Is COX-2 a ‘collateral’ target in cancer prevention?

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Abstract
NSAIDs (non-steroidal anti-inflammatory drugs) prevent colon and other cancers. The fact that NSAIDs inhibit the eicosanoid pathway prompted mechanistic drug-developmental work focusing on COX (cyclo-oxygenase) and its products. The increased prostaglandin E2 levels and the overexpression of COX-2 in colon and many other cancers provided the rationale for clinical trials with COX-2 inhibitors for cancer prevention or treatment. However, one COX-2 inhibitor has been withdrawn from the market because of cardiovascular side effects, and there are concerns about a class effect. Evidence suggests that COX-2 may not be the only, or the ideal, target for cancer prevention; for example, COX-2 is not expressed in human aberrant crypt foci, the earliest recognizable pre-malignant lesion in the colon; COX-2 is expressed in less than half of the adenomas; in vitro data show that NSAIDs do not require the presence of COX-2 to prevent cancer; in familial adenomatous polyposis, the COX-2 inhibitor, celecoxib, had a modest effect, which was weaker than that of a traditional NSAID; and COX-2-specific inhibitors have several COX-2-independent activities, which may account for part of their cancer-preventive properties. The multiple COX-2-independent targets, and the limitations of COX-2 inhibitors, suggest the need to explore targets other than COX-2.

Introduction
Cancer prevention, at present a better option than cancer treatment, is entering an era when it appears to be a realistic possibility. The seminal epidemiological observation that NSAIDs (non-steroidal anti-inflammatory drugs) prevent colon, and possibly other, cancers has led to the unambiguous demonstration that aspirin does prevent colon cancer. Two randomized interventional studies using polyp recurrence as a general end point demonstrated the chemopreventive effect of aspirin [1,2]. The relative risks following administration of aspirin ranged between 0.59 and 0.96, depending on the specific end point and aspirin dose. Although specific aspects of this effect appear unclear at this point, these studies, nevertheless, constitute proof-of-principle for pharmacological cancer prevention. However, NSAIDs are ill-suited for widespread application as chemopreventive agents. Their two prohibitive limitations concern their safety (among patients using NSAIDs, up to 4% per year suffer serious gastrointestinal complications) and efficacy (NSAIDs can prevent at best 50% of colon cancer) (reviewed in [3]). To these, one should add the need to have more stringent criteria for safety and efficacy for chemoprevention, as opposed to chemotherapy, when one deals with a life-threatening cancer.

Considerations of safety and efficacy have prompted the search for a ‘better NSAID’, with coxibs, selective inhibitors of COX-2 (cyclo-oxygenase-2), being the most notable outcome. Coxibs have been developed based on the notion that inhibition of COX-2, the induced isoform of COX, will diminish the pro-inflammatory activities of COX, whereas sparing COX-1, the constitutive isoform of COX, will diminish the gastrointestinal, and perhaps other, side effects of NSAIDs [4]. Recent concerns on the safety of coxibs, especially after their long-term use, justify a re-examination of the fundamental tenet underlying their use in cancer, namely that COX-2 is central to the pathogenesis of several cancers, and that its inhibition would prevent them and regress those already established.

The rationale for COX-2 as a molecular target for cancer prevention
The initial response of many investigators, including ourselves, to the epidemiological data showing that NSAIDs are associated with a decreased incidence of cancer, was that NSAIDs act by inhibiting COX, an important enzyme in the eicosanoid cascade that ultimately leads to PGs (prostaglandins) and related compounds [4]. Thus we demonstrated that, in human colon cancers, PGE2 levels were strikingly increased compared with uninvolved mucosa [5]. Subsequently, Tunni and DuBois [6] demonstrated overexpression of COX-2 in 45% of colon adenomas and 85% of colon carcinomas. COX-2 is overexpressed to varying degrees in several more human cancers, including gastric, breast, lung, oesophageal and hepatocellular carcinomas. Additional mechanistic studies showed that PGE2 increases colon cancer cell proliferation [7] and suppresses apoptosis [8]. The role of eicosanoids in carcinogenesis has been expanded further by studies demonstrating that, in certain cases, LOX (lipoxygenase) products may also play a role in carcinogenesis [9] (Figure 1). The conclusion that inhibition of COX-2 would arrest carcinogenesis has been supported by a constellation of cell culture, animal and human studies, culminating in...
celecoxib receiving FDA (U.S. Food and Drug Administration) approval for cancer prevention in patients with FAP (familial adenomatous polyposis).

Studies using genetically modified animals have indicated that COX-2 may be required for tumorigenesis. Deletion of COX-2, and, importantly, of COX-1 as well, decreased significantly the number of intestinal tumours in Apc<sup>−/−</sup> mice [10]. Overexpression of the human COX-2 gene in the mammary glands of female mice led to focal-mammary-gland hyperplasia, dysplasia, and transformation into metastatic tumours [11]. Overexpression of COX-2 in basal epidermal cells of transgenic mice was either insufficient for tumour induction (although it sensitized the tissue to carcinogens) [12] or, rather surprisingly, protected them from developing tumours that were induced by an initiation/promotion protocol [13]. Alternatively, numerous animal studies have shown that coxibs prevent tumours arising from a variety of tissues [6].

The limitations of current coxibs

The APPROVe (Adenomatous Polyp Prevention on Vioxx) study was designed to evaluate the efficacy of rofecoxib in preventing colon cancer. During the trial, which involved 2600 subjects with a history of colorectal polyps, 3.5% of rofecoxib recipients and 1.9% of placebo recipients suffered myocardial infarctions or strokes. This led to the termination of this and all related trials and the permanent withdrawal of rofecoxib. It is still controversial whether other coxibs share this side effect, but concerns for a ‘class (side) effect’ have been voiced [14].

To explain this side effect, it was suggested that inhibition by coxibs of COX-2, the principal enzyme involved in the production of PGI2 (prostacyclin), tips the balance towards platelet aggregation and vasoconstriction [14]. As discussed below, this may constitute a limiting side effect of coxibs for their required long-term application in cancer prevention.

Is COX-2 overexpression central to carcinogenesis?

Several observations suggest that it may be worth reassessing the notion that COX-2 is central to the pathogenesis of several cancers, and therefore its inhibition should be the prime target of cancer chemoprevention. Below, we outline data that are at variance with this notion.

The pattern of COX-2 expression

Taking colon carcinogenesis as an example, it is apparent that the pattern of COX-2 expression is not entirely consistent with the idea that COX-2 is central to carcinogenesis. COX-2 expression is absent in aberrant crypt foci, the earliest recognizable pre-malignant lesion in the colon [15], and commences only at the adenoma stage (45% of them), increasing in frequency (85%) in carcinomas [6]. An unconventional look at the data may suggest that COX-2 expression is the result of, and not a dominant contributor to, carcinogenesis. In support of this idea is the finding that targeted overexpression of human microsomal PGE synthase-1 (mPGES) in the alveolar type II cells of transgenic mice, accompanied by highly elevated PGE2 production (12.2-fold over control), failed to induce lung tumours [16].

NSAIDs and COX-2 in cancer prevention

NSAIDs do not require the presence of COX-2 to prevent cancer [17]. This was based on our finding that in vitro
Coxibs have only limited clinical efficacy in cancer prevention

In FAP patients, celecoxib reduced the mean number of colorectal polyps by 28% and the polyp burden by 30.7% (the respective placebo reductions were 4.5% and 4.9%) [18]. Rofecoxib had a statistically significant, but marginal, effect on the number of polyps in FAP patients (6.8% reduction from baseline values) [19]. In contrast, the NSAID sulindac had a more pronounced effect on colorectal polyps in FAP patients, being around twice as effective as celecoxib and far more effective than rofecoxib [20].

Coxibs have several COX-2-independent activities

This may account, at least in part, for coxibs’ cancer-preventive properties (Figure 2). For example, celecoxib inhibits the growth of various cancer cell lines [21], including haematopoietic cell lines that are COX-2-deficient. Interestingly, celecoxib also inhibited the growth of COX-2-deficient colon cancer xenografts in nude mice [22]. Moreover, a selective COX-2 inhibitor reduced tumour growth and angiogenesis in COX-2-positive pancreatic cancer, but in COX-2-negative pancreatic cancer, it increased angiogenesis and tumour growth [23]. Thus the chemopreventive effect of COX-2-specific inhibitors may be due to their effect on these targets and not on COX-2.

The expression of COX-2 is not restricted to tumour cells

While COX-2 is undetectable in most tissues in the absence of stimulation, it is induced in cells such as monocytes, macrophages, neutrophils and endothelial cells [4]. A study in healthy humans suggests that COX-2 is a major source of systemic PGI2 biosynthesis [24]. Importantly, atheromatous lesions contain both COX-1 and COX-2, co-localizing mainly with macrophages of the shoulder region and lipid core periphery, whereas smooth-muscle cells show lower levels [25]. Inhibition of vascular COX-2 may shift the delicate balance between TxA2 (thromboxane A2) and PGI2, which have opposite effects on platelets and vascular tone. Shifting this balance in the wrong direction (reduction of PGI2) could have catastrophic effects. Indeed, this may account for the cardiovascular side effects of coxibs. If such a mechanism is proven, it may be of great importance to chemoprevention, in which a chemopreventive agent against cancer will be administered on a long-term basis to older subjects, i.e. those likely to have atheromatous lesions.

Inhibition of COX may shift its substrate fatty acid to a non-COX pathway and generate a pro-carcinogenic end product

For example, inhibition of COX-2 could shift arachidonic acid to the LOX pathway, some of whose products have pro-tumorigenic activities. Although this possibility has not been systematically explored, a recent study suggests that it may not be that unlikely; in humans, under physiological conditions, oral celecoxib increased leukotriene B4 production in the lung micro-environment [26].

Time to search for targets beyond COX-2

It is apparent that the central concept of a dominant role of COX-2 in cancer prevention may have significant limitations that make necessary its re-examination. If the cardiovascular toxicity of coxibs is in fact due to their COX-2 effects, then it may be difficult to envision their practical long-term administration to individuals who, in the context of atherosclerosis, may have endothelial COX-2 overexpression. Thus alternative approaches should focus on targets beyond COX-2.

The two main reasons justifying the search for targets other than COX-2 are the following: first, NSAIDs prevent colon and other cancers and do this sub-optimally, probably by modulating several molecular targets in addition to COX-2. NSAIDs are not reasonable candidates for chemoprevention, owing to their safety and efficacy limitations. Secondly, coxibs have limited clinical efficacy; it is likely that they achieve their clinical effect by modulating targets other than COX-2, and they may have limiting side effects.

At this time, strategies incorporating these considerations may lead to the next, and, one hopes, final, stage in our efforts to prevent cancer. Such an approach appears to be both rational and promising.

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References


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