Under the Microscope

Cyclooxygenase 2 Inhibitors and Colon Cancer

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Colorectal cancer (CRC) accounts for nearly 12% of newly diagnosed cancers in the United States and is the second leading cause of cancer deaths (1,2). Multidisciplinary research in molecular biology, laboratory animal models, and epidemiology during the last 2-3 decades has contributed to our understanding of the etiology of CRC. The hypothesis that inhibition of cyclooxygenase (COX) enzymes by nonsteroidal anti-inflamatory drugs (NSAIDs) might prevent the occurrence or inhibit the growth of CRC arose in the mid-1970s.

Several epidemiological studies have reported significant decreases in the risk of CRC in individuals who used aspirin regularly (2,3). These findings prompted research in animal models, which provided convincing evidence that the administration of aspirin, piroxicam, ibuprofen, sulindac, and other NSAIDs can be used as inhibitors of colon carcinogenesis in humans (4,5). The Table summarizes different COX inhibitors used in the studies for prevention or inhibition of colon carcinogenesis.

Although the mechanism(s) by which NSAIDs reduced the risk of colon carcinogenesis is not fully understood, literature published during the past 30 years has suggested involvement of the arachidonic acid metabolites in different stages of carcinogenesis in a number of ways: a) alterations in cell growth and differentiation; b) tumor promotion and metastasis; c) formation of the endogenous mutagen, malondialdehyde, by spontaneous and enzymatic breakdown of prostaglandin H2 (PGH2); d) activation of carcinogens by the COX-mediated peroxidase activity; e) immunosuppressive effects of PGE2; and f) inhibitory effects of NSAIDs on experimental carcinogenesis.

Two isoforms of COX (also termed as prostaglandin endoperoxide H synthetases) have been identified: COX-1 and COX-2. Both of these enzymes are encoded by separate genes located on different chromosomes and catalyze the conversion of arachidonic acid and other fatty acids to prostaglandins (Figure1). Evidence has revealed that even though both COX-1 and COX-2 catalyze the same reaction, COX 1 produces metabolites that play a central role in normal physiologic functions, including platelet aggregation and gastric cytoprotection. On the other hand, COX-2 is an inducible enzyme expressed in response to a variety of physiological stimuli such as inflammation, wound healing, and neoplasia.

Studies have demonstrated that colonic epithelial cells overexpressing the COX-2 gene resist undergoing apoptosis and show altered adhesion and angiogenic properties (4,6). These findings suggest that COX-2 may be involved in the progression of CRC. Furthermore, COX-2 is elevated in 40% of colon adenomas and 90% of colon carcinoma but not in normal colonic epithelium (2,7). Using human colon carcinoma cell lines, investigators showed that COX-2 induces local immunosuppression by increasing prostaglandin E2, a potent inhibitor of T lymphocyte proliferation, enabling colon cancer cells to escape host immune defenses (8).

COX-2 Inhibitors in Prevention of Colon Carcinogenesis

A number of cell culture and animal studies have provided strong scientific rationale for the therapeutic use of COX-2 inhibitors for the prevention or treatment of colon cancer. In more than 80% of spontaneous colorectal cancers, mutations occur in the adenomatous polyposis coli (APC) tumor suppressor gene. In vitro studies have shown that NSAIDs can stimulate apoptosis in APC-deficient cells (6). One study conducted by Oshima et al. in 1996 demonstrated that treatment of APC delta716 knockout mice with a COX-2-specific inhibitor reduced the polyp number

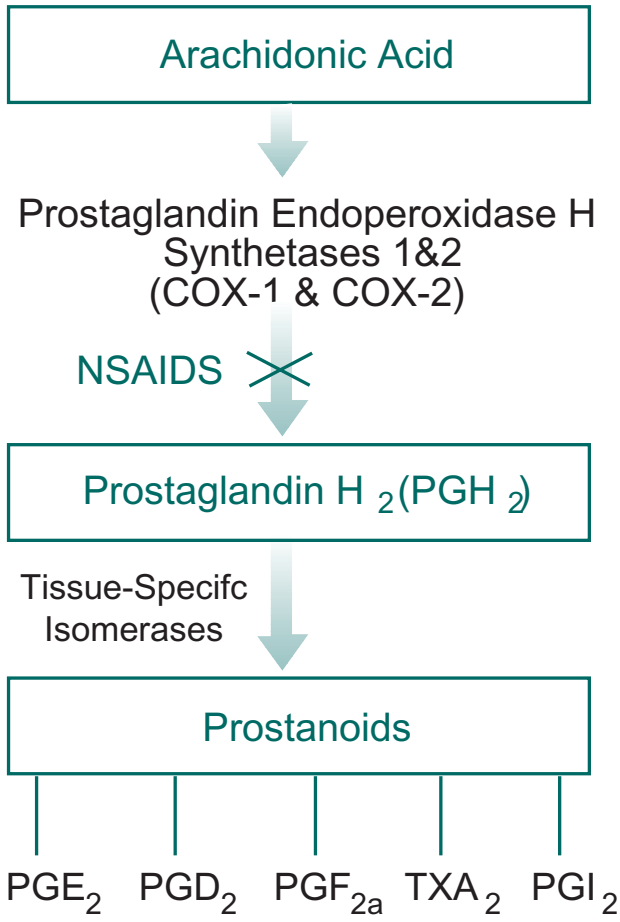

Figure. Metabolic transformation of arachidonic acid to prostaglandins. PG= prostaglandin; Tx= thromboxane

Table. COX Inhibitors Used in the Studies for Prevention of Colon Carcinogenesis.

Agent	Inhibitory Action	
	COX-1	COX-2
Aspirin	+++	+
Indomethacin	+++	+
Ketorolac	+	+
Etodolac	+	+
Diclofenac	+	+
Meclofenamic Acid	+	+
Mefenamic Acid	+	+
Sulindac	+	+
Piroxicam	+	++
Naproxen	+	+
Nimesulide	-	+
Zomepirac	+	+
Nabumetone	+	+
Ketoprofen	+	+
Ibuprofen	+	+
Oxaprozin	+	+
Celecoxib	-	++
Rofecoxib	-	+++
Valdecoxib	-	++
Etoricoxib	-	++
NS-398*	-	++
SC-58125*	-	++
APHS*	-	++

+ = inhibitory effect; * = experimental agent

more significantly than with the nonselective inhibitor sulindac (9). Furthermore, they also showed that COX-2 mutations in mice dramatically reduced the number and size of intestinal tumors in those mice, providing direct genetic evidence that COX-2 played a key role in colorectal polyposis and neoplasia. A number of subsequent studies have confirmed these findings (6). For example, the COX-2-specific inhibitors, SC-58635 and celecoxib, have been shown to significantly suppress azoxymethane-induced colonic aberrant crypt foci and tumor formation in F344 rats (10,11). In two other studies, selective COX-2 inhibitors, nimesulide and NS-398, have been shown to inhibit chemically induced colon carcinigenesis in rodents (2). There are also studies suggesting that the chemopreventive effects of NSAIDs can be potentiated by coadministration of these drugs with other agents such as inhibitors of nitric oxide synthase or lovastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor clinically used as a cholesterol-lowering drug (12,13).

In addition to demonstrating the inhibitory activity of COX-2 inhibitors during the initiation and post-initiation stages of carcinogenesis, animal studies also indicated that these COX-2 selective inhibitors can inhibit tumor growth during the promotion/progression stage of carcinogenesis when premalignant lesions have developed. In one study, F344 rats were continuously treated with celecoxib 14 weeks after treatment with the carcinogen azoxymethane. The drug significantly inhibited the incidence and multiplicity of adenocarcinomas of the colon and suppressed colonic tumor volume (4). This suggests that the chemopreventive effects of COX-2 inhibitors on colon tumor development can also be achieved even when the treatment is delayed. These results prompted the clinical use of COX-2 inhibitors in secondary prevention of colon cancer in patients with familiar adenomatous polyposis (FAP) and sporadic polyps.

Evidence from a randomized clinical trial has demonstrated that celecoxib can suppress the growth of adenomatous polyps

and induce regression of existing polyps in patients with FAP (14). This was a double-blinded placebo-controlled study with patients assigned to two different doses of celecoxib (100 or 400 mg twice daily). After 6 months, the patients receiving the higher dose had a 28% reduction in the mean number of colorectal polyps (P=0.003) and 30.7% reduction in the polyp burden (P=0.001) compared with placebo. No significant regression was observed in patients receiving the lower dose. The study suggested that selective inhibition of COX-2 may help to control the process of FAP.

The inhibitory effect of NSAIDs on colon carcinogenesis in humans may not be specific to COX-2 inhibitors alone. Sulindac, a nonselective COX inhibitor, has been shown to inhibit the development and growth of FAP in a number of randomized clinical trials (6). In one study, sulindac (150 mg, twice daily) treatment for 9 months decreased the number of colorectal adenomas by 56% and their size by 65%. In another placebo-controlled study, sulindac completely regressed the polyps in six out of nine patients, whereas the number of polyps increased in the placebo group. Studies with aspirin and ibuprofin have suggested that 81 mg and 300 mg, respectively, are likely to be the lowest effective doses to prevent colon cancer. More than ten clinical trials are currently in progress using COX-1 and -2 inhibitors not only in FAP, but also in other conditions where COX is over-expressed.

While clinical studies continue despite uncertainty about the mechanism(s) and long-term safety of COX inhibitors, the major focus of the laboratory studies has been to explore the molecular pathways by which these agents prevent or inhibit the development of colorectal cancer. Recently, investigators at Vanderbilt University have identified genes whose expression is affected by the treatment of colon carcinoma cells with NS-398, a selective COX-2 inhibitor (15). Two genes involved in the regulation of cell proliferation (cyclin K and p-100) showed decreased levels of expression. Other genes confirmed to be differentially expressed were human FAT and proto-cadherin-7, which are involved in regulation of cell adhesion, and Dynamin 2, Pdcd4, and LIP.1, which are involved in signaling pathways that regulate apoptosis. These findings suggest that the inhibitory effects of COX-2 inhibitors, especially NS-398, on colon carcinogenesis may be associated with programmed cell death, cell proliferation, and cell-to-cell communication.

COX-2 Inhibitors in the Treatment of Colon Cancer

The results obtained from animal studies suggest that COX-2 inhibitors may be useful in the treatment of colorectal cancer. Nude mice, implanted with transformed human colon cancer cells (HCA-7) which express high levels of COX-2 protein, showed reduced tumor formation by 85% to 90% when treated with SC-58125, an experimental selective COX-2 inhibitor (16). On the other hand, the human colon cancer cells that lacked COX-2 expression were refractory to the inhibitory effect of the drug, suggesting that there was a direct link between colorectal cancer and COX-2. There is evidence that SC-58125 is cytostatic in vivo and exerts its effect presumably through inhibition of progression of the cell cycle at the G(2)/M phase (17).

A number of studies suggest that COX-2 blocking agents may possess chemopreventive activity against other cancers where COX-2 expression is increased. Elevated expression of COX-2 has been shown in human gastric adenocarcinoma tissues, human esophageal adenocarcinoma cells, and human pancreatic neoplasms (9). Dietary administration of celecoxib (1500 ppm) has been shown to decrease the incidence, multiplicity, and volume of 7,12-dimethyl-benz(a)anthracene-induced breast tumors in Sprague Dawley rats (18). The relevance of COX-2 inhibitors in the treatment of cancers is further supported by a cross-sectional study showing that COX-2 expression in colorectal cancer was directly associated with the stage and size of the tumor and inversely associated with patient survival (19).

Risk of Chronic NSAIDs Therapy for Cancer Prevention

Long-term use of aspirin and other conventional NSAIDs, even if used in low doses, may result in serious side effects, especially in elderly patients. Gastrointestinal side effects are serious and include dyspepsia, peptic ulcer disease, and significant bleeding with associated increased mortality. Other side effects include renal toxicity enhanced by the use of nephrotoxic medications, and unreversible platelet dysfunction associated with the use of anticoagulants (20). The side effects associated with nonselective NSAIDs and aspirin are thought to result from cytoprotective COX-1 inhibition. Therefore, the use of a selective COX-2 inhibitor with reduced gastrointestinal and other side effects should help to prevent the development and progression of CRC without the side effects from COX-1 inhibition (21).

Conclusions

Despite the surmounting evidence that NSAIDs, particularly the selective COX-2 inhibitors, are potentially useful anticancer drugs for the prevention or treatment of colorectal cancer, a number of questions regarding safety, efficacy, and mechanisms of action still remain.

The development of COX-2 inhibitors that are highly effective as anticancer agents requires complete understanding of their mechanism of action. This is complicated by a small number of studies suggesting that the anticancer effects of COX inhibitors may be independent of COX-inhibitory activity (22). For example, sulindac sulfone, which does not inhibit COX, prevents colorectal cancer in experimental animals as effectively as sulindac.

Cell culture and animal studies have contributed a great deal to our understanding of the potential of COX inhibitors as chemopreventive agents in colon cancer. Similar studies will continue to be important in screening new compounds for anticancer activity and in developing more effective and safer drugs through the identification of the molecular pathways of their action. Animal studies may also contribute to the characterization of colorectal cancer genes in humans to enable us to identify high-risk populations.

It is also important to achieve the delicate balance between risks and benefits associated with COX inhibitor therapy, particularly when a large number of high-risk individuals must be treated prophylactically for disease prevention. Once the efficacy and the safety of the experimental drug in question are established in the animal models, epidemiologic studies and carefully designed randomized clinical trials should help to identify drugs with proven clinical application for cancer prevention and treatment.

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