of the transcriptional start site and 5'flanking regulatory sequences. *Arch. Biochem. Biophys.* 1992, 293, 391–400.
6. Xie, W., Fletcher, B. S., Andersen, R. D., and Herschman, H. R. v-src induction of the TIS10/PGS2

- prostaglandin synthase gene is mediated by an ATF/CRE transcription response element. *Mol. Cell. Biol.* 1994, 14, 6531-6539.
7. Arias-Negrete, S., Keller, K., and Chadee, K. Proinflammatory cytokines regulate cyclooxygenase-2 mRNA expression in human macrophages. *Biochem. Biophys. Res. Commun.* 1995, 208, 582-589.
  8. Inoue, H., Yokoyama, C., Hara, S., Tone, Y., and Tanabe, T. Transcriptional regulation of human prostaglandin-endoperoxide synthase-2 gene by lip polysaccharide and phorbol ester in vascular endothelial cells. Involvement of both nuclear factor for interleukin-6 expression site and cAMP response element. *J. Biol. Chem.* 1995, 270, 24965-24971.
  9. Tsuji, S., Tsujii, M., Kawano, S., and Hori, M. Cyclooxygenase-2 upregulation as a perigenetic change in carcinogenesis. *J. Exp. Clin. Cancer Res.* 2001, 20, 117-129.
  10. Kosaka, T., Miyata, A., Ihara, H., Hara, S., Sugimoto, T., Takeda, O., Takahashi, E., and Tanabe, T. Characterization of the human gene (PTGS2) encoding prostaglandin- endoperoxide synthase 2. *Eur. J. Biochem.* 1994, 221, 889- 897.
  11. Tazawa, R., Xu, X. M., Wu, K. K., and Wang, L. H. Characterization of the genomic structure, chromosomal location and promoter of human prostaglandin H synthase-2 gene. *Biochem. Biophys. Res. Commun.* 1994, 203, 190-199.
  12. Chandrasekharan, N.V., Dai, H., Roos, K.L.T. et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: Cloning, structure, and expression. *Proceedings of the National Academy of Sciences of the United States of America*, 200299(21):13926-31,
  13. Hawkey, C. J., COX-2 inhibitors, *Lancet*, 1999, 353, 307-314.
  14. Vane, J. R. and Botting, R. M., Mechanism of action of anti-inflammatory drugs. *Scand. J. Rheumatol.* (Suppl. 102), 1996, 25, 9-21.
  15. Dubois, R. N., Abramson, S. B., Crofford, L., Gupta, R. A., Simon, L. S., Van de Putte, L. B. A. and Lipsky, P. E., Cyclooxygenase in biology and disease, *FASEB J.*, 1998, 12, 1063-1073.
  16. Sawdy, R., Slater, D., Fisk, N., Edmonds, D. K. and Bennett, P., Use of a cyclo-oxygenase type-2-selective non-steroidal anti-inflammatory agent to prevent preterm delivery. *Lancet*, 1997, 350, 265-266.
  17. Wallace, J. L., selective COX-2 inhibitors: is the water becoming muddy, *TIPS*, 1999, 20,4-6.
  18. Willoughby, D. A., Moore, A. R. and Colville-Nash, P. R., COX-1, COX-2, and COX-3 and the future treatment of chronic inflammatory disease. *Lancet*, 2000, 355,646-648.
  19. [Picot, D., Loll, P. J. and Garavito, R. M., The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1., *Nature*, 1994, 367, 243- 249.
  20. Luong, C. L., Miller, A., Barnett, J., Chow, J., Ramesha, C. and Browner, M.F., Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. *Nat. Struc. Biol.*, 1996, 3, 927-933.
  21. Kurumbail, R. G., Stevens, A. M., Gierse, J. K., McDonald, J., Stegeman, R. A., Pak, J. Y., Gildehaus, D., Miyashiro, J. M., Penning, T. D., Seibert, K., Isakson, P. C. and Stallings, W. C., Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*, 1996, 384, 644-648.
  22. Gierse, J. K., McDonald, J., Hauser, S., Rangwala, S. H. and Siebert, K., A single amino acid difference between COX-1 & COX -2 specific inhibitors. *J. Biol. Chem.*, 1996, 271, 15810-15814.
  23. Yagamata K, Andreasson KI, Kaufmann WE, et al. Expression of a mitogen-inducible cyclooxygenase in brain neurons: regulation by synaptic activity and glucocorticoids. *Neuron*. 1993, 11:371-386.
  24. Cao C, Matsumura K, Yamagata K, et al. Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in rat brain: its possible role in the febrile response. *Brain Res.* 1995, 697:187-196.
  25. Jensen BL, Kurtz A. Differential regulation of renal cyclooxygenase mRNA by dietary salt intake. *Kidney Int.* 1997; 52:1242-1249.
  26. Komhoff M, Grone HJ, Klein T, et al. Localization of cyclooxygenase-1 and -2 in adult and fetal human kidney: implication for renal function. *Am J Physiol.* 1997, 272:F460-468.
  27. Seibert, K., Zhang, Y., Leahy, K., Hauser, S., Masferrer, J. & Isakson, P. Distribution of cox-1 and cox-2 in normal and inflamed tissues. *Adv. Exp. med. Biol.*, 1997, 400A, 167-170.
  28. Miyamoto, T., Ogino, N., Yamamoto, S. & Hayaishim, O. Purification of prostaglandin endoperoxide synthetase From bovine vesicular gland microsomes. *J. Biol. Chem.*, 1976, 251, 2629 - 2636.
  29. Hemler, M. & Lands, W.E. Purification of the cyclo-oxygenase that forms prostaglandins. Demonstration of two forms of iron in the holoenzyme. *J. Biol. Chem.*, 1976, 251, 5575 - 5579.
  30. Dewitt, D.L. & Smith, W.L. Primary structure of prostaglandin G/H synthase from sheep vesicular gland determined from the complementary DNA sequence. *Proc. Natl. Acad. Sci. U.S.A.*, 1988,85, 1412 - 1416.
  31. Dewitt, D.L. Prostaglandin endoperoxide synthase: regulation of enzyme expression. *Biochim. Biophys. Acta.*, 1991, 1083, 121 -134.
  32. Fu, J.Y., Masferrer, J.L., Seibert, K., Raz, A. & Needleman, P. The induction and suppression of prostaglandin H2 synthase (cyclo- oxygenase) in human monocytes. *J. Biol. Chem.*, 1990, 265, 16737-16740.
  33. Masferrer, J.L., Zweifel, B.S., Seibert, K. & Needleman, P. Selective regulation of cellular cyclo-oxygenase by dexamethasone and endotoxin in mice. *J. Clin. Invest.* 1990, 86, 1375 - 1379.
  34. Rosen, G.D., Birkenmeier, T.M., Raz, A. & Holtzman, M.J.. Identification of a cyclooxygenase- related gene and its potential role in prostaglandin formation, *Biochem. Biophys. Res. Commun.*, 1989, 164,

- 1358 - 1365.
35. Holtzman, M.J., Turk, J. & Shornick, L.P. Identification of a pharmacologically distinct prostaglandin H synthase in cultured epithelial cells. *J. Biol. Chem.*, 1992, 267, 21438 -21445.
  36. Xie, W.L., Chipman, J.G., Robertson, D.L., Erikson, R.L. & Simmons, D.L. Expression of a mitogen- responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc. Natl. Acad. Sci. U.S.A.* 1991, 88, 2692- 2696.
  37. Kujubu, D.A., Fletcher, B.S., Varnum, B.C., Lim, R.W. & Herschman, H.R. TIS10, a phorbol ester tumor- promoter inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclo-oxygenase homologue. *J. Biol. Chem.* 1991, 266, 12866 - 12672.
  38. O'banion, M.K., Winn, V.D. & Young, D.A. CDAN cloning and functional activity of a glucocorticoid-regulated inflammatory cyclo-oxygenase. *Proc. Natl. Acad. Sci. U.S.A.*, 1992, 89, 4888 - 4892.
  39. Wu, K.K. Inducible cyclo-oxygenase and nitric oxide synthase. *Adv. Pharmacol.* 1995, 33, 179 - 207.
  40. Vane, J.R., Bakhle, Y.S. & Botting, R.M. Cyclo-oxygenase-1 and 2. *Ann. Rev. Pharmacol. Toxicol.*, 1998, 38, 97 -120.
  41. Ristimaki, A., Garfinkel, S., Wessendorf, J., Maciag, T. & Hla, T. Induction of cyclo-oxygenase-2 by interleukin-1 $\alpha$ . Evidence for post- transcriptional regulation. *J. Biol. Chem.* 1994, 269, 11769 -11775.
  42. Geng, Y., Blanco, F.J., Cornelisson, M. & Lotz, M. Regulation of cyclo- oxygenase-2 expression in normal articular chondrocytes. *J. Immunol.* 1995, 155, 796 -801.
  43. Xie, W. & Herschman, H.R. Transcriptional regulation of prostaglandin synthase 2 gene expression by platelet-derived growth factor and serum. *J. Biol. Chem.* 1996, 271, 31742 -31748.
  44. Han, S.W., Lei, Z.M. & Rao, C.V. Up-regulation of cyclo- oxygenase-2 gene expression by chorionic gonadotrophin in mucosal cells from human fallopian tubes. *Endocrinology*, 1996, 137, 2929 - 2937.
  45. Stroebel, M. & Goppelt-Struebe, M. Signal transduction pathways responsible for serotonin-mediated prostaglandin- in G/H synthase expression in rat mesangial cells. *J. Biol. Chem.* 1994, 269, 22952-22957.
  46. Hughes, A.K., Padilla, E., Kutchera, W.A., Michael, J.R. & Kohan, D.E., Endothelin-1 induction of cyclo-oxygenase- 2 in rat mesangial cells. *Kidney Int.* 1995, 47, 53- 61.
  47. Barry, O.P., Kazanietz, M.G., Practico, D. & Fitzgerald, G.A. Arachidonic acid in platelet microparticles up- regulates cyclo-oxygenase-2 dependent prostaglandin formation via a protein kinase C/mitogen-activated protein kinase-dependent pathway. *J. Biol. Chem.* 1999, 274, 7545 - 7556.
  48. Klein-Nulend, J., Burger, E.H., Semeins, C.M., Raisz, L.G. & Pilbeam, C.C. Pulsating fluid flow stimulates prosta- glandin release and inducible prostaglandin G/H synthase mRNA expression in primary mouse bone cells. *J. Bone Miner. Res.* 1997, 12, 45-51.
  49. Kato, T., Ishiguro, N., Iwata, H., Kojima, T., Ito, T. & Naruse, K., Up- regulation of COX2 expression by uni-axial cyclic stretch in human lung fibroblast cells. *Biochem. Biophys. Res. Commun.* 1998, 244, 615 - 619.
  50. Tetradis, S., Pilbeam, C.C., Liu, Y. & Kream, B.E.. Parathyroid hormone induces prostaglandin G/H synthase-2 expression by a cyclic adenosine 3',5'-monophosphate-mediated pathway in the murine osteoblastic cell line MC3T3-E1. *Endocrinology.* 1996, 137, 5435- 5440.
  51. Vane, J.R., Mitchell, J.A., Appleton, I., Tomlinson, A., Bishop-Bailey, D., Croxtall, J. & Willoughby, D.A. Inducible isoforms of cyclo-oxygenase and nitric oxide synthase in inflammation. *Proc. Natl. Acad. Sci. U.S.A.* 1994, 91, 2046 - 2050.
  52. Kang, R.Y., Freire-Moar, J., Sigal, E. & Chu, C.Q. Expression of cyclo- oxygenase-2 in human and an animal model of rheumatoid arthritis. *Br. J. Rheumatol.* 1996, 35, 711 -718.
  53. Marcheselli, V.L. & Bazan, N.G. Sustained induction of prostaglandin endoperoxide synthase-2 by seizures in hippocampus. Inhibition by platelet-activating factor antagonist. *J. Biol. Chem.* 1996, 271, 24794 - 24799.
  54. Planas, A.M., Soriano, M.A., Rodriguez-Farre, E. & Ferer, I. Induction of cyclo- oxygenase-2 mRNA and protein following transient focal ischaemia in the rat brain. *Neurosci. Lett.* 1995, 200, 187-190.
  55. Beiche, F., Scheurer, S., Brune, K., Geisslinger, G. & Goppelt-Struebe, M. Up- regulation of cyclo-oxygenase-2 mRNA in the rat spinal cord following peripheral inflammation. *FEBS Lett.* 1996, 390, 165 -69.
  56. Feng, L., Xia, Y., Garcia, G.E., Hwang, D. & Wilson, C.B. Involvement of reactive oxygen intermediates in cyclo-oxygenase-2 expression induced interleukin-1, tumor necrosis factor- $\alpha$ , and lipopolysaccharide. *J. Clin. Invest.* 1995, 95, 1669 - 1675.
  57. Yamamoto, K., Arakawa, T., Ueda, N. & Yamamoto, S. Transcriptional roles of nuclear factor- $\kappa$ B and nuclear factor- interleukin-6 in the tumour necrosis factor- $\alpha$ - independent induction of cyclo-oxygenase-2 in MC3T3-E1 cells. *J. Biol. Chem.* 1995, 270, 31315 -31320.
  58. Hayakawa, M., Jayadev, S., Tsujimoto, M., Hannun, Y.A. & Ito, F., Role of ceramide in stimulation of the transcription of cytosolic phospholipase A2 and cyclo- oxygenase 2. *Biochem. Biophys. Res. Commun.* 1996, 220, 681 - 686.
  59. Hwang, D., Jang, B.C., Yu, G. & Boudreau, M. Expression of mitogen- inducible cyclo-oxygenase induced by lipopolysaccharide: mediation through both mitogen- activated protein kinase and NK- $\kappa$ B signaling pathways in macrophages. *Biochem. Pharmacol.* 1997, 54, 87 - 96.
  60. Miller, C., Zhang, M., He, Y., Zhao, J., Pelletier, J.P., Martel-Pelletier, J. & Di Battista, J.A. Transcriptional induction of cyclo-oxygenase-2 gene by okadaic acid inhibition of phosphatase activity

- in human chondrocytes: co-stimulation of AP-1 and CRE nuclear binding proteins. *J. Cell. Biochem.* 1998, 69, 392 - 413.
61. Kim, Y. & Fischer, S.M., Transcriptional regulation of cyclo-oxygenase-2 in mouse skin carcinoma cells. Regulatory role of CCAAT/enhancer-binding proteins in the differential expression of cyclo-oxygenase-2 in normal and neoplastic tissues. *J. Biol. Chem.* 1998, 273, 27686 -27694.
  62. Masferrer, J.L., Reddy, S.T., Zweifel, B.S., Seibert, K., Needleman, P., Gilbert, R.S. & Herschman, H.R., In vivo glucorticoids regulate cyclo-oxygenase-2 but not cyclo-oxygenase-1 in peritoneal macrophages. *J. Pharmacol. Exp. Ther.* 1994, 270, 1340 -1344.
  63. Morisset, S., Patry, C., Lora, M. & De Brum-Fernandes, A.J. Regulation of cyclo-oxygenase-2 expression in culture by interleukin-1 $\alpha$ , tumor necrosis factor- $\alpha$ , glucocorticoids, and 17 $\beta$ - estradiol. *J. Rheumatol.* 1998, 25, 1146 -1153.
  64. Spencer, A.G., Woods, J.W., Arakawa, T., Singer, I.I. & Smith, W.L. Subcellular localization of prostaglandin endoperoxide synthases-1 and -2 by immunoelectron microscopy. *J. Biol. Chem.* 1998, 273, 9886 - 9893.
  65. Li, Y., Smith, T., Grabski, S. & Dewitt, D.L., The membrane association sequences of the prostaglandin endoperoxide synthases-1 and -2 isozymes. *J. Biol. Chem.* 1998, 273, 29830 -29837.
  66. Williams, T.J. & Peck, M.J., Role of prostaglandin-mediated vasodilatation in inflammation. *Nature.* 1977, 270, 530- 532.
  67. K-B Tan, T C Putti, Cyclooxygenase 2 expression in nasopharyngeal carcinoma: immunohistochemical findings and potential implications, *Journal of Clinical Pathology* 2005;58:535-538.
  68. Giovannucci, E., Egan, K.M., Hunter, D.J., Stampfer, M.J., Colditz, G.A., Willet, W.C. & Speizer, F.E., Aspirin and the risk of colorectal cancer in women. *N. Engl. J. Med.* 1995, 333, 609- 614.
  69. Dubois, R.N., Abramson, S.B., Crofford, L., Gupta, R.A., Simon, L.S., Van De Putte, L.B.A. & Lipsky, P.E., Cyclo-oxygenase in biology and disease. *FASEB J.* 1998, 12, 1063-1073.
  70. Tocco, G., Freire-Moar, J., Schreiber, S., Sakhi, S., Aisen, P. & Pasinetti, G. Maturational regulation and regional induction of cyclo-oxygenase-2 in rat brain: implications for Alzheimer's disease. *Exp. Neurol.* 1997, 144, 339 - 349.
  71. R. C. Harris, M.-Z. Zhang and H.-F. Cheng, "Cyclooxygenase-2 and the renal renin-angiotensin system" *Acta Physiologica Scandinavica* 2004, Volume 181 Issue 4, Pages 543 - 547
  72. Yong I. Cha, Seok-Hyung Kim, Diane Sepich, et al. G-protein-coupled EP4 receptor during vertebrate gastrulation Cyclooxygenase-1-derived PGE2 promotes cell motility via the, *Genes & Dev.* 2006, 20: 77-86.
  73. Cheng Jr-Gang and Stewart C. L., Loss Of Cyclooxygenase-2 Retards Decidual Growth But Does Not Inhibit Embryo Implantation Or Development, *BIOLOGY OF REPRODUCTION* 2003, 68, 401-404.
  74. Gye Young Park and John W. Christman, Involvement of cyclooxygenase-2 and prostaglandins in the molecular pathogenesis of inflammatory lung diseases. *Am J Physiol Lung Cell Mol Physiol* 2006, 290: L797-L805.
  75. H K Baumgartner, O T Starodub, J S Joehl, L Tackett, M H Montrose Cyclooxygenase 1 is required for pH control at the mouse gastric surface. *Gut.* 2004; 53(12):1751-7.
  76. Sang-Ho Choi, Robert Langenbach, and Francesca Bosetti, "Cyclooxygenase-1 and -2 enzymes differentially regulate the brain upstream NF- $\kappa$ B pathway and downstream enzymes involved in prostaglandin biosynthesis" *J Neurochem.* 2006; 98(3): 801-811.
  77. J.E. Puzas, R.J. O'Keefe, and E.M. Schwarz, X. Zhang, Pharmacologic modulators of fracture healing: The role of cyclooxygenase inhibition, *J Musculoskel Neuron Interact* 2003; 3(4):308-312.
  78. Hongning Zhou, Vladimir N. Ivanov, Joseph Gillespie, Charles R. Geard, Sally A. Amundson, David J. Brenner, Zengliang Yu, Howard B. Lieberman and Tom K. Hei Mechanism of radiation-induced bystander effect: Role of the cyclooxygenase-2 signaling pathway *PNAS*, 2005, 102 (41): 14641-14646.
  79. R Bolli, K Shinmura, XL Tang, E Kodani, YT Xuan, Y Guo, B Dawn Discovery of a new function of cyclooxygenase (COX)-2: COX-2 is a cardioprotective protein, *Cardiovasc Res* 2002, 55: 506-19.
  80. Chakraborty, I., Das, S.K., Wang, J. & Dey, S.K. Developmental expression of the cyclo-oxygenase-1 and cyclooxygenase-2 genes in the peri-implantation mouse uterus and their differential regulation by the blastocyst and ovarian steroids. *J. Mol. Endocrinol.* 1996, 6, 107-122.