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Cyclooxygenase-2: How good is it as a target for cancer chemoprevention?

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Abstract

There is now substantial evidence for a role for cyclooxygenase-2 (COX-2)-mediated prostaglandin (PG) signalling during carcinogenesis in a number of tissues and selective COX-2 inhibitors (coxibs) were considered attractive candidate chemoprevention agents. However, recent concerns over the toxicity of systemic selective COX-2 inhibition and the realisation that COX-1 may also contribute to carcinogenesis have cast some doubt on COX-2 inhibition as a safe and effective chemoprevention strategy. This review will describe the available evidence relating to the known benefits (preventive efficacy in rodent tumorigenesis models and limited human data from small randomised, controlled trials and epidemiological studies) and risks (cardiovascular and renal toxicity) of coxib therapy for cancer chemoprevention. Potential, alternative strategies for inhibition of COX-PG signalling that minimise or avoid systemic selective COX-2 inhibition will also be discussed.

Keywords: Cancer; Chemoprevention; Coxib; Cyclooxygenase; Non-steroidal anti-inflammatory drugs; Prostaglandins

1. Introduction

Discussion of the relative chemopreventive efficacy and safety of selective cyclooxygenase (COX)-2 inhibition requires knowledge of the biochemistry and molecular biology of the COX enzymes, as well as other upstream and downstream factors controlling prostaglandin (PG) synthesis and bioactivity. Detailed analysis of these aspects of PG biology is beyond the scope of this review. Therefore, I have summarised current knowledge on PG signalling directly pertinent to this review and the interested reader is directed to a number of excellent reviews, from which primary references can be obtained [1–4].

2. Cyclooxygenases and other regulators of prostaglandin signalling

COX (or prostaglandin (PG) G/H synthase) catalyses the conversion of arachidonic acid (AA), which is derived from membrane phospholipids by phospholipase A_2 [5], into an unstable PG intermediate PGH_2 [2] (Fig. 1(a)). There are two main COX isoforms: the 'constitutive' isoform COX-1 and 'inducible' isoform COX-2 [1]. The two isoforms have similar catalytic activity but are believed to utilise different intra-cellular pools of AA, which may be related to differences in subcellular localisation [6]. Old dogma stated that COX-1 is constitutively expressed and involved in the synthesis of PGs for tissue homeostasis, while COX-2 expression is inducible and related solely to production of PGs in pathological conditions. Old notions of COX isoforms are becoming increasingly out-dated with publication of evidence that COX-1 can promote tumorigenesis in certain

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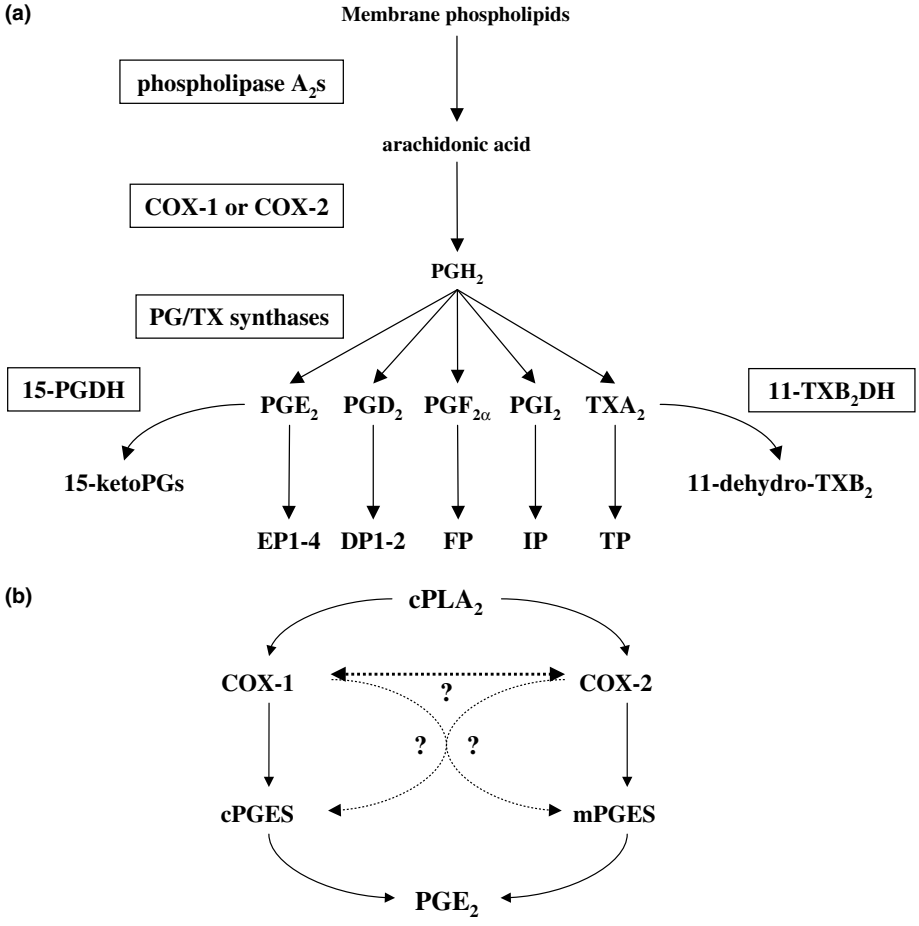

Fig. 1. (a) Prostanoid synthesis and PG-mediated cell-surface receptor signalling. The immediate preferred substrate for COX is arachidonic acid (AA), which is derived from membrane phospholipids by a family of phospholipase A_2 enzymes (AA is also the substrate for the lipoxygenase pathway for leukotriene biosynthesis). AA is converted into PGH_2 by the separate cyclooxygenase and peroxidase activities of COX-1 or COX-2. A family of specific PG or thromboxane (TX) synthases generate five main prostanoids (PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2 and TXA_2), which each signal through specific G-protein-coupled receptors (EP1-4, DP1-2, FP, IP and TP, respectively). A derivative of PGD_2, 15d-PGJ_2 and PGI_2 also act as ligands for peroxisome proliferator-activated receptors γ and δ, respectively. PGE_2, $PGF_{2\alpha}$, PGI_2 and, to a lesser extent, PGD_2 are substrates for type I 15-hydroxy-PG dehydrogenase (15-PGDH), which converts PGs into 15-keto-PG derivatives that have markedly reduced biological activity. TXA_2 spontaneously converts to TXB_2, which is then catabolised to 11-dehydro-TXB_2 by 11-hydroxy-TXB_2 dehydrogenase (11-TXB_2DH). (b) Functional coupling of COX and PGE synthase (PGES) isoforms. The inducible isoform of PGES microsomal (m)PGES preferentially utilises COX-2-derived PGH_2 and cytosolic (c) PGES couples to COX-1 (solid lines). Whether either COX or PGES isoform can compensate for the loss of function of the other is unclear (dotted lines). It is also not understood whether these complementary pathways lead to different downstream PGE_2 activities or whether COX-1 and COX-2 pathways contribute to the same 'pool' of PGE_2 in health and/or disease.

situations [7] and COX-2 is responsible for physiological PG production in the blood vessel wall and the kidney [8]. Recently, a splice variant of COX-1, termed COX-3, which is expressed predominantly in the central nervous system has also been described [9].

Although the reaction catalysed by COX is acknowledged to be the rate-limiting step in PG biosynthesis, the profile of the five major downstream products of the COX pathway (PGE_2, PGD_2, $PGF_{2\alpha}$, PGI_2 and thromboxane [TX] A_2) synthesised by a given cell type is dependent on expression of specific PG or TX synthases [10]. It is known that some PG and TX synthases could link functionally to the separate COX isoforms to affect PG synthesis [11] (Fig. 1(b)). Individual PGs and TXA_2 act in an autocrine and/or paracrine manner *via* specific cell-surface and nuclear G protein-coupled receptors (GPCRs), whose individual expression patterns and downstream second messenger signalling pathways contribute to the complexity of cell-specific PG signalling [12]. Alternatively, certain PGs (15d-PGJ_2 [a dehydration product of PGD_2] and PGI_2) can activate intracellular peroxisome proliferator-activated receptor transcription factors (γ and δ, respectively) [13,14].

The role of PG catabolising enzymes such as 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in controlling prostanoid levels and deregulation of PG signalling in cancer has only recently started to be addressed [15,16].

3. COX-2 expression is up regulated in multiple human cancers and pre-malignant precursor lesions

Cloning of *COX-2* in the early 1990s occured on a background of emerging epidemiological data showing that regular non-steroidal anti-inflammatory drug (NSAID) use reduced cancer risk, particularly in the colon [17]. Therefore, emphasis was placed on the role of COX-2 during carcinogenesis particularly in light of the observation that COX-2 expression was inducible by tumour promoter phorbol esters and known mitogenic growth factors *in vitro* and unlike COX-1, levels of COX-2 mRNA and protein levels were elevated in many solid tumours [18]. In the last decade, an enormous body of evidence has accrued showing up regulation of COX-2 expression in many human cancers including: gastrointestinal and gynaecological malignancies and tumours of the breast, prostate and lung (reviewed in detail in [19,20]). Of particular relevance to its potential role as a chemoprevention target, up regulation of COX-2 has also been reported in a number of benign pre-malignant lesions and pre-disposing, background (often chronic inflammatory) conditions, particularly in the gastrointestinal and urogenital tracts (Table 1).

3.1. Control of COX-2 expression during carcinogenesis

In addition to inhibition of COX-2 activity, negative regulation of *COX-2* gene expression represents an alternative chemoprevention strategy. The human *COX-2* gene is small (spanning 8.3 kilobases) and a 1690-bp sequence upstream of the transcriptional start site contains a TATA box and multiple transcription factor binding sites (CRE, C/EBP, NFκB, AP-2, PEA-3, Sp-1 and GATA-1), which is typical of promoters for highly inducible genes [36–38]. It is clear that transcriptional control occurs in a cell-specific manner. For example, RAS and MAPK signalling contribute to COX-2 up regulation in cancer cells [37,39,40]. Conversely, p53 negatively regulates COX-2 expression in transformed cells [41]. However, NFκB signalling may be more important for macrophage COX-2 up regulation stimulated by lipopolysaccharide (LPS) [42,43] and the transcription factor C/EBPβ is involved in mouse macrophage COX-2 expression, but not regulation of COX-2 in mouse fibroblasts [44]. Co-operative signalling *via* more than one pathway is likely to be important for expression of such a highly inducible gene. Indeed, it appears that *COX-2* gene is not a significant direct downstream target of β-catenin/TCF signalling alone (which usually occurs after loss of function of the Adenomatous polyposis coli tumour suppressor protein). However, combined de-regulation of β-catenin/TCF and RAS signalling has been demonstrated to drive increased COX-2 expression in human hepatocellular cancer cells [40].

Expression of COX-2 is also tightly controlled at the post-transcriptional level [20]. The 3′ un-translated region of COX-2 mRNA contains several *cis*-elements that control transcript stability and translational efficiency, such as AU-rich elements [20]. The role of *trans*-acting mRNA binding factors such as HuR, expression of which is increased in colorectal cancer, in modulating COX-2 mRNA expression is now beginning to be explored [45]. In certain circumstances,

Table 1
Pre-malignant lesions and tissue-specific background pathologies, associated with increased cancer risk, in which COX-2 expression is up-regulated

Organ	Pre-malignant lesion and/or predisposing pathology	Comments/references
Gastrointestinal tract		
Oesophagus	Barrett's oesophagus	Associated with adenocarcinoma [21]
Stomach	Chronic active gastritis (leading to atrophy and intestinal metaplasia)	Caused by *Helicobacter pylori* infection [22]
Liver	Hepatitis	[23]
Pancreatico-biliary tree	Chronic pancreatitis	[24]
	Cholangitis	[25]
Small intestine	Inflammatory bowel disease (Crohn's disease)	[26]
Colorectum	Colorectal adenoma	'Sporadic' and in FAP[a] [27]
	Inflammatory bowel disease	Ulcerative colitis and Crohn's disease [28]
Urogenital tract		
Prostate	Proliferative inflammatory atrophy	[29]
Bladder	'Sporadic' dysplasia	[30]
	Chronic schistosomiasis	
Miscellaneous		
Oral cavity	Leukoplakia	[31]
Skin	Actinic keratosis	[32]
	Ultra-violet light-induced inflammation	[33]
Lung	Scarring	A rare cause of lung cancer [34]
Breast	Ductal carcinoma *in situ*	[35]

[a] Familial adenomatous polyposis.

enhanced COX-2 transcript stability may act in concert with increased *COX-2* gene promoter activity in order to increase intracellular levels of functional COX-2 protein [40].

Consideration of cell type-specific differences in regulation of COX-2 expression is relevant to COX-2 inhibition during early stage carcinogenesis as COX-2 is often localised to host stromal immune cells and fibroblasts, as well as epithelial cells in several of the benign precursor lesions and predisposing conditions noted in Table 1 [46]. Extracellular factors driving COX-2 up regulation also represent potential targets for therapies aimed at decreasing COX-2 expression. Stimuli for COX-2 expression that have been described include LPS [42,47], colonic luminal content [48], bile acid [48–50], bacteria, *e.g.*, *Helicobacter pylori* [51] and mucins [52]. Cellular factors that may drive COX-2 induction in tumours, in a paracrine manner, include epidermal growth factor receptor (EGFR) ligands [53] and COX-2 itself [54]. The above data have generally been obtained from *in vitro* experiments on cultured human epithelial cells. The importance of individual extrinsic and intrinsic stimuli for COX-2 expression at early stages of carcinogenesis *in vivo* has yet to be determined. Elucidating the factors regulating COX-2 expression at stages relevant to cancer chemoprevention will obviously increase with improved understanding of tissue-specific pathologies predisposing individuals to carcinogenesis.

3.2. Pro-tumorigenic activity of COX-2

Several mechanisms of the neoplastic activity of COX-2 have been delineated (Table 2), some of which are likely to be more relevant to later stages of cancer progression (*e.g.*, cell motility and adhesion) than tumour initiation and promotion at earlier stages of carcinogenesis. The majority of mechanistic insights into the pro-tumorigenic activity of COX-2 have come from *in vitro* studies of intestinal epithelial and endothelial cells. The relative contribution of each of these pro-tumorigenic activities to the neoplastic activity of COX-2 is likely to vary in a tissue-specific manner, depending on for example, the profile of PG synthesis and cell-surface PG receptors in a given tumour microenvironment.

Table 2
Mechanisms of the pro-tumorigenic activity of COX-2

Increased invasiveness and decreased adhesion of epithelial cells [55,56]
Resistance to apoptosis [55]
Angiogenesis [57]
Modulation of host immune surveillance (favouring a switch from Th1 to Th2 polarisation) [58]
Increased DNA mutagenesis (by malondialdehyde and oxygen free radicals) [59]
Xenobiotic carcinogen activation by peroxidase activity [60,61]

There is only limited evidence from transgenic mouse models that up regulation of COX-2 expression and activity alone can initiate tumorigenesis. Induction of human COX-2 in mouse mammary tissue using the murine mammary tumour virus promoter has been shown to promote dysplasia and progression to carcinoma [62]. However, COX-2 over-expression in mouse skin under control of a bovine keratin 5 promoter [63] and in gastric mucosa by a cytokeratin 19 promoter [64] only resulted in hyperplasia, not dysplastic change.

It is conceivable that COX-2 is most relevant to the post-initiation phase of tumour promotion *via* mechanisms such as angiogenesis [57] and subversion of the host anti-tumour immune response [58]. The role of COX-2 in promotion of tumour-associated angiogenesis is an area receiving much attention at the present time [65]. It will be important to determine the relevance of anti-angiogenic therapies for cancer chemoprevention (as opposed to cancer treatment) at the same time as increased understanding of direct and indirect (*via* VEGF [66,67]) mechanisms of the angiogenic activity of COX-2.

It is also unclear whether the functional consequences of COX-2 over expression differ depending on the cellular source of COX-2. If downstream PG products of COX-2 act similarly in either a paracrine or autocrine manner then the cellular source of COX-2 (epithelial or stromal) within tumours is probably not crucial (although this makes the unlikely assumption that the PG production profile of different cell types is similar, *i.e.*, epithelial *versus* stromal). Ko and colleagues have demonstrated paracrine COX-2-mediated pro-tumorigenic signalling from macrophages to intestinal epithelial cells that has similar effects to COX-2 over expression in epithelial cells themselves [54,55], suggesting that autocrine and paracrine activity of COX-2 may be similar in the context of intestinal tumorigenesis.

4. Evidence that COX-2 inhibition is anti-neoplastic

4.1. Animal studies

There is a substantial body of evidence that genetic deletion or pharmacological inhibition of COX-2 abrogates tumorigenesis. Experiments using pharmacologically selective COX-2 inhibitors can not exclude COX-2 independent activity of such agents so constitutive genetic deletion of *COX-2* currently provides the strongest evidence that COX-2 inhibition abrogates tumorigenesis. In this way, the *COX-2*-null genotype has been demonstrated to be associated with reduced tumorigenesis in the intestine [7,68,69] and skin [70]. In addition, antisense against *COX-2* has recently been demonstrated to increase apoptosis and decrease tumour growth and angiogenesis of PC-3ML prostate cancer cell tumours

[71]. Particularly strong evidence of the effects of Cox-2 knock-out on tumorigenesis comes from studies with mouse models of familial adenomatous polyposis (FAP) such as $Apc^{Min/+}$ and $Apc^{\Delta 716}$, in which the number and size of small intestinal and colonic polyps were decreased in $Cox\text{-}2^{-/-}$ animals compared with wild-type littermates [7,68,69]. Conditional knock-out of the *Cox-2* gene mirroring pharmacological inhibition of COX-2 activity has not been described. Therefore, the question of whether lack of COX-2 prevents tumour initiation and/or abrogates tumour promotion/progression has not been addressed fully by genetic studies.

Additionally, a large number of rodent studies have confirmed the preventive efficacy of selective COX-2 inhibitors in carcinogen-induced carcinogenesis models, recent examples of which include models of: oesophagus [72], stomach [73], intestine [68,69,74], pancreas [75], prostate [76,77], head and neck [78,79], skin [80] and breast [81,82] carcinogenesis.

4.2. Human data

Epidemiological evidence suggesting that regular NSAID use reduces cancer risk in a number of organs [17] has been put forward as evidence that COX-2 inhibition is anti-neoplastic. However, COX-independent mechanisms of action of NSAIDs may, at least partly, explain the anti-cancer activity of NSAIDs [17,83]. Therefore, this kind of evidence is at best still thought as indirect. A similar argument can be levelled at recent human observational studies exploring neoplastic risk in users of the coxib class of selective COX-2 inhibitors [84,85]. Recently, the selective COX-2 inhibitor celecoxib has been demonstrated to inhibit AKT signalling and induce apoptosis of human colorectal and prostate cancer cells *in vitro* in a COX-2-independent manner by a mechanism involving direct inhibition of phosphoinositide-dependent kinase-1 (PDK-1) [86,87]. Whether this activity is relevant to the anti-neoplastic activity of celecoxib *in vivo* is not known at present.

The first study to address possible anti-neoplastic activity of the coxib class of selective COX-2 inhibitors in humans was a randomised, placebo-controlled trial of celecoxib in patients with FAP [88]. In this study, a significant reduction in colorectal polyp multiplicity (28%) and burden (sum of polyp diameters; 30.7%) was seen only in those individuals taking celecoxib at a dose (400 mg twice daily), which is significantly higher than that used in arthritis patients (typically 100–200 mg twice daily). On the basis of this study, the US FDA approved celecoxib for use as adjunctive therapy for FAP patients receiving standard endoscopic and surgical management. Whether the requirement for high dose celecoxib [88] was a consequence of the relatively low bio-availability of celecoxib or suggests that COX-independent activity (such as PDK-1 inhibition) contributes to the anti-neoplastic activity remains unclear. More recently, a statistically significant maximum 6.8% reduction in polyp number and 9.9% reduction in polyp size was seen in the rectum of FAP patients receiving rofecoxib 25 mg daily [89]. In both of the above studies, it was not possible to distinguish the effects of the coxib on regression of existing polyps from inhibition of polyp development. However, a small open-label study has also been performed analysing the effect of rofecoxib 25 mg daily on polyp recurrence in FAP patients [90]. This study demonstrated a mean 60% reduction in annual polyp recurrence rate during rofecoxib therapy lasting between 18 and 30 months.

There are also epidemiological data supporting the chemopreventative efficacy of coxibs, although these are limited by the relatively short period of time that these drugs have been widely available. Case-control studies based on data from a Canadian Government health database have demonstrated a reduced risk of colorectal neoplasia (adenoma and cancer) and oesophageal cancer in individuals taking celecoxib and rofecoxib compared with non-users [84,85]. In these studies, the risk reduction associated with coxib use was similar in magnitude to that associated with traditional NSAID use.

Currently, a number of clinical trials of the preventative efficacy of coxibs are underway, including those investigating secondary prevention of sporadic (non-familial) colorectal polyps [reviewed in [91], transitional cell bladder cancer, breast cancer, cervical intra-epithelial neoplasia, lung cancer, skin cancer and oral leukoplakia [92,93]. Some of these trials may have been disrupted following recognition of the cardiovascular toxicity of coxibs (see below). However, useful data, with which the chemopreventative efficacy of the coxibs can be further assessed, should still emerge from these studies in the near future.

5. Potential drawbacks to inhibition of COX-2 as a cancer chemoprevention target

The efficacy of selective COX-2 inhibition for cancer chemoprevention could potentially be compromised by lack of COX-2 expression in a proportion of target lesions. It is clear that COX-2 expression is variable in human pre-malignant tissues that are targets for cancer chemoprevention. For example, COX-2 protein is absent in a significant proportion of human colorectal adenomas [27], Barrett's oesophagus [94] and skin cancer precursors [32]. It will be important to analyse COX-2 expression in neoplastic lesions and background 'normal' tissue from patients in the coxib- and placebo-arms of the ongoing phase II/III trials in order to ascertain whether 'resistance' to selective COX-2 inhibition is related to differences in COX-2 expression.

Alternatively, the existence of alternative pathways of COX-PG signalling could compensate for a lack of COX-2 activity following pharmacological inhibition. It remains unclear to what extent COX-1-dependent PG production can compensate for lack of COX-2 activity or whether COX-1- and COX-2-dependent PG synthesis pathways are mutually exclusive (Fig. 1(b)). Recent data suggest that COX-1, as well as COX-2, contributes to intestinal tumorigenesis [7,69,95] and phorbol ester-induced skin carcinogenesis [70], although *COX-1* gene deletion does not affect ultraviolet light-induced mouse skin carcinogenesis [96]. A significant contribution to carcinogenesis from both COX isoforms implies that non-selective COX inhibitors may have advantages over selective COX-2 inhibitors. In the future, the chemopreventive efficacy of coxibs should be compared carefully with that of traditional non-selective COX inhibiting NSAIDs.

Aside from pharmacological considerations of the potential limitations of selective COX-2 inhibitors for cancer chemoprevention, it is also likely that the financial cost of coxib therapy in healthy individuals will outweigh heavily that of alternative agents such as aspirin [97], which also have other beneficial effects (*e.g.*, cardioprotection) on overall population health that may not be shared by coxibs [97].

5.1. Safety of selective COX-2 inhibitors

Development of the coxib class of selective COX-2 inhibitors was based on the hypothesis that COX-2 mediates PG synthesis in pathologies such as arthritis, unlike COX-1, which is responsible for production of PGs involved in normal tissue homeostasis (*e.g.*, gastric mucosal protection). Therefore, it was expected that selective COX-2 inhibition would combine anti-inflammatory efficacy with reduced toxicity compared with traditional NSAIDs, which non-selectively inhibit both COX isoforms. There is now sufficient data to be able to state that coxibs have comparable anti-inflammatory activity to existing traditional NSAIDs [98]. Three large, relatively short (up to 12 months) trials in arthritis patients have also reported that rofecoxib, celecoxib and lumiracoxib have decreased upper gastrointestinal toxicity compared with NSAID comparators [99–101]. However, the emergence of the complete data set from the CLASS study revealed no significant difference in the primary end-point of incidence of complicated (bleeding or perforated) peptic ulcer between individuals taking celecoxib and the NSAID comparators diclofenac or ibuprofen (possibly due to the permitted use of low-dose aspirin in this trial) [100,102]. The very low peptic ulcer rates seen in arthritis patients without other ulcer risk factors and in healthy volunteers taking coxibs indicate that coxibs appear to have little, if any, inherent ulcerogenic activity [103]. However, coxibs are not spared the renal toxicity associated with NSAID therapy [8,104] and it has now become apparent that these agents have a significant vascular side-effect profile [105–107].

Building on earlier data that suggested likely increased cardiovascular risk in coxib users [8,108], conclusive evidence that long-term rofecoxib or celecoxib use is associated with an enhanced risk of serious vascular events (including cardiac sudden death, myocardial infarction and stroke) has emerged from two large, placebo-controlled, colorectal adenoma secondary prevention studies [105,106] and a randomised, controlled trial involving post-operative cardiac surgery patients [107]. The APPROVe (Adenomatous Polyp Prevention on Vioxx) study demonstrated an increase in serious thrombotic events (predominantly myocardial infarction) from 0.78 events per 100 patient years in the placebo group to 1.50 events per 100 patient years in those taking rofecoxib 25 mg daily (relative risk 1.9 (95% CI 1.2–3.1)) with the difference only becoming apparent after 18 months of therapy [106]. Data from the APC (Adenoma Prevention with Celecoxib) trial revealed a dose-related increase in specified cardiovascular end-points in those assigned to celecoxib, which was particularly evident at a daily dose of 800 mg (relative risk 3.4 (95% CI 1.4–7.8)) [105]. However, available data from a preliminary analysis of the PreSAP (Prevention of Spontaneous Adenomatous Polyps) study show no excess cardiovascular risk in users of celecoxib 400 mg daily [92,105]. The mechanistic basis of the apparent cardiovascular risk associated with coxib therapy is currently unclear. The fact that there is evidence of increased cardiovascular risk associated with three coxibs [105–107] suggests a drug class-effect related to systemic COX-2 inhibition. Current understanding is that (contrary to the earlier model of the role of each of the COX isoforms) production of anti-thrombotic PGI_2 from the blood vessel wall is COX-2-dependent and that unopposed COX-1-dependent TXA_2 synthesis by platelets, following selective COX-2 inhibition, may lead to a pro-thrombotic state [108]. In the APPROVe study, there was no evidence of a contribution to increased thrombotic risk from the recognised hypertensive properties of rofecoxib [106]. Some pre-clinical data support the concept that COX-2 inhibition may also accelerate atherosclerosis [8]. In the future, it will be essential to determine whether the increased cardiovascular risk associated with coxib therapy is relevant only in 'at-risk' individuals with pre-existing atherosclerotic disease (relevant to chemoprevention of 'sporadic' neoplasia in older individuals, which was tested in the recent colorectal adenoma prevention trials) or whether younger patients, without background vascular disease (a 'high cancer risk' group in which chemoprevention of cancer-predisposition syndromes is relevant), will be at risk *via* accelerated atherosclerosis. Only when armed with this information, combined with knowledge of the

preventive efficacy for specific pre-malignant precursor lesions and cancers, will individual benefit-risk calculations for coxib chemoprevention in different cancer risk groups be feasible.

6. Alternative targets for inhibition of COX-PG signalling during the early stages of carcinogenesis

The realisation that although inhibition of COX-2 has significant chemopreventative activity, prolonged use of selective COX-2 inhibitors may produce an unnecessary risk to otherwise healthy patients with a propensity for malignancy, has increased emphasis on alternative ways of inhibiting COX-PG signalling whilst avoiding unopposed systemic COX-2 inhibition (Table 3).

Delineation of the cardiovascular toxicity of the coxibs will inevitably lead to a closer scrutiny of the cardiovascular side-effects of traditional NSAIDs in the near future, particularly those that have some selectivity for COX-2, rather than COX-1, such as diclofenac [109]. Assuming that these drugs are spared the cardiovascular problems associated with coxibs, one future strategy for cancer chemoprevention by COX inhibition is development of safer derivatives of non-selective COX inhibitors. Perhaps the most promising novel NSAIDs that have been shown to combine anti-neoplastic activity in vitro and in vivo with improved upper gastrointestinal safety are nitric oxide (NO)-NSAIDs, also known as COX-inhibiting NO-donors (CINODs) [110,111].

An exciting, novel concept in cancer chemoprevention is the use of combination therapy, which may allow dose reduction (and hence decreased systemic bioavailability) of drugs such as NSAIDs or coxibs when combined with other anti-cancer agents, *e.g.*, EGFR inhibitors [112].

Alternatively, other steps in PG biosynthesis [10,113] and signalling [69] represent potential targets (Table 3). For example, pharmacological inhibitors of PGE_2-EP receptors, which have anti-neoplastic activity [69], have been generated. Development of specific inhibitors for individual enzymes and receptors will be dependent on better understanding of the roles of particular PGs and their signalling receptors in health and disease.

Finally, inducible expression of COX-2 is tightly controlled at the transcriptional and translational level in a cell-specific manner (see above). Therefore, targeting mechanisms controlling neoplastic COX-2 regulation in stromal and epithelial elements of tumours may provide tumour-specific COX-2 inhibition, whilst avoiding the unwanted effects related to systemic COX-2 inhibition.

Table 3
Possible strategies for inhibition of COX-PG signalling whilst avoiding/minimising systemic COX-2 inhibition

Safer, non-selective COX inhibition, *e.g.*, NO-NSAIDs [110,111]
Reduced systemic bioavailability of selective COX-2 inhibitors
Dose reduction (in the context of combination therapy) [112]
Restricted (local, *e.g.*, topical) drug delivery
Targeting other steps in the PG biosynthesis/signalling pathway
PLA_2s [113]
PG synthases [10]
PG receptors, *e.g.*, GPCRs and PPARs [69]
Inhibition of COX-2 expression (rather than activity) in pre-neoplastic and/or neoplastic tissue

7. Conclusions

There is now ample pre-clinical evidence that inhibition of COX-2 represents a valid target for prevention of carcinogenesis in a number of tissues. However, at the present time, there are limited data on the preventive efficacy of the coxib selective COX-2 inhibitors in humans that are restricted to small randomised trials in patients with FAP and preliminary epidemiological observations. It is expected that the larger phase III trials testing coxibs in the context of more common 'sporadic' carcinogenesis will start to report their experience in the next few months. Recently, it has become apparent that coxibs have greater (particularly cardiovascular) toxicity than was initially expected. Only when data on the preventive efficacy for particular cancers are combined with accurate information on the side-effect profile for relevant patient groups will the benefit-risk ratio for cancer chemoprevention by selective COX-2 inhibitors be evaluable, leading to comparisons with more established NSAIDs.

Conflict of interest statement

Professor Hull has received two International Medical School Grants from Merck Sharpe & Dohme Ltd. for clinical trials on rofecoxib and has also obtained two travel grants from the same Company.

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