Cyclooxygenases in Cancer: Chemoprevention and Sensitization to Conventional Therapies

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Abstract: We have focused on cyclooxygenase, the key enzyme in prostaglandin synthesis, from our basic knowledge regarding the enzyme, to its clinical application in the field of oncology. We will present evidence that this enzyme is intimately associated with carcinogenesis, invasion, metastasis, and the response of tumors to current therapeutic modalities in a variety of human malignancies. We will also discuss the applications of cyclooxygenase inhibitors to chemoprevention and to the sensitization of tumors to conventional anti-cancer therapies.

INTRODUCTION

According to global statistics, there are 10.9 million new cancer cases and 6.7 million cancer-caused deaths worldwide in 2002 [1]. This suggest that novel approaches to cancer prevention and new techniques for improving the efficacy of conventional treatment modalities continue to be of great importance in the field of oncology.

Chemoprevention involves the use of natural or synthetic compounds to reverse, suppress, or prevent the process of carcinogenesis. In this regard, cyclooxygenase (COX) is considered to be one of the most promising target, recent studies have reported that the enzyme is closely associated with carcinogenesis in a variety of human malignancies. In addition, the relationship of COX with tumor response to current therapeutic modalities implies that COX inhibitors might be employed as sensitizing agents in the treatment of cancer. Here, we focus on a COX inhibition strategy, designed to prevent or delay the development of human malignancies, and to sensitize existing tumors to the currently utilized anti-cancer therapies.

PROSTAGLANDINS AND CYCLOOXYGENASE

Prostaglandins (PGs), potent bioactive lipid messengers which are derived from arachidonic acid (AA), were initially extracted from semen, prostate, and seminal vesicles by Goldblatt and von Euler in the 1930s [2]. They have shown to lower blood pressure and cause the contraction of smooth muscles. The arachidonic acid is enzymatically cyclized and oxygenated *via* the COX reaction, resulting in the formation of endoperoxide containing prostaglandin G_2 (PGG_2) which was first characterized by Hamberg and Samuelsson [3]. Cyclooxygenase has also been shown to reduce a hydroperoxyl in PGG_2 to a hydroxyl, resulting in the formation of PGH_2 *via* a separate peroxidase active site on the enzyme. Using PGH_2 as a substrate, a variety of prostaglandin isomers are generated as is shown in Fig. (**1**).

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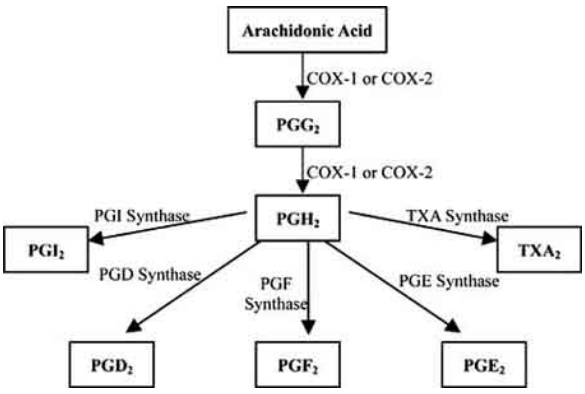

Fig. (1). Arachidonic acid (AA) cascade. AA is metabolized by cyclooxygenases to prostaglandin G_2 (PGG_2) and prostaglandin H_2 (PGH_2). PGH_2 is converted to prostaglandin I_2 (PGI_2), prostaglandin D_2 (PGD_2), prostaglandin F_2 (PGF_2), prostaglandin E_2 (PGE_2), and thromboxan A_2 (TXA_2) by specific synthases.

After the initial purification of COX in 1976 [4, 5] several studies suggested the existence of multiple COX [6-10]. In the early 1990s, several researchers cloned the inducible enzyme which is now referred to as COX-2 [11-15]. Two isoforms of COX, dubbed as COX-1 and COX-2, are different in many respects. Cyclooxygenase-1 is constitutionally expressed in the majority of tissues, and appears to be involved in the cytoprotection of the gastric mucosa, vasodilation in the kidneys, and the control of platelet aggregation. While COX-2 is induced rapidly by a variety of stimuli, including mitogens, cytokines, growth factors, and tumor promoters [16].

GENES AND STRUCTURE OF COX-1 AND COX-2

The human gene which encodes the COX-1 enzyme (*PTGS1*) was first cloned in 1989 by Yokoyama and Tanabe [17]. Gene *PTGS1* is located on chromosome 9 (9q32 – 9q33.3), harbors 11 exons and encompasses a 40 kb span [18]. The gene which encodes for COX-2 (*PTGS2*) is located on chromosome 1 (1q25.2 – 1q25.3), harbors 10 exons and spans 7.5 kb, with a 4.5 kb transcript [19].

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The tertiary and quaternary structure of COX-1 was initially elucidated by Picot *et al*. in 1994 [20]. The crystallographic structure of COX-2 was determined in the presence of a selective inhibitor by Luong *et al*. [21], and comparative computer models of the X-ray crystal structure of the compound have supported the design of a COX-2 inhibitor [22]. These studies revealed that the structure of COX-2 is similar to that of COX-1, and this is most vividly displayed by the ModBase Predicted Comparative 3D Structure shown in Fig. (**2**).

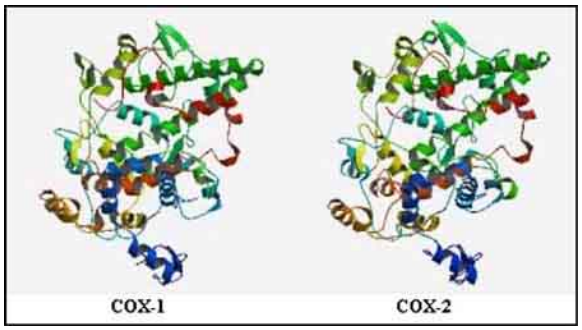

Fig. (2). ModBase Predicted Comparative 3D Structure of human COX-1 and COX-2. These were obtained from http://modbase.compbio.ucsf.edu/modbase-cgi-new/search-form.cgi.

The COXs exhibit a similar primary structure, which consists of a signal peptide, dimerization domain, membrane binding domain, and a catalytic domain [23]. This catalytic domain can be divided into two active sites; the cyclooxygenase active site and the peroxidase active site. The cyclooxygenase active site is a narrow tunnel, approximately 8 Å wide and 25 Å long, which opens into the membrane binding domain [20]. This site accepts the arachidonic acid (AA) liberated from the membrane by cellular phospholipases. The peroxidase active site is globular, and possesses two distinct intertwining lobes which form a shallow cleft on the surface of the enzyme furthest from the membrane to which heme is bound [2]. The structural difference between the active sites of COX-1 and COX-2 lies in the substitution of isoleucine 523 in COX-1 for a valine in COX-2. This substitution results in the opening of a hydrophobic outpocketing in COX-2, which can be accessed by some COX-2 selective drugs [24].

SELECTIVE COX-2 INHIBITOR, CELECOXIB AND ROFECOXIB

Celecoxib is a 1,5-diaryl pyrazole-based compound, which inhibits COX-2 with a 375-fold selectivity [25, 26]. According to the drug data sheet provided by the manufacturer, it is absorbed with a t_{max} of about 3 hours, and is eliminated at a half life of 11.2 hours. Celecoxib achieves its analgesic and anti-inflammatory effects against arthritis *via* selective COX-2 inhibition, but has manifested no gastric mucosal injury related or platelet related side effects, both of which have been previously associated with COX-1 inhibition by NSAIDs [27].

Rofecoxib, a methylsulphonylphenyl derivative, also selectively inhibits COX-2, with no adverse effects on gastric mucosa, and is effective with a daily regimen of 25 or 50 mg, due primarily to its long-acting nature [28, 29]. This drug, however, was voluntarily withdrawn from the global market in 2004, due to an increased number of thromboembolic events occurring in persons participating in colorectal polyp prevention trials. The structures of several selective COX-2 inhibitors are shown in Fig. (**3**).

NSAIDs AND NEOPLASIA

In 1981, Narisawa *et al*. reported that indomethacin inhibited the development of colonic tumors in rats, and suggested that the drug might be used to prevent the development of colonic cancer in patients who are at high risk of colonic adenomatosis or carcinomatosis [30]. McCormick *et al*. also suggested that the inhibition of prostacyclin synthase might be a useful method for the chemoprevention of mammary cancer in rats who consume diets high in fat [31]. Similar investigations into pancreatic cancer, lung cancer, and bladder cancer, using animal models has been reported [32-34]. During the last two decades, numerous animal model studies were published, and the majority of them concerned colon cancer and nonsteroidal anti-inflammatory drugs (NSAIDs) [35-46]. These studies indicated that NSAIDs significantly reduced the incidence and multiplicity of colon tumors, and suggested that NSAIDs can suppress the formation of tumors in rodents during initiation and/or progression.

After the reports of Waddell *et al*., which demonstrated the regression of rectal polyps in familial adenomatous polyposis (FAP) patients, in response to sulindac [47, 48], a number of epidemiological studies, as well as clinical trials, were undertaken [49-58]. These studies suggested that NSAIDs induce the regression of adenomas in some polyposis patients, although studies involving young FAP patients revealed that standard doses of sulindac did not prevent adenoma development [59]. As the result of this data, despite the known side effects of NSAIDs, numerous studies have been conducted into a variety of human malignancies [60-71]. These studies failed to indicate a consistent beneficial effect of NSAIDs on chemoprevention, although they also suggested the need for a more thorough evaluation of the risks and benefits of the routine use of these drugs.

Recent clinical trials regarding the prevention of colorectal adenoma using the selective COX-2 inhibitors, APPROVe (Adenomatous Polyp Prevention on Vioxx) and APC (Adenoma Prevention with Celecoxib), were terminated due to an observed increased risk of death due to cardiovascular events [72, 73]. However, another large study, called the PreSAP (Prevention of Spontaneous Adenomatous Polyps) trial, in which patients received one 400mg dose of Celecoxib once per day, whereas the patients in the APC trial received 200mg or 400mg twice daily, revealed no evident cardiovascular risk factors in a preliminary evaluation by the safety committee. In addition, the National Cancer Institute of USA has not yet closed the numerous cancer prevention and treatment clinical trials involving the effects of Celecoxib on bladder, breast, cervical, colorectal, esopha-

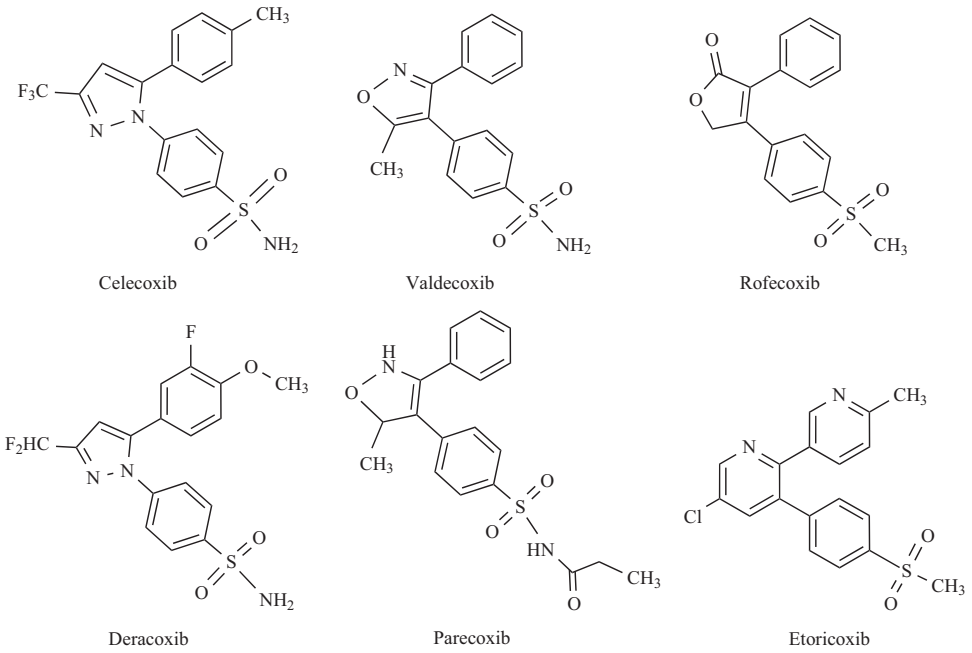

Fig. (3). Structure of several selective COX-2 inhibitors.

geal, head and neck, skin, lung, oral, prostate, pancreatic, and ovarian cancers.

CYCLOOXYGENASE, PROSTANOIDS AND CANCER

Within the last two decades, lots of studies have shown that COX-2 levels tend to be increased in both premalignant and malignant human tissues. These studies have involved such tissues and systems as the gastrointestinal tract, liver, pancreas, head and neck, lung, breast, urinary bladder, uterine cervix, endometrium, and skin [74-92]. Although the exact functional role(s) played by these increased COX-2 levels in tumor tissues have yet to be fully elucidated, several proposed mechanisms have been suggested to explain the role of tumor derived prostanoids.

In 1997, Seed *et al*. reported that diclofenac exerted a suppressive effect on the growth of COX-2 positive colon-26 cells in nude mice, *via* the blockage of angiogenesis [93]. Majima *et al*. also demonstrated that angiogenesis enhanced by basic fibroblast growth factor (bFGF) or epidermal growth factor (EGF) could be inhibited by indomethacin or the selective COX-2 inhibitor, NS-398 [94]. Tsujii *et al*. used a coculture system of endothelial cells with colon carcinoma cells, in order to verify the role of COX in endothelial cell migration and angiogenesis [95]. In this study, the over expression of COX-2 in colon cancer cell lines stimulated tube formation and the extension of cocultured endothelial cells, and this effect could be blocked by both aspirin and the COX-2 specific inhibitor, NS-398. They also concluded that COX-1 regulates angiogenesis in endothelial cells, as tube formation was suppressed when COX-1 activity/expression was inhibited by the treatment of endothelial cells with aspirin or with a COX-1 antisense oligonucleotide. Cyclo-oxygenase-2 expression upregulation can be induced by several factors – EGF, transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), and hypoxia [96-99]. Increased eicosanoid production as the result of COX-2 expression promotes the generation and release of vascular endothelial growth factor (VEGF), a potent angiogenic growth factor [100]. Each of the prostanoids plays a role in angiogenesis. Thromboxan A_2 stimulates endothelial migration and fibroblast growth factor-induced corneal angiogenesis [101]. Also, the inhibitory effects of COX-2 on endothelial cell migration and angiogenic responses can be reversed *via* treatment with the TXA_2 agonist, U46619 [101]. Prostaglandin E_2 regulates VEGF production in a more direct manner. In an experiment involving cultured rat Muller cells, Cheng *et al*. suggested that PGE_2 induced the generation of VEGF *via* the activation of protein kinase A [102]. PGE_2 also promotes the translocation of hypoxia-inducible factor-1α from the cytosol to the nucleus, and consequently stimulates VEGF production [103]. PGI_2 appears to be related to both endothelial sprouting and VEGF-induced vascular permeability [104, 105], or induces VEGF in a direct manner [106].

Early studies have reported the inhibition of cell proliferation induced by NSAIDs in cultured tumor cell lines, as well as the induction of cell proliferation by PGE_2 [107, 108]. Recently, Sheng *et al*. suggested that the effects of PGE_2 on human colorectal cancer cells – most notably, increased motility and changes in cell shape – might be transduced *via* the prostaglandin EP_4 receptor signaling pathway, and also revealed an activation of the phosphatidylinositol 3-kinase/protein kinase B pathway by PGE_2 [109]. Mutoh *et al*. treated EP receptor knockout mice with azoxymethane, a colon carcinogen, and demonstrated that the aberrant crypt foci, putative preneoplastic lesions in

the colon, were decreased only in EP_4-knockout mice [110]. Using colon and gastric cell lines, Pai *et al*. reported that PGE2 transactivates the epidermal growth factor receptor (EGFR) *via* phosphorylation, and induces the extracellular signal-regulated kinase 2 (ERK2) signaling pathway, which might lead to an increase in cell proliferation and the promotion of carcinogenesis [111].

Another contributor to the tumorigenic process is resistance to apoptosis, also known as programmed cell death. This characteristic of tumor cells which exhibit elevated COX-2 and PGE_2 expression appears to be mediated by elevated Bcl-2, the antiapoptotic protein [112-114]. Another prosurvival COX-2-related signaling pathway is the Akt system, and the inhibition of COX-2 is known to induce apoptosis in a Bcl-2-independent manner, *via* the activation of Akt [115-117].

An early study conducted by Tsujii *et al*. revealed that the expression of COX-2 in colon cancer cells might induce the activation of metalloproteinase-2 and increased levels of RNA for the membrane-type metalloproteinase [118]. Matrix metalloproteinases comprise a family of zinc dependent endopeptidases, which are known to be involved in both tumor and vascular invasion. The primary implication of this work is that the expression of COX-2 can alter both the metastatic potential and/or invasiveness of a given tumor. A number of studies regarding COX-2 expression and tumor invasiveness, metastatic potential, and prognosis in conjunction with a variety of malignancies were then undertaken [119-134].

Many investigations, from epidemiologic studies to molecular-level investigations, support the intimate relationship of COX and prostanoids with the carcinogenesis, invasion, metastasis, and prognosis of diverse human malignancies. Based on the results of these works, a host of studies have been initiated into cancer chemoprevention, and several studies have also focused on an increase in the efficacy of cancer therapy with the use of COX inhibitors in both the general population, and in cancer patients.

COX INHIBITORS IN CHEMOPREVENTION AND CANCER TREATMENT

As had been cautioned by Mukherjee *et al*., who indicated the risk of cardiovascular events associated with the use of COX-2 inhibitors [135], recent clinical trials into the prevention of colorectal adenomas using the selective COX-2 inhibitors, APPROVe and APC, were terminated due to the discovery that these inhibitors induced an increased risk of death from cardiovascular events [72, 73]. The National Cancer Institute of the USA, however, continued to conduct cancer prevention trials, on the condition of notification of risks to the principal investigators and the renewal of participant consent after the full disclosure of information. Ongoing cancer prevention trials involving COX inhibitors are listed in Table **1**.

Lots of studies have been conducted in an attempt to enhance the efficacy of conventional cancer therapies including both chemotherapy and radiotherapy using COX inhibitors. In a study involving the use of Sulindac and 5-fluorouracil in 15 advanced colorectal cancer patients, one patient exhibited a partial response, and 3 patients evidenced a stabilization of their disease with tolerable levels of toxicity [136]. In cases of early-stage non-small-cell lung cancer (NSCLC), the preoperative use of Celecoxib with Paclitaxel and Carboplatin also yielded encouraging results [137]. In fact, a number of studies have been conducted

Table 1. Active Chemopreventive Clinical Trials Using COX Inhibitor as of July 2005 (Available online www.cancer.gov)

Study title	Protocol ID
Phase IIB/III Randomized Chemoprevention Study of Celecoxib in Patients With Superficial Transitional Cell Carcinoma of the Bladder at High Risk for Recurrence	MDA-ID-99368
Phase III Randomized Chemoprevention Study of Eflornithine and Sulindac in Patients With a History of Adenomatous Polyps of the Colon	UCIRVINE-UCI-2002-2261
Phase IIb Randomized Study of Celecoxib in Patients With High-Grade Squamous Intraepithelial Lesions of the Cervix	SWOG-S0212
Phase II Study of Exemestane Alone or in Combination With Celecoxib in Postmenopausal Women at High Risk for Invasive Breast Cancer	NCI-04-C-0044
Phase II Randomized Study of Celecoxib in Patients With Cervical Intraepithelial Neoplasia 3	GOG-0207
Phase II Randomized Study of Celecoxib as a Chemopreventive Agent Inhibiting Ultraviolet-Induced Erythema and Biomarkers of Cutaneous Carcinogenesis in Participants With Fitzpatrick Type I-IV Skin	CPMC-U19-CA81888-01-UV
Phase II Randomized Study of Celecoxib With or Without Eflornithine For the Prevention of Colorectal Cancer in Participants With Familial Adenomatous Polyposis of the Colorectum	MDA-ID-00109
Phase II Randomized Pilot Chemoprevention Study of Celecoxib in Former Heavy Smokers At High Risk of Primary or Second Primary Lung Cancer	UCLA-0108074
Randomized Study of Celecoxib and EKB-569, as Monotherapy or in Combination, For Prevention of Oral Cancer in Patients With Aneuploid Oral Leukoplakia	MDA-2003-0824

regarding the treatment of NSCLC with COX-2 inhibitors, because COX-2 is over expressed in the majority of adenocarcinomas and squamous cell carcinomas [138]. Concurrent radiotherapy or chemotherapy coupled with Celecoxib in NSCLC patients yielded quite good results [139, 140], and numerous lung cancer studies involving a variety of combinations are currently underway [138]. Early results of studies involving the combined use of COX inhibitors and conventional cancer therapies on a variety of malignancies have shed some light on the improvement of current therapeutic modalities [141-144]. In addition, dozens of clinical trials involving the use of COX inhibitors to increase the efficacy of treatment in several cancers, including pancreatic, breast, ovarian, non-small cell lung, and other solid tumors, are currently underway (available online at www.cancer.gov).

SUMMARY

After the initial purification of COX was reported in 1976, studies into both COX itself and COX inhibitors have been continuous, and our knowledge has expanded immensely. The relationship between COX and human malignancies constitutes one of the most thoroughly investigated regions in modern oncology. Within the last two decades, both preclinical and clinical data have provided a sufficient quantity of evidence to suggest that COX inhibition might prevent carcinogenesis, retard the growth of established tumors, and enhance the responses of tumors to conventional cancer therapies. In the near future, we may see the development of a host of valuable cancer treatment agents as the result of the many ongoing COX-2 inhibitor trials. However, it should be noted that the precise mechanisms by which COX-2 inhibitors manifest their effects on malignant tissues currently remain to be fully elucidated.

ACKNOWLEDGEMENT

This work was supported by a grant of the National Cancer Center Control R&D Program 2003, Ministry of Health & Welfare, Republic of Korea (800-2003-0394).

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Received: July 27, 2005 **Revised: February 21, 2006** **Accepted: February 22, 2006**