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COX-2 inhibitors: chemoprevention of head and neck cancer

Inibidores de COX-2: quimioprevenção de tumor de cabeça e pescoço

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Abstract

Objective: In addition to the well-established pathophysiological role that COX-2 plays in inflammation, recent evidence implies that this isoform may also be involved in multiple biologic events throughout the tumorigenic process. Many epidemiological studies demonstrate that nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the risk of a wide range of tumors. Further, COX-2 is chronically over expressed in many premalignant, malignant, and metastatic human cancers, and levels of over expression have been shown to significantly correlate to invasiveness, prognosis, and survival in some cancers. **Conclusions**: This article presents a broad overview of the growing evidence that COX-2 plays a pivotal role throughout oncogenesis and summarizes the rationale to explore the use of COX-2 inhibitors for the prevention or treatment of cancer as a single agent or in combination with current anticancer modalities. Epidemiological data and preclinical studies have generated compelling interest in the potential use of COX-2 inhibitors in chemoprevention and chemotherapy of human tumours.

Keywords: Cycloxygenase. Prostaglandins. Oncogenesis.

Resumo

***Objetivos**: Além do papel fisiopatológico bem definido que a COX-2 desempenha na inflamação, a evidência recente sugere que essa isoforma também pode estar envolvida em eventos biológicos múltiplos durante o processo de tumorigênese. Vários estudos epidemiológicos demonstram que anti-inflamatórios não esteroides*

(AINEs) reduzem o risco de uma grande variedade de tumores. Além disso, sabe-se que há sobre-expressão crônica da COX-2 em muitos tumores humanos pré-malignos, malignos e metastáticos, tendo sido demonstrada correlação dessa sobre-expressão com a invasão, o prognóstico e a sobrevida de alguns tumores. ***Conclusões****: Este artigo apresenta uma visão ampla da crescente evidência de que a COX-2 desempenha papel fundamental na oncogênese e resume os fundamentos para explorar o uso de inibidores COX-2 para a prevenção ou o tratamento do câncer como um único agente ou em combinação com atuais modalidades anticancerígenas. Dados epidemiológicos e estudos pré-clínicos têm gerado grande interesse no uso potencial de inibidores COX-2 na quimioprofilaxia e quimioterapia de tumores humanos.*

Palavras-chave*: Ciclo-oxigenase. Prostaglandinas. Oncogênese*.

Introduction

The cyclooxygenases are responsible for the conversion of arachidonic acid to prostaglandins (PGs), and their metabolites play a pivotal role in multiple physiologic and pathophysiologic processes. Cyclooxygenase- 1 (COX-1) is constitutively expressed in most tissues and is responsible for maintaining physiologic processes such as gastric and renal protection and platelet function. In contrast, cyclooxygenase-2 (COX-2) is induced in response to growth factors (1, 2) (i.e., endothelial growth factor [EGF], vascular endothelial growth factor [VEGF], fibroblast growth factor [FGF-2], cytokines (e.g., tumor necrosis factor-TNF], interleukin-[IL], and interleukin-1 [IL-1]), and tumor promoters (e.g., v-src, v-Ha-ras, HER-2/neu, and Wnt) (3, 4). COX-2 is expressed in macrophages, synoviocytes, and endothelial cells in response to inflammation and cellular activation (5-7). Conventional NSAIDs inhibit both COX-1 and COX-2; hence they also disrupt COX-1 dependent homeostatic functions. In addition to the well-studied role of COX-2 in acute inflammatory processes, recent work clearly suggests COX-2-derived metabolites contribute at multiple points throughout tumorigenesis, including premalignant hyperproliferation, transformation, and maintenance of tumor viability, growth, invasion, and metastatic spread.

Epidemiological evidence

COX-2 and Cancer Epidemiological studies provided the first evidence that COX may be involved in the pathogenesis of cancer. Several reports indicate NSAIDs can prevent the development of various human tumours, including colon, breast, lung, gastric and oesophageal neoplasias (7, 8).

COX-2 expression in human tumors

COX-2 was consistently overexpressed in premalignant lesions such as oral leukoplakia, actinic keratosis, prostatic intraepithelial neoplasia, and carcinoma in situ of the bladder and breast (8, 9).

In general, COX-2 is up-regulated throughout the tumorigenic process, from early hyperplasia to metastatic disease, neoplastic epithelium, inflammatory cells, and vasculature within and adjacent to tumor nests, and is expressed in 40% to 80% of neoplastic cells in human cancers. Moreover, well- and moderately-differentiated cancers have significantly higher COX-2 expression than poorly differentiated cancers. COX-2 is also detected in noncancerous cells immediately adjacent to tumor cells (< 2 mm) and in the angiogenic vasculature within tumors and in pre-existing blood vessels adjacent to tumors (6).

Mechanisms of COX-2 – associated tumorigenesis

COX in humans can have a chemopreventive effect (5). The importance of the COX-2 isoform in tumorigenesis was first demonstrated by the observation that, in rodent models of familial adenomatous polyposis, a genetic disease leading to GI cancer, loss of COX-2 activity by either genetic deletion or selective enzymatic inhibition suppressed intestinal polyp formation (9, 10). Celecoxib, a COX-2 inhibitor, has been approved in humans for adjunctive therapy in this population. Preclinical studies using selective COX-2 inhibitors

have demonstrated chemopreventive activity in animal models of colon, bladder and breast cancer. Additional evidence for the importance of COX-2 in tumorigenesis was reported by Hia et al., who showed that selective COX-2 overexpression in the mammary gland of transgenic mice led to tumorigenesis. Taken together, these data provide strong evidence for the importance of COX-2 enzyme activity in oncogenesis and as a target of NSAID antitumor action. COX-2 is overexpressed in oncogenesis and is likely to be a key player in a number of biologic pathways leading to cancer. Current evidence indicates that COX-2 promotes tumor-specific angiogenesis, inhibits apoptosis, and induces proangiogenic factors such as VEGF (9, 10) inducible nitrogen oxide synthetase promoter (iNOS) (11). IL-6, IL-8. In addition, the direct product of COX-2, PGH2, can isomerize by both enzymatic and nonenzymatic reactions to form the potent mutagen malondialdehyde, which can induce frame shifts and base pair substitutions (12). Additional free radical damage may occur via the peroxidative activity of COX-2, which can efficiently oxidize aromatic and heterocyclic amines and dihydrodiol derivatives. Increased prostaglandin levels may be particularly important during the progression of breast cancer. PGE2 has recently been shown to stimulate aromatase transcription, leading to supraphysiologic local estrogen levels, which in turn leads to the subsequent release of growth factors and enhanced proliferation (13). In addition to increasing aromatase transcription, COX-2-induced PGE2 also promotes angiogenesis, which is required for tumor growth and metastasis.

Inhibition of cell cycle progression by COX -2 inhibitors

Transitions between the various phases of the cell cycle are controlled by various cyclins, cyclin-dependent kinases (CDKs), and cell cycle inhibitors. Treatment of various tumour cell lines with celecoxib induces G1 – phase arrest, which is accompanied by the decreased expression of cyclins A, B, and D; the increased expression of cell cycle inhibitors p21 waf1 and p27 kip1; and the loss of CDK activity.

PKB (protein kinase B) regulates cell cycle progression by its ability to phosphorylate, and thereby inactivate, the CDK inhibitors p21 waf1 and p27 kip1. Inactivation of the CDK inhibitors leads to the activation of various cyclin – CDK complexes and to the activation of proliferating cell nuclear antigen; these activities promote DNA replication and cell proliferation.

Inhibition of PKB by celecoxib prevents the cell proliferation – promoting effects of PKB and could be one mechanism by which celecoxib induces a cell cycle block. Furthermore, celecoxib inhibits various CDK – cyclin complexes in cell-free assay systems (14).

Ornithine decarboxylase is another enzyme that is inhibited by celecoxib (15). This enzyme converts L-ornithine to the polyamine putrescine. Increased polyamine levels are associated with increased cell proliferation, decreased apoptosis, and increased expression of genes affecting tumor invasion and metastasis.

Induction of apoptosis by COX-2 inhibitors

Many studies have shown that celecoxib exerts its anticarcinogenic effect in various cancer cell lines by inducing apoptosis (16). Apoptosis, or programmed cell death, can be induced by the extrinsic pathway through activation of death receptors or by the intrinsic pathway by means of the release of cytochrome *c* from the mitochondria. Both pathways require the activation of various caspases, which cleave various proteins and activate DNases, leading to DNA fragmentation. Evidence that the intrinsic apoptotic pathway appears to be activated by celecoxib includes the observations that expression of the antiapoptotic proteins Bcl-2, Bcl-xL, Mcl-1, and survivin decreases after treatment of cancer cells with celecoxib, whereas expression of the proapoptotic protein Bad increases (17).

PKB induces antiapoptotic effects by phosphorylating and then inactivating the proapoptotic protein BAD (i.e., the Bcl-2 or Bcl-X antagonist), by phosphorylating procaspase 9 to prevent its cleavage to active caspase 9, or by phosphorylating the apoptosis signal – regulating kinase 1, which inhibits the stress-activated protein kinase pathway and other kinases (18). Inhibition of PKB by celecoxib reduces all of these activities and promotes apoptosis. Another target of celecoxib seems to be the sphingolipid pathway. As discussed above, an increase in ceramide is also associated with the induction of apoptosis. For instance, ceramides play a major regulatory role in apoptosis by inducing the

release of proapoptotic proteins from the mitochondria that may occur via the formation of ceramide channels in the mitochondrial outer membrane (19). Celecoxib treatment also inhibits the activity of the Ca 2+ ATPase located in the endoplasmic reticulum of PC-3 human prostate cancer cells, so that the reuptake of Ca 2+ from the cytosol is prevented, which elevates the free intracellular concentration of Ca 2+ (19). This activity is highly specific for celecoxib and is not associated with other COX inhibitors, including rofecoxib (19). By use of microsome and plasma membrane preparations from human prostate cancer cells, only Ca 2+ ATPases located in the endoplasmic reticulum have been shown to be direct targets of celecoxib (19). The concentration of Ca2+ plays a central role in apoptosis, because it is involved in the activation of Ca2+ -sensitive proteases, endonucleases, and caspases. Moreover, opening the mitochondrial permeability transition pores, which releases cytochrome *c*, is sensitive to the concentration of Ca2+ (20, 21). Consequently, celecoxib induced inhibition of Ca2+ ATPases in the endoplasmic reticulum may provide a plausible link with the apoptosis inducing activity of celecoxib. Celecoxib inhibits the activity of carbonic anhydrases I, II, IV, and IX. Carbonic anhydrases are widespread enzymes that catalyze the reversible hydration of carbon dioxide. Several isozymes have been identified; some are cytosolic (carbonic anhydrases I, II, III, VII, and XIII), and others are membrane bound (carbonic anhydrases IV, IX, XII, and XIV). The expression of carbonic anhydrase IX is elevated by hypoxia and is involved in acidification of hypoxic tumors that are characterized as having more aggressive behavior and poorer prognosis than tumors without such expression (22). Furthermore, carbonic anhydrase IX is also involved in cell – cell adhesion and cell proliferation (23). At nanomolar concentrations, celecoxib or valdecoxib specifically inhibits various carbonic anhydrase isozymes (carbonic anhydrases I, II, IV, and IX). The crystal structure of the carbonic anhydrase II – celecoxib complex indicates that the sulfonamide group of celecoxib binds to the catalytic zinc of carbonic anhydrase II, whereas rofecoxib, which contains a methyl sulfone group, does not inhibit carbonic anhydrase activity (24). Carbonic anhydrases II and IX appear to play a role in tumor growth and development (25, 26) and are potential biomarkers for various tumor types (e.g., biliary tumors, colorectal tumors, gastric tumors, and renal clear-cell carcinoma) (27-30). However, to our knowledge, no study clearly shows that inhibition of carbonic anhydrases plays a role for the anticarcinogenic activity of celecoxib.

Inhibition of angiogenesis and metastasis by COX-2 inhibitors

Early tumor growth can be divided into two stages: one in which malignant cells form small tumors of limited size because of an inadequate supply of oxygen (hypoxia) and the other in which hypoxia triggers a dramatic change in gene expression, leading to the formation of new blood vessels and a switch in energy metabolism, from respiration to glycolysis (26).

Overexpression of COX-2 in tumor cells affects angiogenesis by the production of COX-2 – derived eicosanoids (i.e., thromboxane A2 and prostaglandins I2 and E2), which stimulate endothelial cell migration and angiogenesis by increasing the expression of vascular endothelial growth factor (VEGF) and stimulating endothelial cell proliferation (31, 32).

Both mechanisms contribute to the formation of new blood vessels. Inhibition of COX-2 activity by celecoxib or rofecoxib reduces all these effects and leads to inhibition of angiogenesis and decreased tumor growth (33). COX-2 – independent mechanisms that contribute to the antiangiogenic effects of celecoxib have also been described. Celecoxib inhibits the activation of the early growth response factor Egr-1. Egr-1 is a transcription factor that is rapidly activated by many extracellular agonists (such as growth factors and cytokines) and environmental stress such as hypoxia, vascular injury, and UV radiation (34). Egr-1 plays a role in the transcriptional regulation of the fibroblast growth factor and various cytokines and receptors that are involved in angiogenesis and promotion of tumour development (35). Thus, inhibition of Egr-1 gene activation by celecoxib counteracts the different proangiogenic stimuli and inhibits angiogenesis.

How safe are COX-2 inhibitors?

Cox-2 selective inhibitors were developed to reduce the risk of gastrointestinal ulceration caused by non-selective NSAIDs. By selectively inhibiting

COX-2, they reduced the risk of upper gastrointestinal bleeding associated with other NSAIDs. In studies of rofecoxib and lumiracoxib, the absolute risk of serious upper gastrointestinal ulceration and bleeding is reduced by 50-60% or more compared to other NSAIDs (36, 37). In the VIGOR (vioxx gastrointestinal outcomes research) study it was concluded that only 41 patients would need to be treated with rofecoxib rather than naproxen to avert one upper gastrointestinal event in a one-year period (36). This figure was calculated from all patients in the trial and the number should be even smaller in patients who are at risk of upper gastrointestinal adverse reactions. This risk increases in patients with a history of peptic ulcer or bleeding, those taking anticoagulants and possibly patients taking oral glucocorticosteroids. Conventional NSAIDs are known to impair renal function, sometimes to the point of renal failure. This effect is observed particularly when the drugs are used preoperatively in older and sicker patients and in patients with already impaired renal function. In these situations maintenance of renal perfusion and function relies on renal prostaglandin synthesis. The possibility that COX-2 selective inhibitors might not manifest this adverse reaction has unfortunately not turned out to be the case. The risks for renal impairment are less severe to those of other NSAIDs and increase with the dose of COX-2 selective inhibitor if taken for a longer time. If NSAIDs, including COX-2 selective inhibitors, are prescribed for patients with renal impairment, cardiac failure or hypertension, each patient should be monitored closely (38, 39). This should include eliciting symptoms and signs of heart failure, measuring weight and blood pressure and monitoring plasma creatinine and electrolytes soon after starting the drug (for example 2-4 weeks) and at regular reasonable intervals depending on the individual case. Individuals with a history of myocardial infarct, angina, coronary artery stents or known risk factors such as hypertension, hyperlipidaemia, smoking, diabetes or obesity are at risk of arterial thrombosis. The approve study was a three-year randomised controlled trial to see if rofecoxib 25 mg/day could suppress the recurrence of colonic polyps. Among the 2600 patients enrolled, 45 taking rofecoxib and 25 taking placebo suffered confirmed, serious adverse thrombotic events. This difference was only apparent after 18 months. The relative risk is about 2.0, but the extent to which this risk of myocardial infarction or stroke has been proven is currently unclear because of the absence of detailed published information.

In vitro studies indicate that celecoxib is somewhat less COX-2 selective than rofecoxib and may therefore be safer in patients at risk of thrombosis. There has not been as strong a signal for thrombotic risk with celecoxib (40, 41) but further studies are clearly required as placebo-controlled trials of the size and duration of approve are not yet available.

Until more data are available, the COX-2 selective inhibitors should only be used for short periods as in cancer chemotherapy with con current radiotherapy. Low-dose aspirin or other anti-thrombotic treatment should be continued in patients at risk of thrombosis. As there are no drugs with potential side effects; dose modulation has to be done in head and neck cancer patients according to the patient's age, immunity, and systemic and metabolic demands. While there are potential side effects to COX-2 inhibiting drugs, some cancer patients accept this small risk in exchange for the anticancer benefit. Since the COX-2 enzyme appears an excellent target for pharmacological intervention. Long-term administration of celecoxib at 1.500 ppm did not induce any toxic side effects, such as body weight loss, gastrointestinal ulceration, or bleeding. It is also note worthy that a pilot endoscopic study showed no difference in gastroduodenal mucosa damages between celecoxib group and placebo group (42). However there are no studies in literature that shows that chemotherapy with COX-2 inhibitors has no side effects, they can be prevented in cancer patients by careful monitoring of the patient. Presently the choice of COX-2 selective inhibitors for a particular patient should be based upon their relative efficacy, toxicity, concomitant drug use, concurrent disease states, hepatic and renal function and relative cost. However, patients should be informed of the potential risks and the lowest possible dose should be used for the shortest possible time.

Prospects

It will be important to determine which direct COX-2 – independent targets for celecoxib and other COX-2 inhibitors participate in the anticarcinogenic effects of these drugs so that new drugs without gastrointestinal or cardiovascular side effects can

be developed for these targets. Currently, celecoxib derivatives have been developed to inhibit PKB/AKT or to disrupt the mitochondrial membrane potential and to have anticarcinogenic activity without inhibiting cyclooxygenases (43). By analyzing these derivatives, it may be possible to identify the chemical moieties that are required for the anticarcinogenic effect of celecoxib and to determine whether modification of these moieties can produce more potent anticarcinogenic drugs. There are many COX-2 – independent mechanisms used by celecoxib to mediate its anticarcinogenic effects. Inhibition of PDK-1 and its downstream substrate PKB/AKT appears to play a central role in the induction of apoptosis and the inhibition of cell cycle arrest, angiogenesis, and metastasis. Such a central target has not yet been described for rofecoxib, and so the COX-2-independent molecular mechanism of rofecoxib remains unclear. Some of the observed anticarcinogenic effects of celecoxib and rofecoxib could result from the inhibition of COX-2 and downstream components. There are also discrepancies between the anticarcinogenic effects of celecoxib and rofecoxib, although rofecoxib is a more potent COX-2 inhibitor than celecoxib. The anticarcinogenic potency of celecoxib thus may be unique among selective COX-2 inhibitors; in addition to COX-2 inhibition, celecoxib must also target other COX-2 – independent proteins. The reasons for these discrepancies are still poorly understood. All COX-2 inhibitors are selective inhibitors of COX-2, but the chemical structures of these compounds are different. Thus, the sulfonamide and the 4-methylphenyl moieties of celecoxib may be particularly important because these chemical moieties also target other proteins, such as the carbonic anhydrases or PDK-1, respectively, as previously discussed.

The many in vivo and *in vitro* studies that have demonstrated the benefit of COX-2 inhibitors in cancer therapy has prompted various researchers to investigate the structural basis of these activities further. New antineoplastic agents that show a higher apoptosis-inducing activity and fewer gastric and cardiovascular side effects (such as ulcerations, stroke, and myocardial infarction) may represent a new class of compounds suitable for tumor prevention and chemotherapy.

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