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REVIEW ARTICLE

Cyclooxygenase inhibitors in the chemoprevention of colorectal cancer

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Summary

The long-term use of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a decreased risk for colorectal cancer. A major molecular target for cancer chemoprevention by these agents is the cyclooxygenase-2 (COX-2) isoenzyme, although other molecular pathways can not be excluded. Data from both human and animal studies suggest that COX-2 is an early event of colorectal carcinogenesis. The NSAIDs, in the particular the newly developed COX-2 selective inhibitors, suppress colorectal tumor development in rodents and significantly reduce the number of colorectal polyps in patients suffering from familial adenomatous polyposis coli.

In this paper current opinions regarding the role of COX inhibitors in colorectal cancer prevention are reviewed. Some perspectives derived from experimental and clinical studies, that might improve future approaches in the prevention of this malignancy, are also considered.

Key words: chemoprevention, colorectal cancer, cyclooxygenase, nonsteroidal anti-inflammatory drugs

Introduction

For many years, investigations in the field of cancer prevention have been fallen behind the investigations of cancer treatment. However, the unsatisfactory outcome of the present treatment modalities and the rising expenses of the new chemotherapeutic agents without important long-lasting effects [1], were signals for changes in the strategies against cancer. So, much emphasis has been placed on developing better approaches in cancer prevention. Currently, the interest in studies concerning the potential of various agents to act in the prevention of cancer is growing rapidly. Data obtained from animal models and humans are promising, and offer a hope in reducing morbidity and mortality associated with cancer.

Colorectal cancer is thought to be an excellent model for studying cancer prevention by means of primary or secondary strategies, since it exemplifies typical progression from normal epithelium through inflammatory, metaplastic, or other intermediate stages, to dysplasia and invasive cancer [2].

The exploration of colon cancer chemoprevention has begun in clinical studies involving patients at increased risk for this malignancy, namely patients with familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNCC), or sporadic adenoma.

One of the most studied and promising approaches in the chemoprevention of colon cancer seems to be the use of NSAIDs, i.e. the inhibitors of the COX enzyme complex. The earliest hints that NSAIDs might be beneficial in colon cancer prevention came from clinical observations that rectal polyps disappeared in patients who had used regularly NSAIDs for a long time [3]. These observations are substantiated additionally by the findings of several retrospective and prospective clinical studies. They showed that chronic intake of aspirin or other NSAIDs led to a 40-50% reduction in the relative risk of developing

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rectal cancer [4-7]. Further investigations have led to the identification of the molecular targets of these agents. Although the exact mechanisms of the inhibitory action of NSAIDs on tumorigenesis are not completely elucidated at present, some of them are certainly related to the inhibition of COX. However, the possibility that NSAIDs exert their effects by modifying other, COX-independent, cellular proteins cannot be excluded.

COX and prostaglandin (PG) synthesis

COX has a key role in the PG synthesis from polyunsaturated fatty acids, such as arachidonic acid. After the release of phospholipids from the cellular membrane upon the enzymic action of phospholipases, free arachidonic acid is converted into PG H_2 by COX. PG H_2 serves as precursor for terminal prostaglandins. Specific synthases catalyze the conversion of prostaglandin H2, and products of a given tissue depend on which of these enzymes are expressed [8,9].

PGs are hormone-like regulatory molecules that have important functions in almost every organic system in the body. As autocrine or paracrine mediators of signal changes, they participate in the regulation of many physiological and homoeostatic processes such as cell growth and differentiation, and immunity [10]. PGs are also involved in various pathological processes including inflammation and autoimmune, rheumatic, and malignant diseases [11,12].

Two isoforms of COX, COX-1 and COX-2, have been identified. They differ in the expression pattern and function within an organism.

The COX-1 isoform, constitutively expressed in almost all tissues, is responsible for normal biosynthesis of PGs.

The COX-2 isoform is rapidly induced by a variety of stimuli, including mitogens, hormones, and a wide spectrum of growth factors and cytokines, in specific pathophysiological conditions [13,14]. Overexpression of COX-2 has been shown to mediate cell-cycle progression and contributes to many diverse processes such as apoptosis [15], angiogenesis [16], and tissue invasion [17].

COX-2 expression in colorectal cancer

Several studies have shown the markedly increased expression of COX-2 in about 85% of human colorectal cancer and in 50% of adenomas, while the expression of COX-1 in malignant tissue was unchanged in comparison to the normal mucosa [18-21]. COX-2 is rarely expressed in normal tissue. An elevated COX-2 expression, even in normal epithelial cells, was associated with more malignant phenotype and resistance to cell death by apoptosis [15]. These findings, coupled with data collected from animal models and epidemiological and clinical studies, suggest that abnormal induction of COX-2 may be one of the early events of colon carcinogenesis [22,23].

Presently, there is no consent about the type of cells expressing COX-2 within the tumor. Several studies in animal models and patients with sporadic human colorectal cancer revealed COX-2 expression in tumor epithelial cells [8,24,25]. In other studies, stromal expression of COX-2 was found [16,26,27]. These differences in COX-2 localization may be an integral part of natural variability between the tumor samples. It is also possible that various cell types within the same tumor express COX-2 [16]. Whether or not the exact localization within the tumor affects the COX-2 action on colorectal tumor growth remains uncertain for the moment.

COX-2 and tumor development

The most direct evidence of COX-2 involvement in colorectal tumorigenesis is derived from genetic studies in mice. In comparison to COX-2 wild type mice, genetically modified COX-$2^{-/-}$ mice have a reduced number and size of intestinal polyps. In addition, treatment of wild type mice with NSAIDs or selective COX-2 inhibitors results in diminished number of polyps [28]. Therefore, both down-regulation of COX-2 expression and specific inhibition of the enzyme activity independently suppress polyp formation. A causal relationship between COX-2 expression and cell transformation and development of premalignant lesions was found in investigations conducted in transgenic mice [29, 30].

PGs derived from COX-2 activation in tumor epithelial cells exert their effect on malignant epithelial cells themselves. Thus, tumor cells expressing COX-2 are more sensitive to the action of specific COX-2 inhibitors than COX-2 negative cells [31]. The COX-2 overexpression in intestinal epithelial cells leads to changes in cellular pathways related to carcinogenesis e.g. elevation of the bcl_2-antiapoptotic protein, or elevation of metalloproteinases. Such changes are associated with increased resistance to apoptosis [15] and increased cell migration and invasion [17]. Similar findings are obtained using other

cancer cell lines [32]. The action of COX-2-derived PGs on the tumor stromal compartment leads to the stimulation of angiogenesis and/or to the suppression of antitumor immune functions [12,33-35].

PGs induced by COX activation exert their effects by binding to cell surface receptors that leads to changes in the level of cellular cAMP and Ca^{++} [36]. Additionally, PGs can modulate cellular responses by direct action on cell nucleus activating the peroxisome proliferator activated receptors (PPAR) family of nuclear hormone receptors [36,37]. However, direct evidence in support of the role of COX-2-induced PG subtypes and their receptors in colorectal cancer progression is limited. In comparison to normal colon mucosa, elevated levels of PGE_2 are found in tumor biopsies [38,39]. The importance of this finding is not completely clear since many PGs are markedly unstable and systematic investigations of COX-2 and PGs receptors in colorectal cancer are lacking. However, some data gained by cancer cell culture experiments support a possible procarcinogenic role of PGs. PGE_2 treatment of different human colon cancer cell lines leads to an increased level of *bcl*$_2$, which was associated with reduced apoptosis and increased proliferation and motility of tumor cells [40,41]. In order to elucidate the exact role of various PGs in colorectal cancer progression, further investigations are needed.

NSAIDs and colorectal cancer: old drugs, new applications

Because of their anti-inflammatory and analgesic effects, NSAIDs are the most widely used drugs in the history of medicine. Among them, aspirin was firstly discovered and synthesized. Several other agents with similar properties, commonly named NSAIDs, have also been introduced in clinical practice. The relationship between NSAIDs and COX activity was observed during the 1970s of the 20th century. The identification of two isoforms of COX enzyme, as well as the linking of gastrointestinal side effects of traditional NSAIDs to the inhibition of COX-1 isoform, promoted the synthesis of a new class of NSAIDs specifically inhibiting COX-2. These agents exhibit equal or better therapeutic effects with lower toxicity than traditional NSAIDs.

After the first promising effects of NSAIDs in colon cancer prevention observed in chronic users of aspirin, encouraging data were collected from several randomized, double-blind clinical trials including patients with FAP. Initially, these patients were treated with traditional NSAIDs, and subsequently, specific COX-2 inhibitors were introduced [42,43]. Furthermore, the effects of NSAIDs on colon tumor cell growth were confirmed in various animal models [22, 44-46]. The antineoplastic properties of these compounds are mainly related to their ability to inhibit COX-2 isoenzyme, although modification of other cellular proteins is also possible.

COX-dependent effects of NSAIDs

Most data support the notion that COX-2 is the main target of the NSAIDs action in colon cancer prevention. COX-2 expression has been established in colon adenoma and carcinoma, both in rodents and humans [18,22,28]. Non-selective or COX-2 selective NSAIDs decrease the incidence and multiplicity of colon tumors in carcinogen-induced neoplasia in rats [23], and adenomas in murine adenomatous polyposis coli (APC) models [45]. Tumor cells expressing COX-2 are more sensitive to the action of COX-2 inhibitors than COX-2-negative cells [31]. The fact that down-regulation of COX-2 expression and specific inhibition of the enzymatic activity by NSAIDs independently suppress polyp formation in mouse model of FAP represents a direct evidence of COX-2-dependent action of NSAIDs in colon cancer prevention.

Since selective COX-2 inhibitors were equal or more active in polyp prevention than the non- selective ones, and since COX-2 inhibitors enabled a wider and safer therapeutic usage, these agents may be advantageous in colon cancer prevention.

Doubts over the selectivity of COX-2 inhibitors have been raised, since high doses of these agents used in some investigations [46, 47] have resulted in high tissue concentrations, indicating loss of their selectivity. However, lower doses of COX-2 inhibitors, which sustain their selectivity, were shown to induce diminished proliferation and increased apoptosis of cancer cell lines [32,48]. On the other hand, an open question remained whether these *in vitro* effects are relevant to *in vivo* effects, since the drug concentrations needed for the induction of cell death in cell cultures are several times higher than serum concentrations needed for tumor growth inhibition in animal models [45, 46].

COX-independent effects of NSAIDs

The NSAIDs may affect cell growth and behavior independently of COX expression, by acting on other cell molecular targets. One of the potential alternative biochemical targets for COX-independent

effects of NSAIDs may be the NF-kB transcription factor. This factor is involved in the regulation of genes controlling the inflammation and growth of several cells and tissues. The NSAIDs may inhibit an enzyme that activates NF-kB signalling [49]. A direct target for NSAIDs may also be the transcription factor PPAR, a member of the nuclear hormone receptors superfamily [50]. Recent evidence suggests a role of the proapoptotic BAX gene in the NSAIDs-induced apoptosis of colon cancer cells [51].

Another mechanism by which NSAIDs may affect tumorigenesis is the inhibition of angiogenesis, acting directly on endothelial cells, without respect to COX expression [52]. Acting on collagenase MMP-2 production, an enzyme necessary for basement membrane degradation, these agents may influence invasion and metastatic potential of malignant cells [53]. It is possible that each of these mechanisms, either independently or associated with diminished PGs synthesis, is involved in the inhibitory action of NSAIDs on tumorigenesis.

According to the current knowledge, it seems that the chemopreventive effects of NSAIDS may only be achieved by their long-term use. The aspirin concentrations needed for its toxic effect on tumor cells e:*in vitro* exceed the *in vivo* tolerable concentrations. Thus, it remains to be resolved the balance between cumulative toxicity and growth inhibition in order to exploit the activity of NSAIDs in cancer prevention.

Clinical potential of NSAIDs in colorectal cancer prevention

Familial adenomatous polyposis (FAP)

FAP is an autosomal dominant hereditary disease, characterized by an increased risk for colorectal cancer. In the general population FAP is responsible for less than 1% of colorectal cancers.

In patients with FAP, a mutation of the adenomatous polyposis gene, located on chromosome 5q21, has been identified [54]. As a consequence, multiple adenomas develop, typically during adolescence. If prophylactic surgical treatment is not carried out, the risk for colorectal cancer is nearly 100%. Even after surgery, patients with FAP remain at risk for intestinal cancer [2]. This is the reason for targeted surveillance and chemopreventive interventions in these patients.

Data obtained from uncontrolled clinical studies showed that traditional NSAIDs induced nearly complete regression of rectal adenomas in FAP patients [55,56]. These findings were not completely confirmed in other placebo-controlled clinical trials, although the effects were undisputed [42,43]. Furthermore, a reversible trend was observed, i.e. adenomas reappeared after termination of NSAIDs therapy. However, the toxicity of traditional NSAIDs limits their long-term application in cancer prevention [57].

Recently, data on the usage of the selective COX-2 inhibitors became available [58,59]. In a double-blind, placebo controlled study by Steinbach et al. [59], 6-month therapy of FAP patients with the COX-2 inhibitor celecoxib induced statistically significant reduction in the size and number of adenomas, without intolerable side effects. The American Food and Drugs Administration (FDA) approved this agent for use in FAP and proposed some recommendations for additional studies. One of them is to investigate the effectiveness of this drug in the prevention of adenomas in young, phenotype-negative population with APC gene mutations. A further recommendation is to describe the clinical benefit of celecoxib in FAP patients comprising a registry of clinical outcomes (FAP-related surgery, colorectal cancer or death) [2]. If the preventive action of celecoxib on the onset of adenoma or its delay in young persons carrying APC mutations might be confirmed, it would be possible to postpone surgical intervention for several years, or to delay secondary surgery in previously operated patients.

Presently, since some polyps remain, even after long-term administration of COX-2 inhibitors, it is unlikely that these agents alone can be used in the treatment of adult FAP patients with developed polyps [59]. It seems that COX-2 inhibitors can be merely useful as adjunct therapy to surgery in the management of this disease.

Another potentially more promising approach in the chemoprevention of colorectal cancer is based on the combination of COX inhibitors with agents targeting other oncogenic pathways. Such a pathway might be the ERBB/HER receptor family. ERBB ligands increase colon cancer cell proliferation [60]. The level of ERBB2 (HER2/neu) expression increases with the stage of colon cancer and correlates with decreased relapse-free interval [61]. In mouse models for human FAP (APC^{min} mice), complete polyp suppression was achieved by concomitant treatment with sulindac and an inhibitor of ERBB kinase [62]. Additive inhibitory effect on colon cancer cell growth was also obtained by the combination of a selective COX-2 inhibitor and anti-ERBB2 monoclonal antibodies [63].

Hereditary nonpolyposis colorectal cancer (HNCC)

HNCC is a familial multiple primary colorectal cancer syndrome, characterized by autosomal dominant mode of inheritance. Adenomas are usually not present, but if they exist, they number only low. The mean age of cancer appearance is about 45 years and the age distribution is similar to sporadic colorectal cancer. As in FAP, in the prevention and management of this malignancy, it is important to recognize a risk, i.e. familial pattern of the onset of the disease. The offspring of affected patients have a cancer risk of nearly 50%. Molecular genetic examinations revealed mutations in one of the HNCC genes in about 85% of the families. If the mutation is identified, offspring of affected patients may be divided into two groups: noncarriers with general population risk, and carriers whose risk approaches 100%. In addition to instability of microsatelite markers and relative paucity of tumor suppressor gene mutations, low expression of COX-2 protein is found in HNCC [2,63]. So, it may be expected that COX-2 inhibition will be less effective than in FAP and sporadic colorectal cancer. Since the incidence of adenomas in HNCC is relatively low, clinical trials are difficult to be conducted in order to show reduction in the incidence of adenomas.

Sporadic colorectal adenoma

Sporadic, nonfamilial adenoma or cancer is a problem more common than FAP and HNCC, although the risk of incident neoplasia is comparatively low. No data regarding the usefulness of COX-2 inhibitors in the prevention of sporadic colorectal cancer are currently available. The encouraging effects of these agents in patients with FAP stimulated the beginning of clinical research in patients with sporadic adenoma. In a recently opened, multicenter study, cited in the article by Lynch [2], patients with histologically verified sporadic adenoma are randomly assigned to receive high-dose celecoxib or placebo. The primary endpoint of this study is adenoma recurrence at 12 and 36 months following study entry. Published data of this study are not available thus far.

Colorectal cancer in inflammatory bowel disease (IBD)

Whether COX-2 inhibitors may be useful in the prevention of colon cancer arising in the setting of IBD is currently unclear. Induction of COX-2 in active foci of IBD in humans and colitis-associated tumor in mice has been reported [27,64,65]. On the other hand, there is evidence indicating a possible protective role of COX-2 in intestinal inflammation, i.e. COX-2 products may modulate immune response to food antigens [66,67].

Conclusion

A growing body of data derived from animal models and clinical trials suggest potential importance of NSAIDs, especially COX-2 inhibitors, in the prevention of colorectal cancer. However, the usefulness of these agents in the treatment of intestinal adenomas and in the prevention of colorectal cancer is currently approved only in patients with FAP. Further investigations are needed to elucidate the exact role of COX-2 inhibitors in the prevention of colorectal cancer in other patient populations at risk. In order to provide safe, effective chemoprevention of this malignancy, special attention should be directed on the combination of COX inhibitors with other agents targeting distinct oncogenic pathways. Molecular genetic investigations in patients at risk for colorectal cancer might be helpful in designing chemopreventive strategies. New insight into molecular biology might also provide clues to identify high-risk populations that are more likely to benefit from chemopreventive approaches.

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