Radiol Oncol 2003; 37(3): 187-94.

review

The role of cyclooxygenase-2 in the malignant tissue and possible applicability of cyclooxygenase-2 inhibitors in the therapy of cancer

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Cyclooxygenase-2 (COX-2), an inducible prostaglandin (PG) synthase, is elevated in many types of malignant and pre-malignant tissues. This enzyme is localized in neoplastic (epithelial) cells, microvascular endothelial cells, and stromal fibroblasts. Through the released PG it enhances carcinogenesis with increasing angiogenesis, inhibiting apoptosis, activating matrix metalloproteinases, suppressing of cell mediated antitumor immune response and protection against damage by cytotoxic agents. Evidences from in vitro studies, studies on animal models as well as first clinical outcomes suggest that the inhibition of COX-2 may suppress carcinogenesis by affecting a number of pathways: inhibiting angiogenesis, invasiveness of tumors and promoting apoptosis. References forecast that COX-2 inhibitors, mostly COX-2 selective inhibitors, may get a role in the therapy of cancer as an adjuvant therapy or as an co-chemotherapeutic agent. The purpose of the present article is to summarize the most important facts about the role of COX-2 in the malignant tissue and discuss possible ways for potential therapeutic place of COX-2 inhibitors in clinical practice.

Key words: neoplasms - drug therapy - physiology; cyclooxygenase inhibitors; apoptosis

About cyclooxygenase

Cyclooxygenase (COX) enzyme is a prostaglandin (PG) H synthase that catalyzes the rate limiting step in the production of PG and tromboxanes. It mediates the insertion of molecular oxygen into arachidonic acid that is liberated from membrane glycerophospholipids and forms unstable intermediate PGG2 that is rapidly converted to PGH2 by the peroxidase activity of COX. Specific isomerases then convert PGH2 into biologically active PGs, such as PGF2 alpha, PGE2, PGD2, PGI2, and thromboxane (TX) A2. PGs have important function in almost every organ system; they regulate diverse physiological processes, such as immunity, reproduction, maintenance of vascular integrity and tone, nerve growth and development and bone metabolism. PGs act as autocrine and paracrine mediators to signal changes within the immediate environment.[1,2]

There are two isoforms of COX: COX-1 and COX-2. They are encoded by different

Received 25 July 2003
Accepted 11 August 2003

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genes and express cell-specific regulation. COX-1 is constitutively expressed in most mammalian tissues and is responsible for normal kidney and platelet function and for the maintenance of gastrointestinal mucosa.[3] On the other hand, COX-2 is not detected in most of normal tissues. It is induced by mitogenic and inflammatory stimuli, which results in an enhanced synthesis of PGs in neoplastic and inflamed tissues.[4]

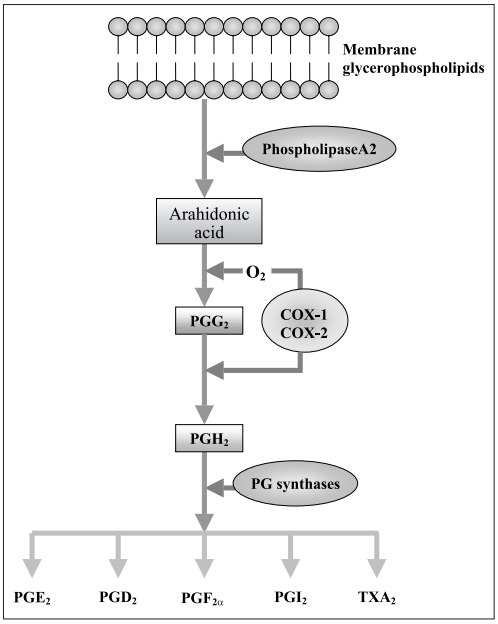

Figure 1. Cyclooxygenase enzymes in prostaglandin synthesis.

COX-2 is overexpressed in several premalignant and malignant conditions

There are growing evidences that COX-2 is commonly overexpressed in malignant tissue. Eberhart *et al.*[5] first noted that COX-2 is upgraded in colorectal cancer. Till today, COX-2 overexpression was found in the colon adenoma and adenocarcinoma, stomac metaplasia and adenocarcinoma, in Barrett's esophagus and carcinoma of the esophagus, chronic hepatitis, hepatocellular carcinoma, cholangiocarcinoma, bill duct hyperplasia, adenocarcinoma and squamous cell carcinoma of the lung, actinic keratose and squamous cell carcinoma of the skin, malignancies and premalignancies of the breast, bladder, pancreas, head and neck.[6-11]

An enhanced expression of COX-2 is the result of increased transcription and stability of COX-2 mRNA[12] due to oncogenes, growth factors, cytokines, chemotherapy and tumor promoters. COX-2 is expressed as an early response[13] due to these factors. One possible mechanism of increased transcription of the COX-2 mRNA is the loss of wild-type p53, an inhibitor of transcription of COX-2 gene.[14]

In oral mucosal lesions, the expression of COX-2 protein increases from hyperplasia to dysplasia and is the highest in squamous-cell carcinoma.[15] Chan *et al.*[11] quantified the levels of COX-2 mRNA by RT-PCR and found that, in comparison to normal controls, COX-2 mRNA was increased 150-fold in the head and neck squamous-cell carcinoma and 50-fold in a normal appearing epithelium adjacent to cancer.

What are the precise COX-2 signaling pathways that promote tumorigenesis

COX-2 in carcinogenesis may include multiple mechanisms that may act at different stages of malignant disease. PGs, especially of the E series, induce cell proliferation, angiogenesis, invasion and metastases. Probably the most important role of COX-2 in tumorigenicity is enhancing angiogenesis of the tumor cells.[2] Angiogenesis is the prerequisite for tumor development and metastasis. Hypoxia, like in-growing tumor tissue, induces in vitro COX-2 expression, thereby also increasing the expression of the proangiogenic growth factor VEGF - vascular endothelial growth factor.[16]

Studies by Cianchi *et al.*[17] on 31 surgical specimens of colorectal carcinoma suggest that VEGF should be considered as one of the

most important factors involved in the stimulation of tumor angiogenesis promoted by COX-2 activity in colorectal cancer. These investigators found a significant correlation between COX-2 and VEGF mRNA levels as well as VEGF protein levels in the colorectal specimens. Gallo[18] showed that the COX-2 activation in epidermal tumor cell lines causes a rapid induction of VEGF mRNA and VEGF production in the neoplastic cells. COX-2 can also directly stimulate endothelial cell migration and growth factor induced angiogenesis with the production of eicosanoid products like TXA2, PGE2 and PGI2. Each of them is capable to stimulate the endothelial cell migration, tube formation, and induction of growth factors.[2]

COX-2 also inhibits endothelial cell apoptosis.[3] The pathogenetic pathway is the stimulation of Bcl-2 transcription.[3] Human microvascular endothelial cells that overexpress Bcl-2 are refractory to the apoptotic and angiosuppressive properties, and participate in more vigorous and sustained angiogenetic response.[19]

There are several studies on animal models that demonstrate these pathogenetic mechanisms. However, the recent clinical studies, where COX-2 expression was examined by immunohistochemistry, and correlated to clinicopathological features, are the most expressive. Tomozawa *et al.*[20] showed that COX-2 overexpression correlated with tumor recurrence and haematogenous metastasizing in colorectal cancer. In the study on esophageal squamous cell carcinomas by Kase *et al.*[21] COX-2 expression was associated with an increased intratumoral microvessel density and suppressed tumor cell apoptosis. In the study on renal cell carcinomas of 131 patients by Miyata *et al.*,[22] COX-2 immunohistochemical expression was significantly associated with various clinicopathological features (like high T, N, M stage in high tumor grade), with microvessel density and metalloproteinase-2 expression, but not with the apoptotic index (p= 0.054). In multivariate analysis, COX-2 expression was not a significant prognostic factor for survival; the disease stage stays the most significant determinant of patient's survival. The same significant positive correlations between COX-2 expression and lymph node metastases as well as histologic grade and tumor size were proved in the patients with breast carcinoma.[23]

COX-2 is also involved in the suppression of cell-mediated anti-tumor immune response. PGE2, probably the most damaging final products of COX-2 enzymatic action, inhibits, in vitro the production of tumor necrosis factor alpha and induces the production of interleukin-10,[24] a cytokine with immunosuppressive effects.

COX-2 also induces matrix metalloproteinase production via PGE2.[13] Matrix metalloproteinase enzymes degrade the type IV collagen of basement membrane and thus increase the invasiveness of tumor cells.

COX-2 may enhance the activation of procarcinogenesis - it can activate several classes of chemical carcinogens (aromatic and heterocyclic amines).[25]

Use of nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2 selective inhibitors in human cancers

In the early 1990s, Thun *et al.*[26] showed in their study that regular aspirin use at low doses may reduce the risk of colon cancer. They speculated that this could be mediated through the inhibition of PG synthesis. Several studies were then performed on the use of other nonsteroidal anti-inflammatory drugs (NSAIDs), which are among the oldest and most widely used drugs. They reported about a 50-percent lower risk of colorectal cancer in people who are continuously taking these drugs.[27-29] Recent epidemiological studies found a significant inverse association be-

tween the intake of NSAIDs and the risk of breast cancer.[30,31] Meta-analysis of 14 epidemiological studies, analyzing the reduction of the risk for breast carcinoma with the use of NSAIDs was studied, showed that the regular use of NSAIDs was associated significantly with an 18-percent reduction in breast carcinoma.[32] Seven patients with head and neck squamous cell carcinoma (stages III and IV) were treated with different doses of indomethacin for 2 to 7 weeks.[33] Five of 7 patients demonstrated tumor regression; in 3 of them, it was significant. The patients who did not receive indomethacin showed no detectable response. The studies led to the identification of a molecular target, COX-2, involved in tumor promotion during colorectal cancer progression.[34-36] It was also discovered that NSAIDs did not suppress COX-2 expression or COX-2 protein level, but reduced its activity and inhibited PGE2 production.[37]

Encouraging results have now also been obtained with selective COX-2 inhibitors. Two different COX-2-selective inhibitors - rofecoxib and celecoxib - are currently available. Reddy *et al.*[34] reported that the administration of celecoxib to rats (male F344 rats with azoxymethane-induced colon carcinogenesis) during either stage of tumorigenesis inhibited the incidence as well as multiplicity of adenocarcinomas of the colon in a dose-dependent manner. It also suppressed colon tumor volume. This study provides the first evidence that celecoxib is very effective if given in the promotion or progression stage of colon carcinogenesis, indicating that chemopreventive efficacy is achieved during the later stages of colon tumor development. Also, the study on mice[35] showed that selective COX-2 inhibitor prevented hematogenous metastases of colon cancer. In addition to studies on colorectal carcinomas, the selective COX-2 inhibitor was used on human oral squamous cell carcinoma cell line (KB cells) implanted on the oral cavity of nude mice.[38] The significant reduction of tumor growth was observed and the number of microvessels, peripheral to the side of the tumor, was reduced. The study of Leahy *et al.*[39] on FGF-2 treated rodent corneas showed that the use of celecoxib at a dose of 30 mg/kg/day per os inhibited angiogenesis by 79%, and PGE2 production by 73%. A 65-percent decrease of proliferation and a 2.5-percent increase of apoptosis were observed.

The treatment with selective COX-2 inhibitors inhibited the COX-2 enzyme selectively and did not lower the gastrointestinal PG levels associated with mucosal protection. Celecoxib 400 mg twice daily effectively decreased the number and size of colon polyps in familial adenomatous polyposis with as little as 6 months of treatment;[40] however the dose of celecoxib 100 mg twice daily was not associated with significant regression in the size and number of polyps. Clinical evidence indicates that COX-2 selective inhibitors offer the therapeutic benefits of traditional NSAIDs with less of the associated toxicity.

Future directions

Recent studies in humans indicate that the therapy with specific COX-2 inhibitors might be an effective approach to cancer prevention and treatment. As the treatment with commonly used NSAIDs inhibit COX-1 and COX-2, the use of these agents may be limited by normal tissue toxicity, particularly that of gastrointestinal tract. Selective COX-2 inhibitors exert potent antiinflammatory activity but cause fewer undesired side effects. In both, the prevention of carcinogenesis and cancer therapy they may be more suitable as anticancer agents than standard NSAIDs. Based on the results of the study by Steinbach *et al.*,[40] US Food and Drug Administration approved celecoxib as adjuvant therapy for the patients with familial adenomatous polyposis (FAP). Similiarities between the biology of FAP and sporadic colorectal cancer suggest

that the strategies effective in FAP might be applicable also in the patients with colorectal adenoma. Several clinical studies are already under way to assess the efficacy of selective COX-2 inhibitors (celecoxib and rofecoxib) in preventing sporadic colorectal adenomas in large population.

Since COX-2 inhibitors protect against the formation of multiple tumor types in experimental animals, the potential utility on various target organs is also being examined. Therefore, cohorts of patients with Barrett's dysplasia, premalignant oral lesions, bronchial metaplasia, basal cell nevi and actinic keratosis are being treated.[1] COX-2 inhibitors could play a role in the chemoprevention of epithelial cancers.[13] COX-2 inhibitors could have an additive role also in the treatment of some breast tumors. In the breast cancer tissue, aromatase activity for the production of estrogens is enhanced via the increased expression of the aromatase CYP19 gene by PGE2,[41,42] which is increased by overexpression of COX-2 in neoplastic breast cells. The discovery that a selective COX-2 inhibitor suppresses aromatase activity would be very important since a large number of postmenopausal women who are at risk of breast cancer chronically use selective COX-2 inhibitors to treat artritis; thus an epidemiologic study should not be problematical.

In preclinical models, a selective inhibitor of COX-2 potentiated the beneficial antitumor effects of ionizing radiation with no increase in normal tissue antitoxicity.[43] A selective COX-2-inhibitor-induced enhancement of tumor radioresponse was associated with a decrease in PGE2 levels, inhibition of neoangiogenesis; however, there was no effect on radiation-induced apoptosis. This opens the possibility for the use of these drugs for the chemoprotection during the courses of ionizing radiotherapy. Recent evidence indicates that COX-2 also increases multidrug resistance protein1 (also known as P-glycoprotein), an efflux pump for chemotherapeutic agents.[44] This effect was prevented by the treatment with a selective COX-2 inhibitor.[44] Although much work is required to establish the clinical significance of this interaction, it is appealing to speculate that selective COX-2 inhibitors will enhance the antitumor activity of cancer chemotherapy by reducing multidrug resistance.[1]

Mohan and Epstein[13] discussed the use of COX-2 inhibitors in the head and neck squamous-cell carcinoma and proposed that this drug may represent a strategy for the prevention of displasia and cancer.

Although, the prevention and treatment with celecoxib seem promising, there are many obstacles that must not be overlooked. Selective COX-2 inhibitors have an excellent safety profile regarding gastrointestinal tract, but concerns have risen about cardiovascular safety.[45] The incidence of myocardial infarction has increased in the groups treated with Vioxx (rofecoxib) comparing with naproxen. By now, it is not certain whether this is a chance event, a pro-thrombotic effect of rofecoxib or a cardioprotective effect of naproxen. Further studies are needed.

Most likely, selective COX-2 inhibitors could become promising adjuvant therapy in the prevention and treatment of certain carcinoma, next to radiation and/or chemotherapy. It is most promising, too, that COX-2 inhibitor will be added to antiangiogenic chemotherapy. That clinical evaluation is urgently warranted. A possible indication for selective COX-2 inhibitor may also include secondary prevention of recurrent disease.

Conclusions

Combining the evidence from many studies, it may be concluded that the inhibition of COX-2 is a viable approach to cancer prevention and treatment. Despite these successes, many questions remain unanswered. Clearly,

research on COX-2 offers more hope of finding new approaches to the treatment of cancer.

Acknowledgement

Author would like to thank Prof. Dr. Andrej Cör for reading of the manuscript and helpful comments.

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