REVIEW ARTICLE

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The Multifaceted Profile of Cox-2 Enzyme and Emerging Therapeutic Uses of Cox-2 Inhibitors

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The enzyme, cyclooxygenase is now well recognized to exist in two isoforms i.e. cyclooxygenase-1 and cyclooxygenase-2. Cyclooxygenase-1 is constitutively expressed serving the so-called 'house-keeping' role while, cyclooxygenase-2 was thought to be expressed in pathophysiological conditions such as inflammation. It is now known that cyclooxygenase-2 plays a key role in a wide range of physiological processes. In the current article the multifaceted profile of cyclooxygenase-2 enzyme in the body is described indicating its potential clinical and diagnostic applications. It is also discussed how the currently available selective cyclooxygenase-2 inhibitors can affect the normal physiological role of cyclooxygenase-2 enzyme.

Prostaglandins (PGs) have been shown to mediate the inflammatory response locally and modulate a variety of physiological responses systematically[1-3]. The conversion of arachidonic acid to prostaglandins is mediated by the two-step action of prostaglandin H synthase (PGHS), commonly known as cyclooxygenase (COX). The first committed step in this process is the oxidative cyclisation of arachidonic acid to PGG_2, which is followed by peroxide reduction to PGH_2 at the second distinct binding site[4]. These moieties are then converted to PGD_2, PGE_3, PGF_2 and PGI_2 or thromboxanes (TBX). The specific prostaglandin produced is determined in part, by the particular cell type under consideration. In the past it was thought that COX was a single enzyme present constitutively in most cells. This led to the widely held notion that inhibition of cyclooxygenase would unavoidably lead to both beneficial and detrimental effects[5,6]. However, recently it was observed that COX activity dramatically increased in inflammatory states and that cellular COX activity could be induced by inflammatory cytokines and endotoxins. This suggested that a second form of COX existed, an inducible form (COX-2), which is expressed during inflammatory conditions. The constitutive form (COX-1), produces physiologically important PG's and is present in tissues such as the gastrointestinal tract and kidney[7-9]. Despite recognition of cyclooxygenase's mechanism of action 30 years ago[6], the enzyme, COX-1 was first cloned[10] only in 1988. The second isoform, COX-2 was reported[9] three years later. Both the isoenzymes have about the same affinity (K_m) and capacity (V_{max}) to convert arachidonic acid to PGH_2[11]. COX-2 is able to metabolize C18 and C20:3 fatty acids, additionally. Protective PGs which preserve the integrity of the stomach lining and maintain normal renal function in a compromised kidney are synthesized by COX-1[12,13]. It is also constitutively expressed in cultured endothelial and vascular smooth-muscle cells. Although, COX-1 is constitutively expressed playing a house-keeping role, in many tissues under basal conditions, its expression may also be regulated[14,15]. Conversely, COX-2 was thought to be an inducible enzyme, generally not present (or minimally so) in most tissues[16]. Rather, its expression was considered to be associated with an inflammatory response and other pathophysiological states. More recent investigations, however, reveal that COX-2 plays a key role in a wide range of physiological processes[16-20] including organogenesis, brain and nerve function[21], reproduction[22], bone metabolism, salt and water handling, renin release[23], angiogenesis and apoptosis[24]. It is present constitutively in the brain and the spinal cord,

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where it may be involved in nerve transmission, particularly that for pain and fever[21]. PG's made by COX-2 are also important in ovulation and in the birth process[25-28]. Thus the dichotomy of a 'constitutive' COX-1, with solely 'house-keeping' functions under physiological conditions, and an 'inducible' COX-2, accounting for prostanoid production in disease states, is an over simplification of biological reality.

During the past few years, selective inhibitors of COX-2 enzyme have emerged as important pharmacological tools for the treatment of pain and inflammation. But the recent findings of the involvement of COX-2 enzyme in some key physiological functions, as described above, have complicated the scene regarding the use of selective/specific COX-2 inhibitors as antiinflammatory agents. At the same time, these findings have opened new doors for the treatment of certain other ailments as well. Further, higher concentrations of COX-2 have been implicated in several other disease states other than in inflammation, such as colonic polyposis[29], various forms of cancer[30,31], Alzheimer's disease[32] and vascular restenosis following angioplasty[33]. Prevalence of higher concentrations of COX-2 enzyme than the normal in these disease states has opened avenues for the possible therapeutic mitigation of these diseases. Involvement of COX-2 expression in these diseases and the role COX-2 inhibitors could play as potential therapeutic agents in future, have been reviewed in this article.

Role of COX-2 In Alzheimer's disease:

Alzheimer's disease (AD) is a progressive neurodestructive process of the human neocortex, characterized by the deterioration of memory and higher cognitive function[34-36]. A progressive and irreversible brain disorder, AD is characterized by three major pathogenic episodes[37] involving (i) an aberrant processing and deposition of β-amyloid precursor protein (β-APP) to form neurotoxic β-amyloid (β-A) peptides and an aggregated insoluble polymer of β-A that forms the senile plaque, (ii) the establishment of intraneuronal neuritic face pathology yielding widespread deposits of agyrophilic neurofibrillary tangles (NFT) and (iii) the initiation and proliferation of a main specific inflammatory response. These three seemingly diverse attributes of AD etiopathogenesis are linked by the fact that proinflammatory microglia, reactive astrocytes and their associated cytokines are associated with the biology of the microtubule-associated protein tau, β-A speciation and aggregation. Specific β-A fragments such as β-A42 can further potentiate the proinflammatory mechanism. Expression of the inducible COX-2 and cytosolic phospholipase A_2 ($CPLA_2$) are strongly activated during cerebral ischemia and trauma, epilepsy and AD, indicating the induction of proinflammatory gene pathways as a response to brain injury[38, 39].

Studies have identified a reduced risk for AD in patients previously treated with NSAIDS for non-CNS afflictions that include arthritis[40]. In a memory test on rats using specific COX-1 and COX-2 inhibitors it was established that COX-2 is a required biochemical component indicating the consolidation of hippocampal-dependent memory[41]. In a study involving 15 subjects examining the onset or progression of AD, NSAIDS treatment prevented or ameliorated the symptoms of AD in fourteen of these subjects[42]. Circumstantial evidence implicated inflammation in the pathogenesis of AD[42]. In a study on mice carrying mutated genes (PS/APP, responsible for causing AD) it was inferred[43] that COX-2 and complement component 1q levels increased in response to the formation of fibrillar β-A in PS/APP mice.

COX 2 in glaucoma:

In patients suffering from primary open-angle glaucoma (POAG) it was observed that there was either a complete loss of COX-2 expression or only a few remaining scattered COX-2 expressing cells[44]. Eyes of patients with either congenital juvenile or angle-closure glaucoma showed COX-2 expression indistinguishable from control eyes. Aqueous humor of eyes with POAG contained significantly less PGE_2 than control eyes. In normal eyes, ocular COX-1 and COX-2 expression were largely confined to the non-pigmented secretory epithelium of the ciliary body. COX-2 expression was completely lost in the nonpigmented secretory epithelium of the ciliary body of the eyes with end-stage POAG, whereas COX-1 expression was unchanged[45,46].

In virus replication:

PGE_2, the product of COX-2 activity, increased transiently by a factor of more than 50 in cultures of human fibroblasts infected with human cytomegalovirus[47]. Both specific and non-specific COX-2 inhibitors could abrogate the virus-mediated induction of PGE_2 accumulation. Levels of COX-2 inhibitors that completely blocked the induction of COX-2 activity, but did not compromise cell viability, reduced the yield of human cytomegalovirus in human fibroblasts by a factor of >100 and the yield could be substantially restored by the addition of PGE_2 together with the inhibitory drugs. This finding[48] argued that elevated levels of PGE_2 were required for efficient replication of human cytomegalovirus in fibroblasts. Another finding[49] suggests that combined expres-

sion of inducible nitric oxide synthase (iNOS) and COX-2 may play an important role in prognosis of hepatitis C virus-positive HCC (hepatocellular carcinoma) patients.

COX-2 IN CANCER

Several lines of evidence suggest that COX-2 enzyme plays an important role in carcinogenesis. Increased amounts of COX-2 are commonly found in both premalignant tissues and malignant cancers of the head and neck, oesophagus and lung[50-52]. COX-2 expression is induced in a variety of cells leading to high levels of prostaglandins production. Such aberrant expressions of COX-2 have been reported in murine and human breast cancer, human colon cancer, lung cancer, head and neck cancer and pancreatic cancer[30, 53-57]. There is extensive evidence, beyond the findings that COX-2 is commonly overexpressed in tumors, to suggest that COX-2 is mechanistically linked to the development of cancer. COX-2 can affect multiple mechanisms that are important in carcinogenesis. It may aid in carcinogenesis by altering the normal cellular processes like angiogenesis, cell proliferation, immunomodulation, carcinogenic metabolism and apoptosis.

Angiogenesis is essential for the proper nourishment and oxygen supply to highly dividing and proliferating cells. Normal blood vessel endothelium expresses COX-1 isoenzyme whereas angiogenic blood vessel endothelium expresses COX-2 isoenzyme[58]. COX-2 and thromboxane A2 receptor dependent signaling pathways have been shown to activate cellular invasion and angiogenesis[59]. Recently, levels of COX-2 were found to correlate with both VEGF expression and tumor vascularisation in head and neck cancers[60]. This finding in human tissues is consistent with prior evidence that overexpression of COX-2 in epithelial tissues leads to enhanced production of vascular growth factors and the formation of capillary-like networks[61]. Evidence has been provided that COX-2 derived prostaglandins contribute to tumor growth by inducing newly formed blood vessels (neoangiogenesis) that sustain tumor cell viability and growth[62]. Prostaglandins appear to increase cell proliferation with the help of biological modifiers like polyamines. Increased polyamine levels are associated with increased DNA synthesis which results from ornithine decarboxylase activity[63]. Overexpression of COX-2 in colorectal tumor cell lines like caco-2 results in increased metastatic potential[30]. The growth of tumors typically is associated with immune suppression[64]. Colony stimulating factors released by tumor cells activate monocytes and macrophages to synthesize PGE_2 which inhibits the production of immune lymphokines, T and B cell proliferation and the cytotoxic activity of natural killer cells. PGE_2 also inhibits the production of tumor-necrosis factor while inducing the production of IL-10, which has immunosuppressive effects[65]. High concentrations of certain prostaglandins like PGE_2 attenuate host's immune response preventing the killing of malignant cells[63,66]. PGE_2 shows a potent immunosuppressive effect by acting as a negative feed-back inhibitor, thereby inhibiting T cell activity, lymphocytic mitogenesis, macrophage activity, antibody production, production of cytokines by immune cells and natural killer cell activity[63,66]. COX-2 inhibitors, when given immediately after tumor transplant in animal models decrease PGE_2 levels and tumor growth[63,66]. The peroxidase component of COX isoenzymes has broad specificity and can oxidize a variety of xenobiotics including certain procarcinogens and carcinogens. Classes of compounds like aflatoxins, halogenated pesticides, polycyclic hydrocarbons, heterocyclic amines, etc. are acted upon by the peroxide component of COX to form mutagenic carcinogens[63,67,68]. The byproducts of the oxidation of arachidonic acid like malondialdehyde, are highly reactive and form adducts with DNA that may initiate cancer[63,67]. COX inhibitors along with antioxidants may play a role to protect the cells and DNA from the damage[69]. Overproduction of prostaglandins like PGE_2 by COX-2 may also send improper signals in the cells thereby stimulating cell growth inappropriately or reducing apoptosis[70]. Bcl-2 gene is an important gene in regulating apoptosis. Murine intestinal cell lines like RIE-S showed that overexpression of COX-2 in cancerous cells was associated with the overexpression of Bcl-2 gene, which prolongs cell's life by inhibiting apoptosis[67]. The exact relation between COX-2 overexpression, production of prostaglandins and expression of Bcl-2 gene in cancer is yet to be investigated. Inhibition of COX isoenzymes results in decreased production of PGs from their substrates like arachidonic acid, leading to the accumulation of the substrate. Arachidonic acid when present in increased concentration in the cells is supposed to stimulate the fragmentation of DNA and conversion of sphingomyelin to ceramide in the cells, which is a known inducer of apoptosis[71].

Selective COX-2 inhibitors have the desired property of interfering with tumorigenesis in experimental systems[72,73]. Inhibition of COX-2 by celecoxib delays tumor growth and metastasis in xenograft tumor models as well as suppresses basic fibroblast growth factor 2 (FGF-2)-induced neovascularisation of the rodent cornea[62, 74].

Cancer of GIT:

Both COX-1 and COX-2 have been detected in appar-

ently normal GI epithelium, raising the possibility that both may contribute to the generation of cytoprotective prostaglandins[75]. Overexpression of COX-2 has been observed in the colon tumors therefore, specific inhibitors of COX-2 activity could potentially act as chemoprotective agents[76]. Epidemiological studies[77-79] have suggested a decreased incidence of cancer of the oesophagus, stomach, colon and rectum in people who use NSAIDs regularly, although a delay of about a decade is seen to realise the outcome. The cancers do recur and regrow when the treatment is curtailed[80]. Two NSAIDs, sulindac and celecoxib, have been found to inhibit the growth of adenomatous polyposis and cause regression of existing polyposis in randomised trials of patients with familial adenomatous polyposis[81] (FAP). Two separate studies[82,83] have indicated protection offered by celecoxib against colorectal carcinoma.

Pancreatic carcinomas:

COX-2 mRNA and protein expression were found to be frequently elevated in human pancreatic adenocarcinomas and cell lines derived from such tumors. Immunohistochemistry demonstrated COX-2 expression in 67 % of pancreatic carcinomas. The level of COX-2 mRNA was found to be elevated in carcinomas relative to histologically normal pancreas from a healthy individual, as assessed by reverse transcription-PCR. Sulindac and NS398, produced a dose-dependent inhibition of cell proliferation in all pancreatic cell lines tested[84]. The findings indicated that COX-2 up-regulation is a frequent event in pancreatic cancers and suggested that NSAIDS may be useful in the chemoprevention and therapy of pancreatic carcinoma [85].

Cancer of gall bladder:

Information suggests that the COX metabolites, the prostanoids, play a role in gall bladder physiology and disease. In a study on human gall bladder cancer cell line it was specifically the COX-2 inhibitor and not the COX-1 inhibitor that decreased mitogenesis and increased gall bladder cell apoptosis[86]. The inhibition of replication of gall bladder cancer cells and the increase in apoptosis produced by the selective COX-2 inhibitor suggests that COX enzymes and the prostanoids may play a role in the development of gall bladder cancer and that the COX-2 inhibitors may have a therapeutic role in the prevention of gall bladder neoplasms[87].

Cholangiocarcinoma:

Cholangiocarcinoma is common in South East Asia in regions endemic for the liver fluke *Opisthorchis viverinii*[88]. There is evidence for a significant relationship between liver fluke infection and cholangiocarcinoma. The tumor promoting effect may be due to permanent irritation of the liver by the parasite. In normal liver, constitutive expression of COX-2 protein was a characteristic feature of hepatocytes whereas no COX-2 immunosignal was detectable in bile duct epithelium, Kupffer and endothelial cells. In cholangiocarcinoma cells, COX-2 was strongly expressed at high frequency. It was concluded in the study[89] that aberrant COX-2 expression seemed to be related to later stages of cholangiocarcinogenesis.

Malignant pheochromocytoma:

COX-2 dependent prostaglandin formation is necessary for normal renal development. COX-2 has been localized to the renal vasculature, the cortical macula densa, and the medullary interstitial cells of the kidney, and its content in these areas increases with age. By contrast, COX-1 is found in the vasculature, the collecting ducts, and the loops of the Henle[90]. There are no histological or chemical markers available that define the malignant behaviour of pheochromocytomas, the tumors of adrenal medulla. So far, only the discovery of metastases reveals malignancy. In a study[91] on patients with malignant and benign pheochromocytomas and normal persons it was found that normal adrenal medulla does not express COX-2 immunohistochemically; however, strong COX-2 protein expression was found in malignant pheochromocytomas, whereas most benign tumors expressed COX-2 only weakly. These findings suggest that negative or weak COX-2 expression in pheochromocytomas favours benign diagnosis.

Malignant thyroid nodules:

Factors contributing to the development of thyroid neoplasia remain poorly understood. A study[92] was undertaken to evaluate whether COX-2 was up-regulated in human thyroid neoplasia. Levels of COX-2 mRNA were significantly increased in thyroid nodule samples compared with adjacent thyroid tissue in the malignant specimens but not in the benign specimens. COX-2 protein expression was higher in 8 of 10 thyroid nodules compared with the adjacent tissue. The data indicated that COX-2 was upregulated in human thyroid cancer, but not in benign thyroid nodules and suggested that COX-2 expression might serve as a marker of malignancy in thyroid nodules.

Lung cancer:

Recent studies have demonstrated that increased expression of COX-2 was observed frequently in human non-

small cell lung cancer (NSCLC), and elevated biosynthesis of PG's was found in NSCLC cell lines[93]. Additionally, recent work[94] also indicated that aspirin might inhibit proliferation of NSCLC cell lines and might reduce the number of lung adenoma induced by the tobacco-specific nitrosamine. NS398, a specific COX-2 inhibitor inhibited PGE_2 synthesis and induced G_1 growth arrest in A549 lung cancer cells. NS398 specifically upregulated cyclin-dependent kinase inhibitor $p27^{K181}$ in a post-translational regulation, whereas the expression of G_1-acting cyclins and cyclin-dependent kinases were not changed. Additionally NS398 effectively suppressed cyclin E-associated kinase activity in A 549 cells[95].

Breast cancer:

Interest in chemoprevention in oncology using suppressants of prostaglandin synthase has been stimulated by epidemiological observation that the use of aspirin and other NSAIDS, has been associated with reduced incidence of some cancers, including cancer of the breast[96]. COX-2 can undergo rapid induction in response to many factors such as bacterial lipopolysaccharides, growth factors, cytokines and phorbol esters. Potential role of COX-2 inhibitors in chemoprevention of mammary carcinogenesis in rats has already been investigated[97]. Using a highly metastatic mammary tumor cell line that expresses both COX isoforms, it was shown[98] that oral administration of either a selective COX-2 inhibitor celecoxib or a selective COX-1 inhibitor SC560 to mice with established tumors results in significant inhibition of tumor growth. Administration of the dual inhibitor indomethacin leads to even better growth control. Pretreatment of tumor cells with COX inhibitors also reduces metastatic success, indicating that tumor cells may be a direct target of action by COX inhibitors. Growth of a second cell line, which does not express COX-2 *in vivo*, is also reduced by celecoxib, implicating both COX-dependent and COX-independent mechanisms. Results of a study[99] indicated that elevated COX-2 expression is more common in breast cancers with poor prognostic characteristics and is associated with an unfavourable outcome. The present findings support efforts to initiate clinical trials on the efficacy of COX-2 inhibitors in adjuvant treatment of breast cancers. Results of another study[100] suggested that NSAIDS and selective COX-2 inhibitors might be useful in the chemoprevention and therapy of human breast cancer.

IMPLICATIONS IN CARDIOVASCULAR DISEASE

COX-1 is constitutively expressed in cultured endothelial and vascular smooth-muscle cells[101]. COX-2 has an important role in the increase in prostacyclin formation that occurs in clinical syndromes of platelet activation. Expression of both COX-2 and COX-1 is upregulated in the foam cells and smooth-muscle cells of atherosclerotic plaques[102]. COX-2 may well be important under physiologic conditions also. For example, COX-2 inhibitors decrease urinary excretion of prostacyclin metabolites in normal subjects[103, 104] indicating that the production of prostacyclin is also decreased[105]. Among the important roles that COX-2 plays in terms of its induction in disease states is the production of prostacyclin associated with atherosclerotic platelet activation[106]. Prostacyclin and COX-2 may thus represent part of a homeostatic defense mechanism that limits the consequences of platelet activation *in vivo*. Deletion of the prostacyclin receptor in mice leads to increased sensitivity to thrombotic stimuli, although it does not result in spontaneous thrombosis[107]. COX-2 may be relevant to other aspects of cardiovascular biology as well. Suppression of COX-2 dependent prostacyclin formation, for example, increases the susceptibility of ventricular myocytes to oxidant injury *in vitro*[108]. Thrombosis has occurred acutely on celecoxib administration to four patients with lupus anticoagulant[109]. Thromboxane (TBX), a COX-1 mediated prostaglandin produced primarily by platelets, is not inhibited by selective COX-2 inhibitors. Given TBX's procoagulant and vasoconstricting effects, long term therapy with COX-2 inhibitors may create a state of chronic 'unopposed' TBX activity with potential deleterious cardiovascular outcomes. As noted recently, selective COX-2 inhibition may offer gastroprotection but not cardioprotection[110].

CONCLUSIONS

COX has been discovered to exist in two isoforms COX-1 and COX-2. Only a part of the arachidonic acid pathway remains common to both these isoenzymes. COX-1 was considered to be expressed constitutively by certain tissues to serve house keeping role while COX-2 was thought to be expressed in certain pathophysiological states in response to certain stimuli. Recent investigations, however, revealed that COX-2 enzyme plays a key role in a wide range of physiological processes. Abnormal concentrations of COX-2 enzyme have been implicated in several disease states such as Alzheimer's disease, primary open-angle glaucoma and in some forms of cancers apart from various inflammatory diseases. COX-2 has important implications in cardiovascular system also. COX-2 levels were also found higher in the brain of those subjects suffering from Alzheimer's disease. COX-2 expression was found essential in cardiovascular system for the formation of prostacyclin which prevents oxidation injury to ventricular myocytes. Giving specific COX-2

medication to a patient may lead to accumulation of TBX which is procoagulant and vasoconstricting in nature. Since progression of Alzheimer's disease and deteriorating cardiovascular profile are characteristic of growing age, medication with COX-2 inhibitors may prove counter productive. COX-2 has been found to be overexpressed in different cancers like cancer of GIT, pancreas, gall bladder, thyroid, lung and breast. COX-2 is mechanistically linked to cancer by altering the normal cellular processes like angiogenesis, cell proliferation, immunomodulation, carcinogenic metabolism and apoptosis. So, in future the most potential use of specific COX-2 inhibitors could be in limiting the progression of various forms of carcinomas.

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