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Review Article

ROLE OF CYCLOOXYGENASE - 2 IN CANCER

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ABSTRACT

Prostaglandins are formed from arachidonic acid by the action of cyclooxygenase (COX) and subsequent downstream synthetases. Two closely related forms of the cyclooxygenase have been identified which are now known as COX 1 and COX 2. Both isoenzymes transform arachidonic acid to prostaglandins, but differ in their distribution and their physiological roles. COX 1, is the pre-dominantly constitutive form of the enzyme. In contrast, the inducible form is expressed in response to inflammatory and other physiological stimuli and growth factors, and is involved in the production of the prostaglandins that mediate pain and support the inflammatory process. Apart from its involvement in inflammatory processes, COX 2 seems to play a role in angiogenesis, colon cancer and Alzheimer's disease, based on the fact that it is expressed during these diseases. Cyclooxygenase 2 (COX 2), an inducible prostaglandin synthase is over expressed in several human cancers. Here, the potential utility of selective COX 2 inhibitors in the prevention and treatment of cancer is considered. The mechanisms by which COX 2 levels increase in cancers, key data that indicate a causal link between increased COX 2 activity and tumorigenesis, and possible mechanisms of action of COX 2 are discussed. In a proof-of-principle clinical trial, Selective COX 2 inhibitors appear to be sufficiently safe to permit large-scale clinical testing and numerous clinical trials are currently under way to determine whether selective inhibitors of COX 2 are effective in the prevention and treatment of cancer.

Keywords: COX 2, Prostaglandins, Tumorigenesis, Selective COX 2 inhibitors.

INTRODUCTION

Multiple lines of evidence indicate that cyclooxygenase 2 (COX 2) is a bona fide pharmacological target for anticancer therapy. Epidemiological studies show that use of non-steroidal anti-inflammatory drugs (NSAIDs), which are prototypic inhibitors of COX, are associated with a reduced risk of several malignancies, including colorectal cancer[1]. Consistent with this, tumor formation and growth are reduced in animals that are either engineered to be COX 2 deficient or treated with a selective COX 2 inhibitor[2–8]. The finding

that NSAIDs inhibit COX suggested that prostaglandins, the products of COX activity, substantially contribute to carcinogenesis. For example, COX-derived prostaglandins have been implicated in angiogenesis[9, 10]. The recent development of selective inhibitors of the inducible form of COX, COX 2, represents another important advance. Importantly, selective COX 2 inhibitors cause fewer serious

adverse effects than traditional NSAIDs[11, 12]. The improved safety profile of selective COX 2 inhibitors makes it realistic to consider their long-term use in individuals at low to moderate risk of cancer. This review focuses on the rationale for using selective COX 2 inhibitors to prevent cancer.

Prostaglandin synthesis

Arachidonic acid, a 20-carbon polyunsaturated fatty acid precursor of prostaglandins, is found almost exclusively as an ester at the 2-position of membrane phospholipids. The first step in prostaglandin synthesis is hydrolysis of phospholipids to produce free arachidonate and this reaction is catalysed by phospholipase A_2 (Figure 1). Next, in a key reaction catalysed by COX, molecular oxygen is inserted into arachidonic acid to produce an unstable intermediate, prostaglandin G2 (PGG_2), which is rapidly converted to prostaglandin H2 (PGH_2) by the peroxidase activity of COX. Specific isomerases then convert PGH_2 to different prostaglandins and thromboxanes. Each product derived from PGH_2 has its own range of biological activities. There are two isoforms of COX: COX 1 and COX 2[13, 14]. COX 1 is expressed constitutively in most tissues and seems to mediate production of prostaglandins that control normal physiological functions, such as maintenance of the gastric mucosa and regulation of renal blood flow. COX 2, on the other hand, is undetectable in most normal tissues. It is induced by proinflammatory and mitogenic stimuli and increases the synthesis of prostaglandins in inflamed and neoplastic tissues[15].

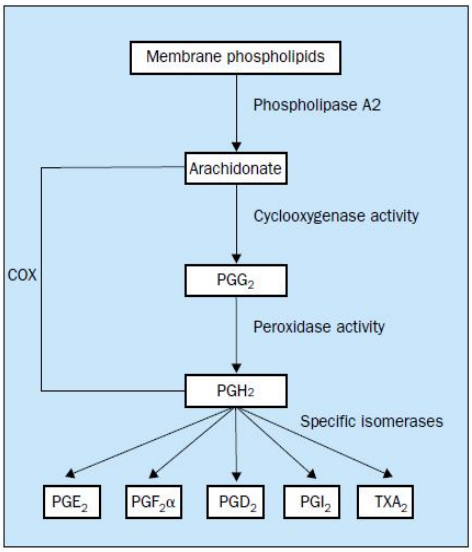

Fig. 1: Prostaglandin biosynthesis. PG: prostaglandin; TX: thromboxanes.

Selective inhibition of COX 2 can be achieved despite the very similar structures of the active sites of COX 1 and COX 2 proteins. A single substitution of isoleucine in COX 1 with valine in COX 2 at the NSAID binding site creates a larger active site with a void volume. Compounds designed to bind in this additional space are potent and selective inhibitors of COX 2[16].

Evidence that COX 2 contributes to carcinogenesis

Increased amounts of COX 2 are commonly found in both premalignant tissues and malignant tumors[17-37] (Table 1).This finding appears to reflect the effects of oncogenes, growth factors, and tumor promoters and other known inducers of COX 2[38-40].

Table 1: COX 2 is overexpressed in various premalignant and malignant conditions in man

• Colorectal adenomas and cancer • Gastric intestinal metaplasia and cancer • Barrett's oesophagus and oesophageal cancer • Chronic hepatitis and hepatocellular carcinoma • Pancreatic cancer • Oral leucoplakia and head and neck cancer • Atypical adenomatous hyperplasia and non-small-

cell lung cancer
- Ductal carcinoma *in situ* and breast cancer
- Prostatic intraepithelial neoplasia and cancer
- Bladder dysplasia and cancer
- Cervical dysplasia and cancer
- Endometrial cancer
- Actinic keratoses and skin cancer
- Glioma

There is extensive evidence, beyond the finding that COX 2 is commonly overexpressed in tumors, to suggest that COX 2 is mechanistically linked to the development of cancer. The most specific data supporting a cause-effect relation between overexpression of COX 2 and carcinogenesis come from genetic studies. Recently, Hla and colleagues[41] bred transgenic mice that overexpressed the human *COX 2* gene specifically in mammary glands. Multiparous females had a high frequency of focal mammary gland hyperplasia, dysplasia, and transformation into metastatic tumors. These observations support the idea that enhanced expression of COX 2 is sufficient to induce tumorigenesis. In a separate study, knocking out the *COX 2* gene in the $Apc^{\Delta 716}$ mouse (a model for human familial adenomatous polyposis) reduced the number and size of intestinal polyps[42]. This effect was gene dose dependent. Thus, an 86% reduction in the number of intestinal polyps occurred in mice lacking *COX 2*, but knocking out a single copy of the *COX 2* gene led to a 66%decrease in the number of polyps. COX 2 deficiency also protects against the formation of other tumor types. *COX 2* knockout mice developed about 75% fewer chemically induced skin papillomas than control mice[43].

Pharmacological evidence also implicates COX 2 in tumorigenesis. Selective inhibitors of COX 2 such as celecoxib and rofecoxib reduced the formation of intestinal, breast, skin, lung, bladder, and tongue tumors in animals[44-51]. In addition to preventing tumorigenesis, selective COX 2 inhibitors suppress the growth of established tumors including head and neck, colorectal, stomach, lung, breast, and prostate tumors[52-57].

Mechanisms by which COX 2 contributes to cancer

COX 2 affects many processes that are important in carcinogenesis, which makes it an attractive therapeutic target. These include xenobiotic metabolism, angiogenesis, apoptosis, inflammation, and immunosuppression:

Xenobiotic metabolism

COX is a bifunctional enzyme that has both cyclooxygenase and peroxidase activities (Figure 1). The cyclooxygenase activity of COX oxidises arachidonic acid to PGG_2 and its peroxidase activity converts PGG_2 to PGH_2. The latter also catalyses the conversion of procarcinogens such as benzo[a]pyrene to carcinogens[58, 59] (Figure 2). In the liver, these types of oxidative reactions are mainly catalysed by cytochrome P450s. However, many tissues outside the liver, such as the colon, have low concentrations of P450s and other mono-oxygenases. In these cases, substantial amounts of xenobiotics can be co-oxidised to mutagens by the peroxidase activity of COX. This activity is probably especially relevant at organ sites such as lung, oral cavity, and bladder, which are exposed to tobacco carcinogens.

Furthermore, the metabolism of arachidonic acid by COX produces mutagens. For instance, by-products of the oxidation of arachidonic acid such as malondialdehyde are highly reactive and form adducts with DNA. There is also evidence that COX 2-derived prostaglandin endoperoxides can be metabolised by selected P450s to malondialdehyde[60]. This pathway could also contribute to genomic instability in developing cancers. In addition to catalysing the synthesis of mutagens, COX 2 can be induced by procarcinogens. For example, benzo[a]pyrene, a polycyclic aromatic hydrocarbon in tobacco smoke and chargrilled foods, can stimulate transcription of COX 2[61]. In turn, COX 2 catalyses the conversion of benzo[a]pyrene-7,8-diol to benzo[a]pyrene-7,8-diol-9,10-epoxide, which binds to DNA (Figure 2). These findings raise the possibility that benzo[a]pyrene mediated induction of COX 2 facilitates its own conversion to benzo[a]pyrene-7,8-diol-9,10-epoxide, thereby amplifying the effect on tumor initiation of a given dose of benzo[a]pyrene. Although the significance of this mechanism is uncertain, it

does suggest that inhibiting COX 2 could be helpful in preventing cancers related to tobacco smoke or other sources of benzo[a]pyrene.

Angiogenesis

The growth of tumors depends on an increase in blood supply. Tumor cells ensure their own growth by secreting growth factors such as vascular endothelial growth factor (VEGF) that stimulate angiogenesis. COX 2 has been implicated in this aspect of carcinogenesis as well. Overexpression of COX 2 in colon cancer cells increases the production of vascular growth factors, the migration of endothelial cells through a collagen matrix, and the formation of capillary-like networks *in vitro*[62]. These effects can be blocked by NS-398, a selective inhibitor of COX 2. Two recent studies also showed the importance of COX 2 in angiogenesis[63].

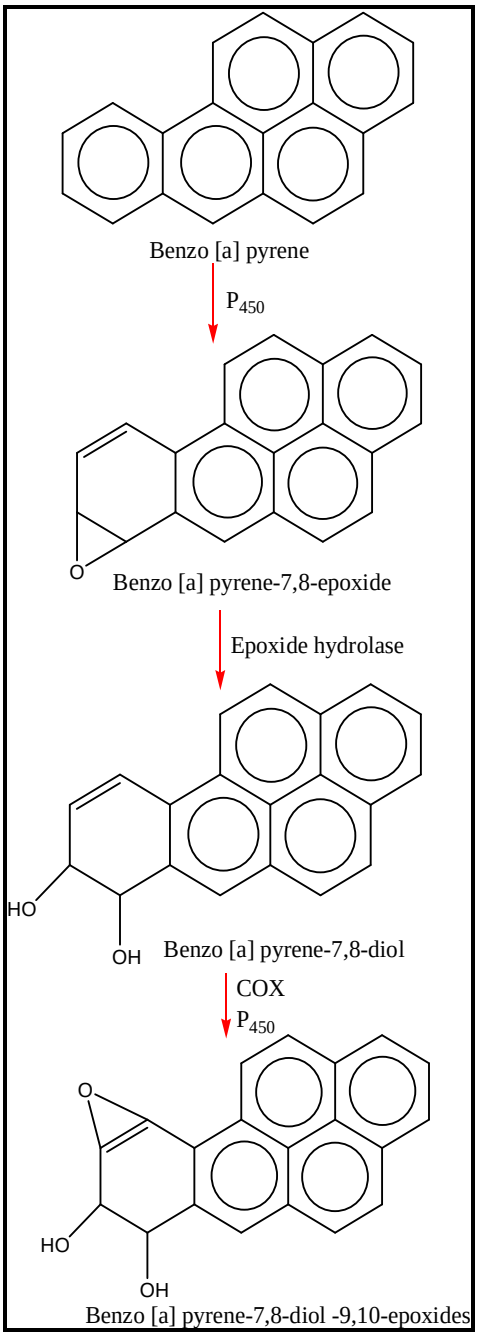

Fig. 2: Schematic illustration of the metabolism of benzo[a]pyrene. COX 2 can convert benzo[a]pyrene-7, 8-diol to the ultimate carcinogen benzo[a]pyrene-7, 8-diol-9, 10-epoxide.

Apoptosis

The size of a cell population depends on the balance between cell proliferation and cell death. Decreased apoptosis has been observed in premalignant and malignant neoplasms. Of

the many factors that regulate apoptosis, there is an inverse relation between levels of BCL 2 and apoptosis. Rat intestinal epithelial cells engineered to overexpress COX 2 stably have increased amounts of BCL 2 and are resistant to butyrate-stimulated apoptosis. Treatment with the NSAID sulindac sulphide reversed the resistance to apoptosis conferred by overexpression of COX 2[64]. As described above, overexpression of COX 2 led to mammary cancer. Decreased amounts of the proapoptotic proteins, BAX and BCL x_L, and increased amounts of the antiapoptotic protein BCL 2, were detected in mammary tumor tissue. Taken together, these data show a clear causal linkage between expression of COX 2 and inhibition of programmed cell death. Possibly, up-regulation of COX 2 prolongs the survival of abnormal cells and thereby favours the accumulation of sequential genetic changes that increase the risk of tumorigenesis.

Inflammation and immunosuppression

Chronic inflammation is a recognised risk factor for epithelial carcinogenesis[65]. Inflammation is associated with increased synthesis of prostaglandins partly through cytokine-mediated induction of COX 2. Therefore, the data reviewed above provide a basis for a cause-and-effect link between chronic inflammation and carcinogenesis via overexpression of COX 2 and thereby suggest a reasonable mechanism by which chronic inflammation increases the risk of cancer. The growth of tumors is typically associated with immune suppression[66]. Colony-stimulating factors released by tumor cells activate monocytes and macrophages to synthesise prostaglandin E_2 (PGE_2), which inhibits the production of immune-regulatory lymphokines, T-cell and B-cell proliferation, and the cytotoxic activity of natural killer cells. PGE_2 also inhibits the production of tumor necrosis factor α while inducing the production of interleukin 10, which has immunosuppressive effects[67]. Selective inhibition of COX 2 is believed to promote antitumor activity by restoring the balance between interleukin 10 and interleukin 12 *in vivo*.

Aromatase activity

Estrogen deprivation is an effective therapy for the prevention and treatment of hormone-dependent breast cancer. The final step in estrogen biosynthesis is catalyzed by aromatase cytochrome P450 (aromatase), the product of CYP19[68]. PGE_2 increases aromatase activity in cells in culture and, thus, should stimulate cell proliferation indirectly by increasing estrogen biosynthesis. In this model (Figure 3), overexpression of COX 2 in neoplastic breast cells leads to increased production of PGE_2, which in turn, stimulates the expression of CYP19 in stromal cells. Consequently, estrogen biosynthesis is enhanced, which leads to increased growth of neoplastic epithelial cells. Consistent with this, there is a positive correlation between expression of CYP19 and the level of COX in specimens of human breast cancer[69]. This implies that inhibiting the production of estrogen in breast tissue using a selective COX 2 inhibitor might be useful for either preventing or treating breast cancer.

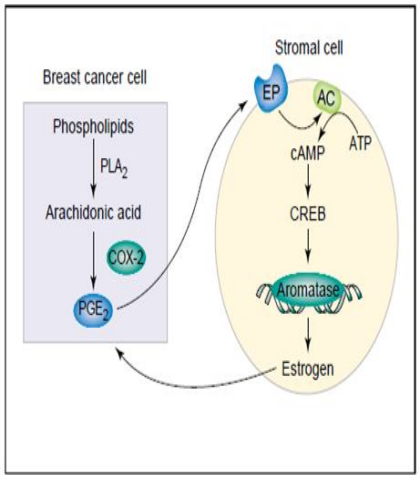

Fig. 3: Prostaglandin E_2 (PGE_2) produced by tumor cells stimulates expression of the gene encoding aromatase (CYP19). Overexpression of cyclooxygenase 2 (COX 2) in breast cancer cells leads to increased PGE_2 synthesis. In turn, PGE_2 stimulates expression of the gene encoding aromatase in stromal cells via a paracrine mechanism. In this mechanism, binding of PGE_2 to its receptor(s) stimulates adenylyl cyclase (AC) activity and increases production of cAMP, which stimulates expression of the gene encoding aromatase via CREB (cAMP response element binding protein). Consequently, estrogen biosynthesis is enhanced, which leads to

increased proliferation of tumor cells.

Abbreviations: EP, prostaglandin E2 receptor(s); PLA_2, phospholipase A_2.

Use of selective COX 2 inhibitors in human cancers

Prevention

Sufficient information is now available to warrant clinical testing of drugs for the prevention of cancers in individuals at low to moderate risk of these diseases. Enthusiasm for the widespread, long-term use of NSAIDs in a healthy population is dampened because of potential toxic effects, i.e. peptic-ulcer disease[70]. On the other hand, the data reviewed above suggest that selective inhibition of COX 2 may be an effective strategy for preventing cancer. Selective COX 2 inhibitors have the desirable property of interfering with tumorigenesis in experimental systems. Moreover, endoscopically controlled studies show that selective COX 2 inhibitors cause less injury to the mucosa of the upper gastrointestinal tract than classical NSAIDs.

The first human trial to evaluate the anti-cancer properties of a selective COX 2 inhibitor is complete. This study was carried out in FAP patients because of the strength of the preclinical data and prior evidence that sulindac, which inhibits COX 1 and COX 2, reduced the number of colorectal polyps in these patients[71].

Familial adenomatous polyposis (FAP) is a rare disease caused by an autosomal dominant genetic change in a tumor suppressor gene, the adenomatous polyposis coli (*APC*) gene. Individuals with FAP account for 1% of colorectal carcinomas detected annually, and they are at increased risk of developing not only colorectal cancer, but duodenal cancer and desmoid tumors. The FAP phenotype, characterised by large numbers of adenomatous polyps, is initially seen during adolescence[72]. In the absence of intervention, polyps continue to develop in the colon and rectum, resulting in a 100% chance of colorectal cancer by age 50. Current disease management consists of prophylactic surgical procedures, periodic surveillance, polypectomy as needed, and occasional radical surgery of the upper gastrointestinal tract. Although FAP is very rare, it is intensively studied because the findings are believed to be directly applicable to sporadic colorectal cancer. The ability of the selective COX 2 inhibitor celecoxib to cause regression of adenomatous polyps in FAP was recently assessed in a randomised, double-blind, placebo controlled study of 77 patients with FAP[73]. Celecoxib was given twice daily at two doses (100 mg and 400 mg) for 6 months. Treatment with 400 mg celecoxib twice daily caused a 28% reduction in the number of colorectal polyps compared with a 4.5% reduction for placebo. Similarly, the total polyp burden (defined as the sum of polyp diameters) was significantly reduced in patients receiving 400 mg celecoxib twice daily (30.7% reduction) compared with those receiving placebo (4.9% reduction). Furthermore, 53.3% of patients randomly assigned to receive 400 mg celecoxib twice daily responded to the treatment (i.e. experienced at least a 25% reduction in polyp number), but only 6.7% of patients receiving placebo responded. Based on the results of this study, celecoxib has been approved as adjunctive therapy for patients with FAP. The fact that celecoxib was useful in patients with FAP is consistent with results from previous studies in which selective COX 2 inhibitors were effective in preventing and treating intestinal tumors in animals.

The majority of colorectal cancers are believed to evolve from adenomas. Hence, treatments that decrease the formation of premalignant adenomas should protect against the development of colorectal cancer.

Promising preclinical findings and the encouraging results of the FAP study have led to several other clinical trials using selective COX 2 inhibitors. COX 2 is overexpressed in Barrett's oesophagus and oral leucoplakia, which are premalignant conditions that predispose to cancers of the oesophagus and oral cavity, respectively. NSAIDs protect against oesophageal and oral cancer in animals[74, 75]. Moreover, in a recent preclinical study, a selective COX 2 inhibitor was highly effective in preventing chemically induced squamous-cell carcinoma of the tongue in rats.

A primary objective of current human studies is to find out whether a selective COX 2 inhibitor induces regression of either dysplastic Barrett's oesophagus or oral premalignant lesions. Given the frequent need for surgical intervention in both conditions, identification of a pharmacological approach to cause either regression or stabilisation of

disease would represent a significant clinical advance. Clinical trials have also been initiated to investigate the potential use of selective COX 2 inhibitors in patients at risk of cancers of the skin and bladder. In one study, the effects of a COX 2 inhibitor on actinic keratoses, a precancerous skin lesion, is being assessed. This is a logical approach because COX 2 is overexpressed in actinic keratoses and selective COX 2 inhibitors protect against ultraviolet-light-induced skin carcinogenesis in mice. Moreover, the nonselective NSAID diclofenac has already been shown to be useful in the treatment of actinic keratoses[76]. The rationale for the bladder cancer prevention trial is clear. In addition to evidence that COX 2 is overexpressed in precancerous and cancerous lesions of the bladder, selective COX 2 inhibitors prevent the formation of bladder tumors in animals. A major objective of the clinical trial is to find out whether a selective COX 2inhibitor reduces the recurrence of bladder cancer in individuals with a history of superficial bladder cancer.

Treatment

Although many studies are under way, it is too soon to know what role selective COX 2 inhibitors will have in cancer therapy. Selective COX 2 inhibitors are being evaluated intensively in conjunction with chemotherapy and radiotherapy in patients with cancers of the colon, lung, oesophagus, pancreas, liver, breast and cervix. Representative trials are described below. Given the strength of the preclinical evidence, there are numerous trials to evaluate selective COX 2 inhibitors in the treatment of colorectal cancer. A Phase II trial is evaluating treatment with celecoxib plus irinotecan, 5-fluorouracil (5-FU) and leucovorin in patients with measurable, incurable, colorectal cancer[77]. In a retrospective study of patients with metastatic colorectal cancer, investigators from M.D. Anderson Cancer Center (Houston, TX, USA) found that adding celecoxib to capecitabine delayed tumor progression and improved overall survival[78]. Because of these findings, the same group plans to launch a Phase II study of capecitabine and celecoxib.

Selective COX 2 inhibitors are also being evaluated in patients with non-small-cell lung cancer (NSCLC). It is observed previously that paclitaxel induces COX 2 and stimulates PG biosynthesis in cells culture, and postulated that this might reduce the efficacy of paclitaxel[79]. Theoretically, coadministering a selective COX 2 inhibitor with paclitaxel should overcome any decrease in paclitaxel efficacy that is related to the induction of COX 2. This hypothesis was tested in Phase II, neoadjuvant trial that used celecoxib in combination with paclitaxel and carboplatin[80]. In this recently completed study, the overall response rate was higher than predicted from historical data, which indicates that addition of a selective COX 2 inhibitor might enhance the response to preoperative paclitaxel and carboplatin. A confirmatory placebo controlled trial is being planned. Other investigators are conducting Phase II trials of celecoxib and docetaxel in NSCLC[81].

CONCLUSION

Although significant progress has been made in defining the link between COX 2 and carcinogenesis, many questions remain. First and foremost, it is important to establish whether selective COX 2 inhibitors are effective in either preventing or treating cancer, and the numerous clinical trials that are under way should provide crucial information about this. Another interesting issue concerns the potential use of selective COX 2 inhibitors to decrease chemotherapy-related side-effects. Theoretically, selective COX 2 inhibitors might be useful in decreasing the myalgias and arthralgias caused by paclitaxel, a known inducer of COX 2. Preliminary evidence indicates that selective COX 2 inhibitors decreases chemotherapy-induced diarrhoea[82].Additional studies are needed to further evaluate these questions.

Genetic studies, using either transgenic or knockout technology, have firmly established the link between COX 2 and tumorigenesis[83]. However, whether inhibition of COX 2 is the sole reason for the anti-tumorigenic effects of pharmacological inhibitors of COX 2 is less certain[84]. For example, high concentrations of either NSAIDs or selective inhibitors of COX 2 suppress the growth of cells in culture that do not express COX 2[85]. It is possible, therefore, that the anti-cancer activity of these compounds might also reflect COX-independent effects. It is important to determine which, if any, other COX-independent effects occur in humans given

clinically relevant doses of a selective COX 2 inhibitor.

Although selective COX 2 inhibitors have an excellent safety profile when given as monotherapy to arthritis patients, recently, concerns have been raised about cardiovascular safety[86]. In the VIGOR (Vioxx Gastrointestinal Outcomes Research) trial, the incidence of myocardial infarction was significantly higher in groups treated with rofecoxib compared with naproxen. However, whether this difference is caused by a chance event, a pro-thrombotic effect of rofecoxib or a cardioprotective effect of naproxen is uncertain. Importantly, it does not appear to be a class effect because similar effects have not been observed in studies of celecoxib[87]. Thus, as well as assessing efficacy, the safety of selective COX 2 inhibitors needs to be monitor carefully in cancer treatment studies. Ongoing placebo-controlled trials, including the colorectal adenoma prevention trials, will provide additional, useful safety data. Although there is no evidence to date that the toxicity of selective COX 2 inhibitors will approach the level normally associated with anti-cancer agents, more experience is needed before we know whether the excellent safety profile established in arthritis patients translates to cancer patients receiving chemotherapy or radiation.

To date, major emphasis has been placed on evaluating the role of selective COX 2 inhibitors in preventing cancer. We should emphasise, however, that there is growing interest in finding out whether these agents are also useful in treating cancer. In most preclinical studies, selective COX 2 inhibitors reduced the growth rate of established tumors rather than causing tumor regression. This suggests that selective COX 2 inhibitors will be most beneficial when administered in combination with standard therapy. This idea is supported by several experimental studies in which the efficacy of chemotherapy or radiotherapy was enhanced by cotreatment with a selective COX 2 inhibitor[88, 89]. Whether the same will prove to be true in human beings awaits further investigation.

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