British Journal of Pharmacology (2001) 134, 1137–1150

www.nature.com/bjp

REVIEW

COX-2 and cancer: a new approach to an old problem

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British Journal of Pharmacology (2001) **134,** 1137–1150

Keywords: Angiogenesis; APC; apoptosis; aspirin; breast; (cancer); carcinogenesis; chemoprevention; chemotherapy; colorectal; (cyclo-oxygenase); cytokines; epidemiology; FAP; HNPCC; lung; Min mouse; NSAID; PPAR; prostate; sulindac; thiazolidinedione

Abbreviations: APC, adenomatous polyposis coli; bFGF, basic fibroblast growth factor; COX, cyclooxygenase; CRC, colorectal cancer; C2I, selective inhibitor of COX-2; EGF, epidermal growth factor; FAP, familial adenomatous polyposis; FOBT, faecal occult blood tests; 5-FU, 5-fluorouracil; HNPCC, hereditary non-polyposis colon cancer; IL, interleukin; LPS, bacterial lipopolysaccharide; Min, multiple intestinal neoplasia; MMR, mismatch repair; NFκB, nuclear factor kappa B; NSAID, non-steroid anti-inflammatory drug; PG, prostaglandin; PGI_2, prostacyclin; $15dPGJ_2$, 15-deoxy-delta 12,14-PGJ_2; PPAR, peroxisome proliferator-activated receptor; RXR, retinoic acid receptor; TNFα, tumour necrosis factor alpha; TxA_2, thromboxane A_2; TZD, thiazolidinedione

Introduction

The proposition that cyclo-oxygenase-2 (COX-2) is causally linked to cancer offers a new approach to extending our knowledge of neoplasia and of improving treatment of the human disease. The identification of an enzyme catalysing fatty acid oxidation as a rate limiting step in the progress from normal cell growth through hyperplasia on to neoplasia has opened up a whole new field of cancer research, far from the usual approaches based on nucleic acid metabolism. The precise interactions and links between lipid metabolism and DNA replication have still to be elucidated and defined but they are already having clinical consequences. In practical terms, this surprising proposition offers a sound scientific basis for the successful prevention of cancer and a real prospect of drug treatment of diagnosed disease without the serious side effects usually associated with cancer chemotherapy. Chemoprevention based on COX-2 has already been achieved in a restricted group of patients (Steinbach *et al*., 2000) and clinical enthusiasm has already been expressed for COX-2 based treatment to be extended as chemoprevention in a wider range of patients or as adjuvant therapy of established disease (Vainio & Morgan, 1998; Lord *et al*., 1999; Cuendet & Pezzuto, 2000; Gately, 2000).

This review will summarize the evidence for the COX-2 and cancer proposition in general. However because the majority of the work, both experimental and clinical, has focused on the relevance of COX-2 to colorectal cancer (CRC), this form of cancer will also be the focus of this review. At the end of the review some selected references to COX-2 in other forms of cancer are given as points of entry into those areas, but with no attempt at a comprehensive coverage.

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Biology of COX-2

First, a brief description of the enzyme, COX-2, concentrating on those of its characteristics particularly relevant to its participation in the processes of carcinogenesis. Further details can be found in a number of reviews (Bakhle & Botting, 1996; Vane *et al*., 1998; Bakhle, 1999; Smith *et al*., 2000a).

Cyclo-oxygenase-2 (COX-2) is also formally called prostaglandin H_2 synthase-2 (PGHS-2), mostly in the American literature. COX-2 and COX-1 are isoforms of an enzyme which catalyses the first stage in the oxidation of arachidonic acid to the prostanoids (Figure 1). Most of the biochemical cascade outlined in this Figure was well characterized for what is now known as COX-1, before a new protein with COX like activity was described in the early 1990s (Xie *et al*., 1991; Kujubu *et al*., 1991). This new protein proved to be a true isoform of COX-1, i.e., it accepts the same substrate and yields the same product, by the same reactions. In addition, the linear sequence and three-dimensional structure of the two isoforms are very similar. Even the active site of the isoforms differs minimally (valine/isoluecine substitutions) at only two positions. The crystal structure of COX-2 (Luong *et al*., 1996; Kurumbail *et al*., 1996) was solved by superimposition on that already described for COX-1 (Picot *et al*., 1994). Nevertheless, inhibitors with high and clinically demonstrable selectivity for the isoforms are available (Smith *et al*., 1998; Chan *et al*., 1999; Talley *et al*., 2000; Riendeau *et al*., 2001).

Although the development of such inhibitors (Vane *et al*., 1998; Bakhle, 1999) will not be discussed here, it is necessary for the rest of this review to note that the clinically used, non-steroid anti-inflammatory drugs (NSAIDs), such as aspirin, indomethacin, ibuprofen or diclofenac, are inhibitors of COX activity. These are either non-selective, acting on both isoforms, or are more effective on COX-1. The term

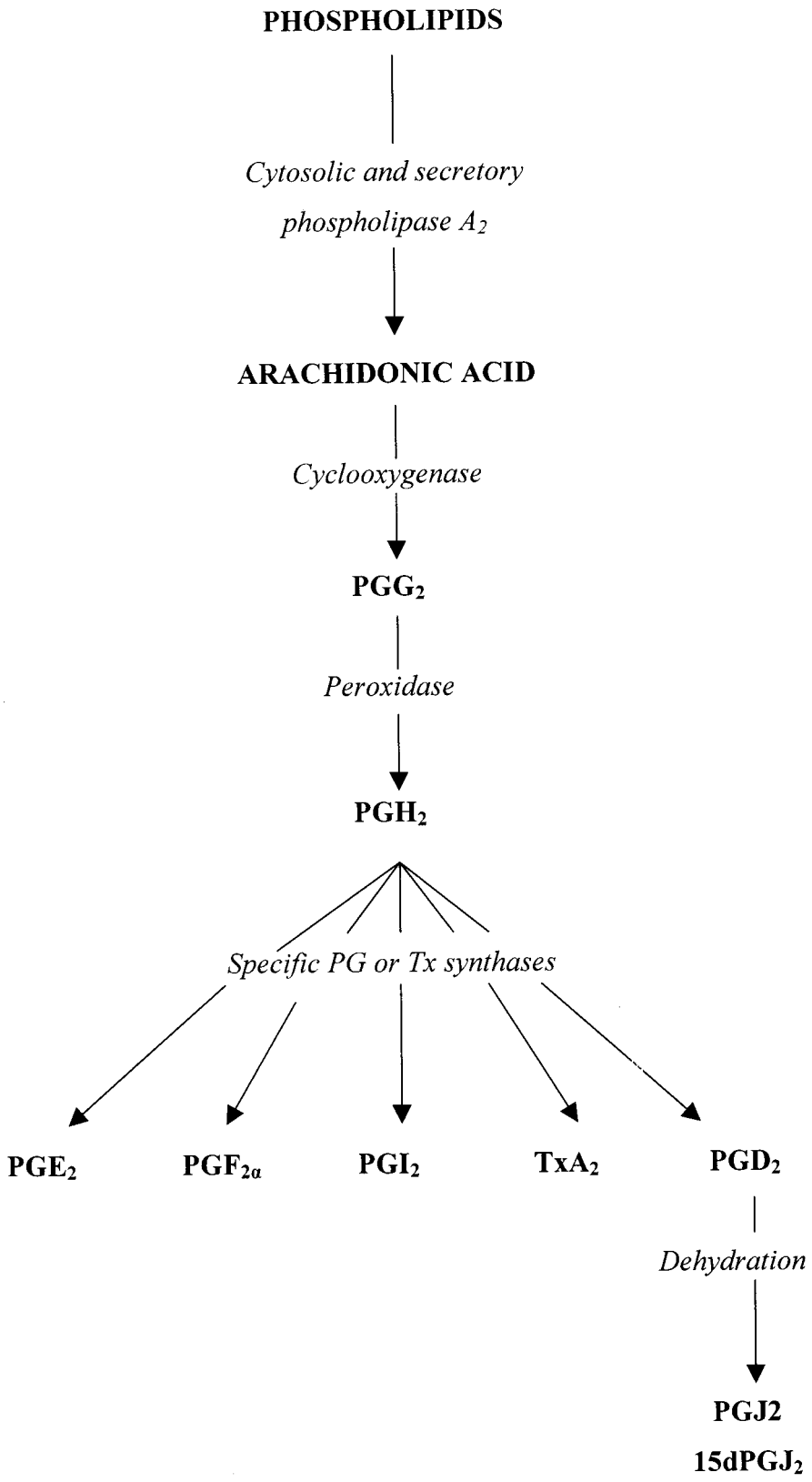

Figure 1 Biosynthesis of prostanoids. The resting levels of free arachidonic acid (AA) in cells are low and PG synthesis is dependent on the provision of AA from phospholipid. The PLA_2 enzymes are the major providers but free AA can be generated indirectly *via* PLC or PLD activities. The COX protein in either isoform exhibits two separate enzymic activities, as shown. Inhibition of the COX activity does not inhibit peroxidase activity. All the final prostanoid products have a common precursor, PGH_2, and separate synthase enzymes. So far only PGE_2 synthase has been found to be inducible, by the same stimuli as induce COX-2. The cyclopentenone PG ligands for the nuclear receptor PPARγ, PGJ_2 and $15dPGJ_2$, are derived only from PGD_2 by ill-defined pathways *in vivo*.

NSAID will be used to denote this type of inhibitor in contrast to the selective inhibitors of COX-2, which will be referred to as C2Is.

The important biological difference between the isoforms is that COX-1 is normally present in most types of cells and is a constitutive, housekeeping enzyme. The latter characteristic is derived from its DNA and RNA structures and in practical terms implies that amounts of COX-1 protein remain virtually constant (about 2–5 fold variation) under either physiological or pathological conditions. By contrast, COX-2 protein is normally absent from most cells – with some notable exceptions – but appears rapidly (2–4 h) in large amounts in a range of pathological, often inflammatory, situations and in many cell types.

In the present context, it is important that COX-2 was first described as being induced by a viral oncogene (Xie *et al*., 1991) or by a tumour promoter (Kujubu *et al*., 1991). Subsequent work has shown it to be inducible by a variety of growth factors and mitogens (Bakhle & Botting, 1996; Smith *et al*., 2000a), making this isoform particularly relevant to the processes of cell growth and carcinogenesis.

One further aspect of COX biochemistry must be emphasised. Both COX-1 and COX-2 form the same product, PGH_2. This PG is the common precursor for the biosynthesis of thromboxane A_2 (TxA_2), prostacylin (PGI_2) and the other prostaglandins PGD_2, PGE_2, $PGF_{2\alpha}$ (see Figure 1). These 'post COX' transformations are catalysed by quite separate enzymes. It is these prostanoids that determine the final biological response to the action of COX. For instance, it is the same isoform of COX that in platelets leads to the formation of the vasoconstrictor and pro-aggregatory TxA_2 but in endothelial cells provides the vasodilator and anti-aggregatory PGI_2. This biological selectivity reflects the absence of PGI_2 synthase in platelets and of TxA_2 synthase in endothelial cells. The importance of the 'post COX' enzymes has recently been underlined by the identification of an inducible isoform of PGE_2 synthase. This isoform is induced, like COX-2, by bacterial lipopolysaccharide (LPS) or interleukin-1 (IL-1), it is located on the perinuclear membrane and it appears to be functionally coupled to COX-2 (Naraba *et al*., 1998; Jakobsson *et al*., 1999; Murakami *et al*., 2000). Thus the final biological effect of COX-2 activity may be predominantly expressed by PGE_2 and susceptible to control by inhibitors selective for the inducible PGE_2 synthase.

Natural history of CRC

CRC provides a significant proportion of cancer deaths in the Western world and is second only to lung cancer in the U.S. Its incidence is age related – hardly any below the age of 40 and rising to about 300 per 100,000 over 65 years. Mortality is high, about 150 per 100,000. It might be reasonable to predict an increase in deaths from CRC, purely on an age-related basis, as other causes of death in the over 50s (mainly cardiovascular at present) are reduced, by the relative success of modern treatment of cardiovascular disease.

Although the great majority of CRC cases are sporadic, i.e. have no clear cause, in a small proportion (5–13%; Emery *et al*., 2001) there is a family history suggesting a heritable susceptibility to the disease. In two very small groups there is clear evidence for an autosomal dominant genetic mutation that leads to CRC. The most clearly defined is the group with familial adenomatous polyposis (FAP) who comprise about 0.5% of all CRC cases. In patients with FAP, hundreds of small polyps occur spontaneously in the colon and rectum by about 20 years of age. These growths are initially benign but within the next 20 years as they grow in size and number, some will become malignant. If untreated, most of the subjects will develop colorectal cancer. The genetic fault is in the *APC* (adenomatous polyposis coli) gene and several mutations are known, giving rise to defective APC proteins (Dubois *et al*., 1996; Syngal *et al*., 2000). The penetrance of this mutation is almost 100%, i.e., all who carry the defective gene will develop CRC (Emery *et al*., 2001). Because of this high penetrance, this small group of subjects have been very important in the development of potential chemopreventive agents and C2Is are already used in this condition (Steinbach *et al*., 2000).

The other group of heritable CRC is hereditary non-polyposis colon cancer (HNPCC) and is less clearly defined than FAP although it is larger in size (about 2% of all CRC) (Syngal *et al*., 2000, Emery *et al*., 2001). The genetic defect here is also less specific, being in a number of genes related to DNA repair including *MLH1, MSH2, PMS1 & 2*. The corresponding proteins identify and repair errors in DNA arising from mismatching during replication (MMR enzymes) and their dysfunction allows errors in DNA to persist into the next cycle of cell division. The penetrance here is significantly less than in FAP and all the genetic mutations associated with HNPCC have still to be identified.

However, it must be emphasised that the large majority of CRC cases (over 80%) are sporadic and exhibit no obvious heritable tendency. Therefore the epidemiological finding that chronic NSAID use decreased CRC incidence (summarized in Thun, 1996; Table 1) applies mainly to sporadic CRC and is not restricted to any genetically determined subgroup of the disease. If, as suggested from experimental work, COX-2 may be a rate-limiting component at several stages in the development of neoplasia (Reddy *et al*., 2000) and in the growth of the fully transformed cell, inhibition of this enzyme would be effective in the genesis of CRC and in its subsequent growth, irrespective of the initiating mechanisms.

Carcinogenesis in CRC Carcinogenesis is a multi-stage process, in which a succession of mutations is needed to progress from the normal cell to the fully neoplastic cell (Kinzler & Vogelstein, 1996; Marks & Furstenberger, 2000; Syngal *et al*., 2000; Emery *et al*., 2001). Such a progress is well exemplified in human CRC and in related experimental systems. The first mutation in colonic epithelial cells is in the gene for APC, a protein that is a significant component of the apoptotic pathway in these cells (Kinzler & Vogelstein, 1996; Marks & Furstenberger, 2000; Fosslien, 2000). This mutation is followed by others and one scheme of the progress *via* hyperplasia to neoplasia in epithelium is summarized in Figure 2. The need for many different gene mutations to accumulate within a cell before transformation to a neoplastic form is compatible with the relatively slow development of CRC from the precursor adenoma and of the adenoma from normal epithelium (CRC is uncommon before 50 years of age). It must be emphasised that although COX-2 induction appears in this scheme there is no suggestion or

Table 1 Epidemiological studies of NSAID use and colon cancer

Country	*Year*	*Numbers*[a]	*Treatment*	*Risk ratio*	*Reference*
Australia	1988	715	Aspirin	0.57	Kune *et al*., 1988
USA	1991	1326	Aspirin	0.5	Rosenberg *et al*., 1991
USA	1993	830	Aspirin	0.4–0.8	Suh *et al*., 1993
UK	1993	40	NSAID*	0.49–0.66	Logan *et al*., 1993
USA	1994	47,900[b]	Aspirin	0.68	Giovannucci *et al*., 1994
USA	1994	97	NSAID*	0.52–0.88	Peleg *et al*., 1994
USA	1994	511	NSAID*	0.32–0.77	Muscat *et al*., 1994
USA	1995	157	NSAID	0.36–0.77	Martinez *et al*., 1995
USA	1996	206	NSAID*	0.31–0.59	Peleg *et al*., 1996

This Table shows some of the earlier epidemiological studies assessing correlations between chronic use of aspirin or other NSAIDs and the incidence of colon cancer. Overall, the risk (ratio) of developing colorectal cancer was decreased by about 50% relative to those who did not take NSAIDs. The number and geographical distribution of the studies strengthens the overall conclusion that ingestion of NSAIDs protected against colon cancer. It is important to note that treatment was, for most studies, at an 'anti-inflammatory' level; it is not known if the 'anti-platelet' level of aspirin (75–100 mg day^{-1}) which is considerably lower, will have any similar protective effect (Giovannucci *et al*., 1995). Treatment with, or chronic self-administration of, aspirin only is shown as Aspirin in this column. NSAID signifies that other aspirin-like, COX-inhibitory, anti-inflammatory agents were used or prescribed. [a]This refers to the numbers of cases surveyed. [b]This is the total number of respondents to the survey. *In these studies, use of paracetamol, as distinct from COX-inhibitory NSAIDs, was identified and was shown to be ineffective in reducing risk of colon cancer.

evidence that the COX-2 gene or the protein itself is mutated in colonic epithelial cells during carcinogenesis.

Genetic models of CRC There are many animal models of genetic faults relating to cancer (Alexander, 2000) and the most important for CRC is a model with mutations in the gene for the APC protein, with a phenotype closely resembling that of FAP (Shoemaker *et al*., 1997; Fodde & Smits, 2001). Such Min (multiple intestinal neoplasia) mice spontaneously developed intestinal polyposis which was prevented by NSAIDs (Boolbol *et al*., 1996; Jacoby *et al*., 1996; Nakatsugi *et al*., 1997). A more direct involvement of COX-2 with this phenotype was found when a strain of the Min mice was cross-bred with mice lacking the gene for COX-2 (Oshima *et al*., 1996). The number and size of the polyps was successively diminished in the Min mice lacking one or both genes for COX-2. The effect of COX-2 deletion on polyposis in the Min mice was comparable to that of treating Min mice with an experimental C2I. Subsequently both the C2Is in present clinical use were shown to decrease polyposis in APC mutant Min mice (Jacoby *et al*., 2001; Oshima *et al*., 2001). A new variation on the theme of polyp prevention in the Min mouse is combination chemoprevention (Torrance *et al*., 2000). Here the NSAID sulindac was used together with inhibitors of the epidermal growth factor (EGF) receptor kinase to give better polyp suppression than either agent used alone. It would be interesting to combine a C2I with the EGF kinase inhibitors in this model.

The close correlation between responses of the Min mouse models and human FAP to NSAIDs and C2Is has strengthened confidence in results obtained in this model, in spite of some differences between the phenotypes of the Min mouse and FAP (Alexander, 2000; Shoemaker *et al*., 1997).

Development of the COX-2 and cancer concept

The correlation between COX-2 and CRC emerged at a uniquely appropriate time, with several factors coming together from quite disparate sources. A number of case study reports showing a correlation of chronic NSAID use and a lower incidence of CRC appeared in the early '90s (Table 1). The epidemiological case for chronic NSAID use protecting against CRC was strong but there was no clear existing scientific explanation of these observations.

At the same time, COX-2 had been characterized as one of the proteins induced during the transformation of cells by the viral oncogene, *v-src*, and as a mitogen inducible protein (Xie *et al*., 1991; 1992; Kujubu *et al*., 1991).

Another highly significant contributory event was the small clinical trial of the NSAID sulindac in patients with FAP (Giardiello *et al*., 1993). Although this trial was short term (only 12 months), the marked reduction in polyp number and size while sulindac was taken together with the increase after the treatment was stopped, showed very clearly that inhibition of either COX-1 or COX-2 or both did have real benefit in this condition.

These independent findings were then synthesized into a testable hypothesis that a COX activity associated with growth and oncogene action was causally related to the protection offered by inhibitors of this enzymic activity against CRC. An early result crucial to the hypothesis was provided by Eberhart *et al*. (1994) who showed the presence of COX-2 in neoplastic tissue from CRC patients and its absence from adjacent histologically normal intestinal tissue. COX-1 was present in both normal and neoplastic tissue equally. In the many laboratory and clinical studies since then the presence and action of COX-2 has been causally related to carcinogenesis.

The strength of the correlation between COX-2 and CRC does not deny the efficacy of many other agents lacking COX-inhibitory actions (Laird *et al*., 1995; Levy, 1997), nor even the actions of NSAIDs and C2Is unrelated to inhibition of PG biosynthesis (Ahnen, 1998; Shiff & Rigas, 1999). The other isoform, COX-1, was also crucial in development of polyposis in Min mice (Chulada *et al*., 2000). Clearly with a multi-stage development of CRC, a similar multiplicity of inhibitory mechanisms could be expected. Nevertheless because the 'COX-2 and cancer' hypothesis provides a new and clearly defined mechanism for, and identifies another stage in, the development of CRC, this hypothesis has become the basis for an important new therapeutic target in tumorigenesis in general.

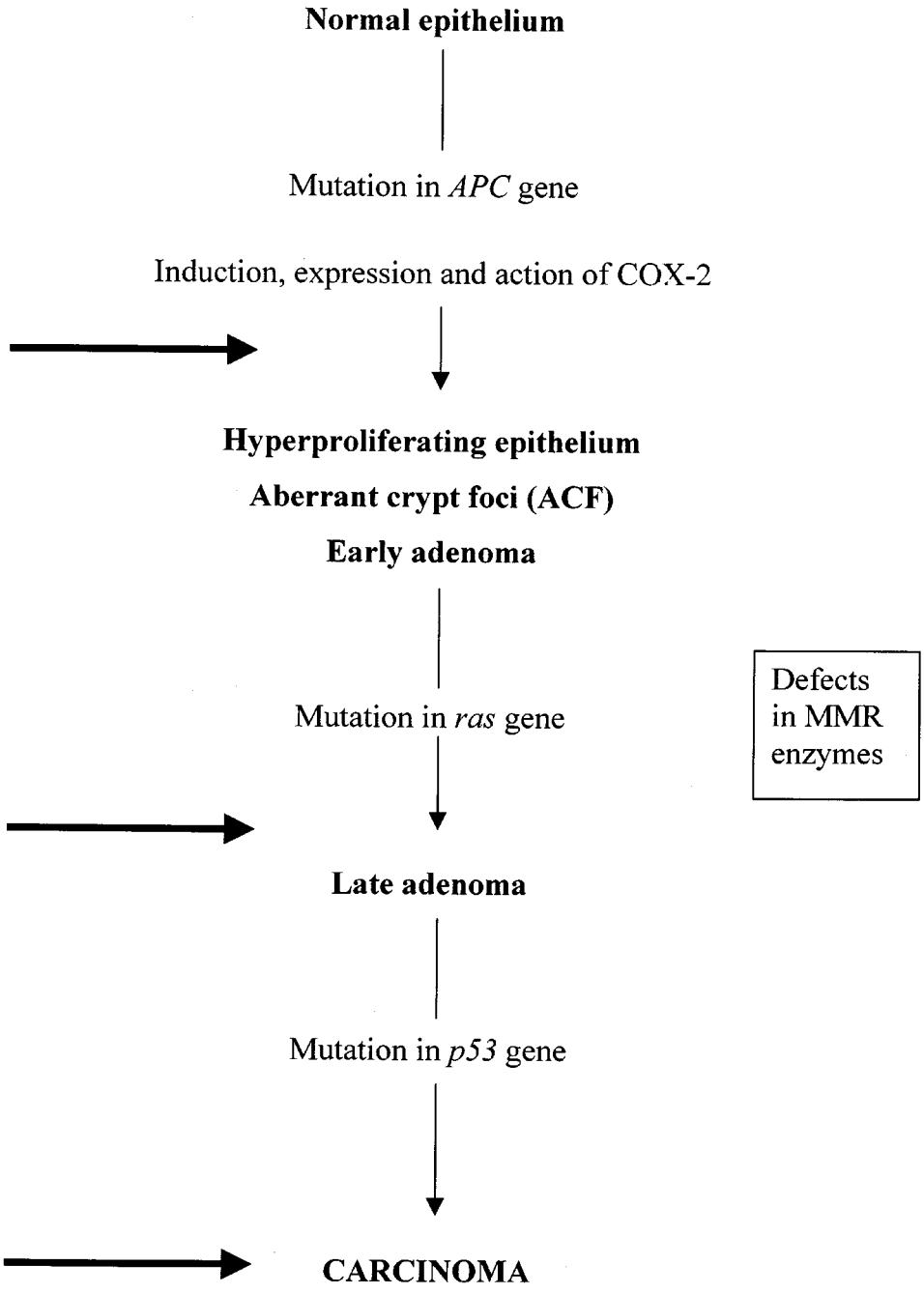

Figure 2 Carcinogenesis in CRC and action of NSAIDs or selective COX-2 inhibitors (C2Is). The progression from normal epithelium to carcinoma has several identifiable stages, some associated with particular genetic mutations with the loss of the related protein and its function. Loss of the APC gene product is accepted as the initiator of the whole process. Expression and consequent action of COX-2 in the APC-deficient cells is required for carcinogenesis to progress. The nature of the link between loss of APC and induction of COX-2 is not clear, e.g., is it direct or coincidental. Further progression from the early adenoma onwards is associated with mutations of RAS and of p53 but these mutations alone, i.e., in the absence of the APC mutation do not lead to carcinoma. At these stages, the defects in the DNA repair enzymes (MMR) appear to be expressed. The heavy arrows show the points at which inhibition of COX-2 will block progression. COX-2 activity is also needed for growth of transformed cells i.e. after carcinogenesis is complete.

Mechanisms and pathways involved in modulation of cancer by COX-2

PG-dependent and COX-2 dependent mechanisms appear to influence both the progression from normality to neoplasia, i.e., carcinogenesis and also the replication of neoplastic cells after transformation, in the epithelial cell. Here I shall discuss first the mechanisms that may influence the neoplastic transformation and then those that may control the growth of the tumour.

Apoptosis Decreased apoptosis of epithelial cells appears to play a crucial role in the genesis of CRC. The 'gate keeping' mutation required for the development of CRC is in the *APC*

gene, which codes for a pro-apoptotic protein (Kinzler & Vogelstein, 1996; Shoemaker *et al*., 1997; Marks & Furstenberger, 2000; Fodde & Smits, 2001). Modulation of apoptosis by APC involves other proteins including β-catenin and E-cadherin (Fosslien, 2000; Marks & Furstenberger, 2000) and the Wnt signalling pathway (Bienz & Clevers, 2000; Polakis, 2000).

In Min mice, intestinal epithelium showed a decreased apoptotic rate, which was reversible by the NSAID sulindac (Mahmoud *et al*., 1997). A more direct connection between COX-2 and apoptosis was shown in rat intestinal epithelial cells (Tsujii & Dubois, 1995). In cultures of these rat cells, apoptosis was decreased after transfection with the COX-2 gene. This was accompanied by increased spontaneous output of PGE_2 and increased levels of Bcl-2, another anti-apoptotic protein, suggesting a possible molecular mechanism for the pro-neoplastic effects of COX-2 action. Inhibition of PGE_2 biosynthesis with sulindac restored the apoptotic rate. There was a direct effect of PGE_2 on Bcl-2 and apoptosis in cell lines derived from human colon cancer samples (Sheng *et al*., 1998). In HCA-7 and HT 29 cell lines, NSAIDs and C2Is inhibited growth and induced apoptosis (Smith *et al*., 2000b).

Another colon cancer cell line, HCT-29, expresses a truncated APC protein and high levels of COX-2 (Hsi *et al*., 1999). After transfection with a normal APC gene, the cells exhibited increased apoptosis, decreased COX-2 and changes in β-catenin signalling pathways. These findings are compatible with the proposed 'self promotion' interaction between APC and COX-2 in which decreased activity of the APC protein increased COX-2 biosynthesis (Prescott & White, 1996).

Peroxisome proliferator-activated receptors (PPARs) There are three different PPARs – α, β (or δ), and γ – and each acts as a transcription factor controlling gene expression as a heterodimer with another nuclear protein, the retinoic acid receptor, (RXR; Figure 3). Each PPAR affects a different range of genes, most of which are generally involved in lipid metabolism (Lowell, 1999; Clarke *et al*., 1999; Kersten *et al*.,

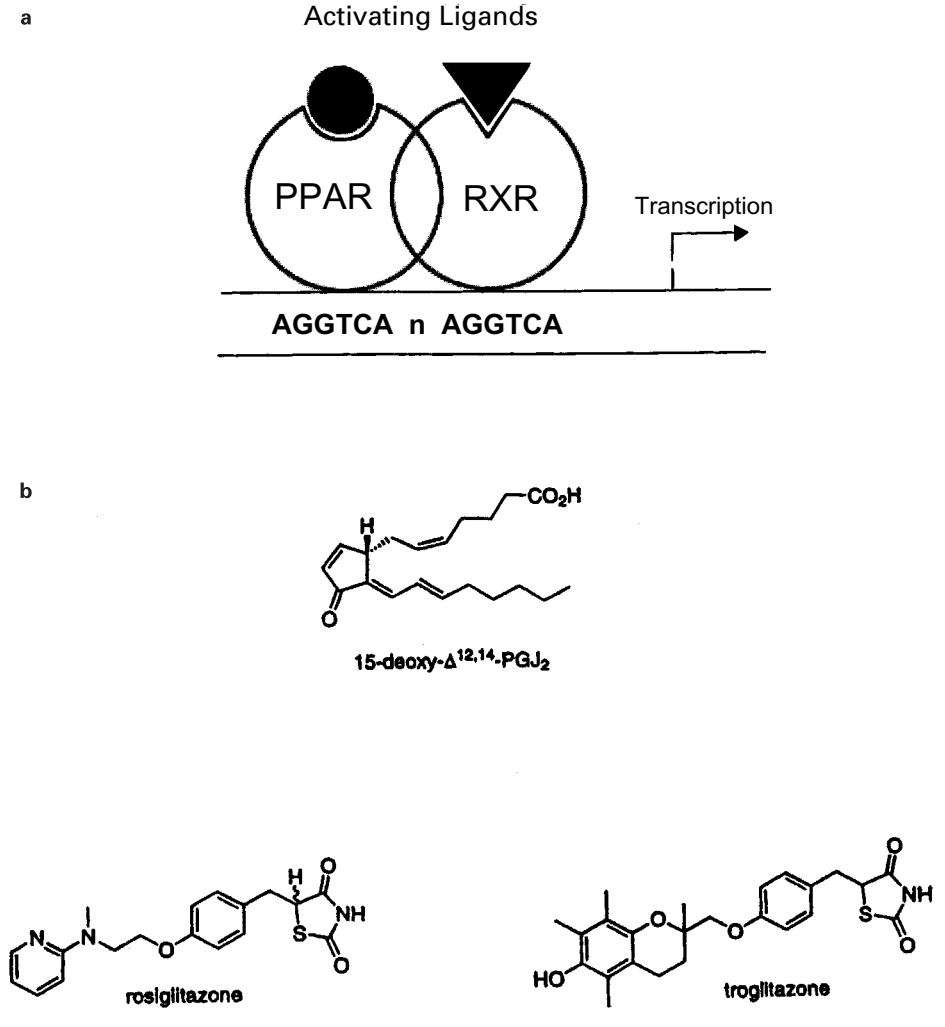

Figure 3 The PPAR nuclear receptor is one part of a transcription factor complex. In (A) the heterodimer of a PPAR and a retinoic acid receptor, RXR, is shown binding to the DNA sequence that functions as a response element for these proteins. Both receptors require activating ligands to bind other co-activating proteins necessary for the transcription of the downstream gene. In (B) two types of activating ligand for the PPARγ are shown. The PGJ_2 derivative is the most potent endogenous agonist known so far; long-chain fatty acids (arachidonate, linoleinate, eicosapentenoate) are also agonists. Several synthetic thiazolidinediones (TZDs) are agonists with sub-micromolar potency and two of those at present used as anti-diabetic therapy are also shown.

2000; Willson *et al*., 2000). In the present context, although PPAR β/δ may be involved (Kersten *et al*., 2000; Gupta *et al*., 2000), most attention has been paid to PPARγ (Debril *et al*., 2001). This PPAR is particularly involved with PG action because of the high potency of the cyclopentenone PGs derived from PGD_2 – PGJ_2 and its further derivatives, particularly 15-deoxy-delta 12,14-PGJ_2 (15dPGJ_2) – as agonist ligands for this nuclear receptor (Kliewer *et al*., 1995; Forman *et al*., 1995).

PPARγ The originally described function of the cyclopentenone PG ligands for PPARγ was to drive the differentiation of adipocytes from precursor cells (Kliewer *et al*., 1995; Forman *et al*., 1995). Many other fatty acids are less potent ligands for PPARγ, including another oxidized metabolite of AA, 15-HETE (Kersten *et al*., 2000). Another group of highly active agonist ligands are the thiazolidinediones (TZDs) such as troglitazone and rosiglitazone, but the link between activation of PPARγ and anti-diabetic action is not clear (Murphy & Holder, 2000).

A pro-carcinogenic role for activation of PPARs and cancer was originally proposed on the basis of the correlation of CRC with dietary fat (Hill, 1999; Reddy, 2000), the latter providing the agonist ligands for the PPARs. Later, work using Min mice showed that two TZD agonists of PPARγ, troglitazone and rosiglitazone, increased the numbers of polyps in the colon (Saez *et al*., 1998; Lefebvre *et al*., 1998). This coupled with the agonist potency of PGs and the anti-carcinogenic effect of C2Is in Min mice suggested that a PG agonist for PPARs activates pro-carcinogenic mechanisms and that the PG concerned is derived from COX-2. A potential positive feedback loop is suggested by the induction of COX-2 by PPARγ agonists and identification of a PPAR-response element in the promoter region of the COX-2 gene in epithelial cells (Meade *et al*., 1999).

An opposing hypothesis that stimulation of PPARγ is anti-carcinogenic also has experimental support. Early reports established the anti-neoplastic and anti-proliferative effects of PGD_2 and PGJ_2 (Fukushima, 1992; Negishi *et al*., 1995) before these PGs were recognized as ligands for PPARγ (Kliewer *et al*., 1995; Forman *et al*., 1995). The TZD troglitazone inhibited growth of several CRC cell lines in culture and of implants of these cells in mice (Sarraf *et al*., 1998). In mammary cancer cell lines, both troglitazone and 15dPGJ_2 inhibited proliferation and induced apoptosis (Clay *et al*., 1999). This action was independent of the oestrogen responsiveness of the cells. Recently, a synthetic, highly selective and potent non-TZD PPARγ agonist, GW7845, decreased tumour incidence, weight, and number in chemically induced mammary cancer in rats (Suh *et al*., 1999).

These conflicting results must be resolved if action at PPARγ is to provide viable explanations for the anti-carcinogenic effects of the C2Is. Further the procarcinogenic effects of TZDs in Min mice raises questions about their long-term effects in the elderly for the treatment of Type 2 diabetes. It is also tempting to speculate that the recent correlation between cancer and obesity (WHO, 2001) could be attributed to stimulation of PPARγ driving both adipocyte formation and cancer growth.

Angiogenesis Most solid tumours require new blood vessels to provide the nutrients necessary to ensure growth and survival (Holmgren, 1996). The provision of this new blood supply – angiogenesis – is also a crucial determinant of metastasis. Angiogenic factors could be secreted either by the tumour cells themselves or by the adjacent host cells and would elicit the new outgrowth of the host blood vessels (Augustin, 1998). Such pro-angiogenic actions of colon cancer cells were shown with HCA-7 or Caco-2 cells over expressing COX-2 [Caco-2 + COX-2], co-cultured with endothelial cells (Tsujii *et al*., 1998). Formation of endothelial cell tubules (an angiogenic response) was increased with [Caco-2 + COX-2] cells and these cells also secreted 4 fold more angiogenic factors than the parent Caco-2 cells. Both tubule formation and secretion of angiogenic factors was inhibited by the addition of C2Is or aspirin. The crucial contribution of the host cells was clearly demonstrated by the marked inhibition of growth of Lewis lung carcinoma cells in mice with COX-2 deletions, whereas COX-1 deletions had no effect relative to the wild type strain (Williams *et al*., 2000). Vascularity of the tumours (Factor VIII staining) in the COX-2 null mice was also reduced by about 30% compared with those in the wild type strain.

Histological analysis of metastases derived from HT29 or Lewis lung carcinoma cells showed COX-2 in the adjacent normal host blood vessels and in the tumour neovasculature but not in the tumour cells themselves (Masferrer *et al*., 2000). Incidence of metastasis from either HT29 or Lewis lung carcinomas was markedly reduced by treatment with a C2I, celecoxib. There is thus strong evidence to support the positive correlation between COX-2 activity, angiogenesis and primary or metastatic tumour growth.

Nevertheless, it is important to remember that angiogenesis in the adult is a normal physiological response, as, for instance, in wound healing or endometrial development during the menstrual cycle, and is driven by the same endogenous angiogenic signal molecules which stimulate tumour angiogenesis. In models of non-tumour angiogenesis, COX-2 has also been implicated. For instance, neovascularisation in rat cornea stimulated by exogenous bFGF was strongly inhibited by a C2I, celecoxib but not by a selective inhibitor of COX-1, SC 560 (Masferrer *et al*., 2000). Healing of gastric ulcers is also delayed by C2Is (Schmassman *et al*., 1998; Jones *et al*., 1999). Inhibition of physiological angiogenesis would then be a possible side effect of COX-2 inhibition in treatment of tumour angiogenesis in CRC or other cancers.

Effects of COX-2 on immunological responses Many cancers are associated with 'immunosuppressed' states, often expressed by changes in secretion of immuno-active cytokines. Thus IL-10 is increased and IL-12, TNF and IL-1 decreased in experimental models of cancer and these changes have been linked to increased synthesis of PGE_2 and COX-2 activity (Kambayashi *et al*., 1995; Shattuck-Brandt *et al*., 2000; Stolina *et al*., 2000). In CRC patients, a PGE_2-mediated immunosuppression has been observed (Balch *et al*., 1984). In this 'immuno-suppressive' mode, PGE_2 or other products of COX-2 may act *via* PPARγ as activation of this nuclear receptor by 15dPGJ_2 and other non-PG agonists reduced output of the inflammatory cytokines, IL-1, TNFα and IL-6 (Ricote *et al*., 1999). Another mode of action would be by interference with the expression of immunological receptors. Expression of HLA antigens was decreased in

samples of human CRC and adenomas (Tsioulias *et al*., 1992; 1993). Exposure of SW 116 cells to PGE_2 downregulated the HLA-DR (class II) antigen and aspirin induced expression of this antigen on HT 29 cells (Arvind *et al*., 1995).

Effects of COX-2 not mediated by PGs Although most of the biological consequences of COX-catalysed oxidation of arachidonate are attributable to the formation of PGs, this may not always be so in carcinogenesis. For instance the oxidation of arachidonate by COX generates other oxidative species and thus raises the overall oxidative state of the cell. One consequence of this is that COX-2 will co-oxidise compounds such as benzo [a] pyrene (Eling *et al*., 1990) to highly carcinogenic derivatives. Direct oxidative damage to DNA is a well recognized mutagenic event and such damage is increased following induction of COX-2 (Nikolic & van Breeman, 2001). Another cellular oxidation-response is the activation of NFkB known to be a transcription factor for COX-2 (Lim *et al*., 2001; Hardwick *et al*., 2001), setting up a possible positive feedback loop to maintain high oxidative levels in the cell. Accumulation of free arachidonate may also be involved since this appeared to be the trigger for apoptosis in HCT 116 cells, experimental mammary tumours or clinical samples of CRC (Chan *et al*., 1998; Trimboli *et al*., 1999; Cao *et al*., 2001). However, all these possible alternative mechanisms would still be susceptible to inhibition of COX-2 activity by selective inhibitors.

Prevention and treatment of CRC

Existing programmes Prevention schemes for CRC are at present based on early detection of polyps or tumours. Typically, they comprise a combination of genetic analysis to identify 'at risk' groups followed by physical screening – faecal blood tests (FOBT), sigmoidoscopy or, if justified, colonoscopy (Midgley & Kerr, 1999; Podolsky, 2000, Dove-Edwin & Thomas, 2001).

The most at risk group consists of the families with FAP; these would be the most intensively screened. A lesser risk group are the first-degree relatives of those with diagnosed HNPCC. A much larger third group (maybe as high as 5% of the population in the U.S.) comprises those with a family history of CRC, but which do not fall into either of the first two categories.

The treatment of diagnosed CRC is still resection of the affected bowel but although macroscopic clearance of tumours is good (80% in colon cancer), recurrence rates are high. Recurrence in the bowel may be treated by further resection in a small proportion of cases (3–5%) but usually the treatment is radiotherapy and chemotherapy. Chemotherapy is also given as an adjuvant therapy immediately after resection to minimise the development of metastases already disseminated (Leen *et al*., 2000). The main chemotherapeutic agent is 5-fluorouracil (5-FU) which interferes with thymidine incorporation into DNA. This and other cytotoxic agents (ralitrexed, oxaplatin and the newer topoisomerase inhibitors such as irinotecan) exert characteristic and unpleasant side effects such as diarrhoea, mucositis, alopecia and neutropaenia (Midgley & Kerr, 1999).

What is the role of COX-2 inhibition in prevention and treatment of CRC? Chemoprevention programmes for CRC have hardly started and any programme based on COX-2 inhibition would reflect the 'at risk' assessments already described above (Steinbach *et al*., 2000; Lynch, 2001). Indeed, the value of COX-2 inhibition has already been recognized for FAP (Steinbach *et al*., 2000) and such inhibitors may be used to decrease polyp number before colectomy or post-operatively to suppress growth of the polyps remaining. Although colectomy will remain the treatment of choice in FAP, it may be possible to delay that intervention, safely, by early 'prophylactic' use of C2Is in those who have the characteristic genetic profile but no physical signs.

Extension of the use of C2Is in the close relatives of those with HNPCC who are symptom-free is certainly worth a clinical trial, bearing in mind the inherently low side effect profile of these compounds. Furthermore, since HNPCC is also associated with tumours elsewhere in the body (Midgley & Kerr, 1999; Emery *et al*., 2001) and COX-2 expression is elevated in cervical and endometrial tumour tissue (Kulkarni *et al*., 2001; Munir *et al*., 2000), as well from many other sites (Table 2), extra-colonic action of C2Is could be beneficial.

Wider, more general chemoprevention of CRC with C2Is would ideally take the form of a prophylactic daily dose in, say, all those over 50 years old. The theoretical justification of this much wider use comes from the clear results from epidemiological analysis of NSAID use and CRC incidence (Table 1). These by themselves would have been enough to initiate clinical trials but for the low tolerability of the NSAIDs in subjects in the appropriate age range but otherwise symptom-free. The much lower potential of the C2Is for gastro-intestinal side effects have already been demonstrated (Simon *et al*., 1999; Bombardier *et al*., 2000). Direct support for a general chemopreventive application should be available from an analysis of those using C2Is for chronic inflammatory disease (rheumatoid or osteoarthritis), by analogy with the analyses already carried out for the NSAIDs (Thun, 1996). Refinement of this 'shotgun', age-based, approach to CRC prevention may also be possible with recent advances in gene analysis, using, for instance, screening for germline mutations in MMR enzymes or other new data from the Human Genome Project.

Treatment For most forms of CRC, resection will remain the preferred treatment. However, there is enough evidence from experimental models to propose a role for C2Is in treatment of diagnosed CRC. At present, the most logical place for C2Is is in adjuvant therapy mixtures given after resection. Several advantages would accrue. First, the low side effect profile of the C2Is would create little or no additional side effect to those already associated with the cytotoxic agents, routinely used. The anti-inflammatory effects of the C2Is may actually alleviate some of these side effects, which have inflammatory aspects (diarrhoea, mucositis). The anti-metastatic effects of C2Is in experimental systems (Masferrer *et al*., 2000) would be valuable in suppressing metastases which are frequently the cause of recurrence after potentially curative resections (Leen *et al*., 2000; Midgley & Kerr, 1999). Finally, there is experimental evidence for radio-sensitization by C2Is (Milas *et al*., 1999; Kishi *et al*., 2000; Gallo, 2000) and such an effect would be beneficial in those recurrences that are treated with radio-therapy. All these would be in addition to any inhibition of adenoma or tumour growth exerted directly by the C2Is.

Table 2 Association of COX-2 with cancer in other tissues

Tissue	*Increased level in clinical samples*	*Effect of COX-2 inhibition Human cell lines*	*Animal models*
Lung	Khuri *et al*., 2001 Hosomi *et al*., 2000 Soslow *et al*., 2000 Marrogi *et al*., 2000	Tsubouchi *et al*., 2000 Hung *et al*., 2000 Hida *et al*., 2000	Eli *et al*., 2001 Yao *et al*., 2000
Breast, mammary	Soslow *et al*., 2000		Alshafie *et al*., 2000 Nakatsugi *et al*., 2000
Prostate	Lee *et al*., 2001 Uotila *et al*., 2001 Madaan *et al*., 2000 Yoshimura *et al*., 2000 Kirschenbaum *et al*., 2000	Liu *et al*., 2000 Hsu *et al*., 2000 Attiga *et al*., 2000	

The references shown here have been restricted to the last 2 years (2000, 2001) for the sake of brevity. They are intended to provide an entry into a particular area and not a full survey. There are, for instance, several references to the detection of COX-2 in experimental models of cancer in earlier years and these should be accessible from the references dealing with the effects of COX-2 inhibition in the particular model. In all the references shown above and in most of the earlier work, there was a positive association of COX-2 with tumour tissues and inhibition of COX-2, either non-selective or selective, decreased tumour growth. Further associations between the level of COX-2 activity and the invasiveness or metastatic potential of tumour tissue are emerging (Dohadwala *et al*., 2001; Gaffney *et al*., 2001; Ryu *et al*., 2000) but will require further substantiation. Other tissues in which COX-2 has been associated with tumour tissue include bladder (Ristimaki *et al*., 2001; Grubbs *et al*., 2000), skin (Kanekura *et al*., 2000; Higashi *et al*., 2000), and liver (Shiota *et al*., 1999).

The first trials of C2Is in treatment of CRC would have to be as an addition to the usual mixture of agents. It may however be possible to reduce the level of cytotoxic agents by combining them with the C2Is to achieve equal or better survival. This may need a careful selection of patients, perhaps by genetic screening to maximise benefit. The consequent reduction in side effect intensity due to the cytotoxic agents would have marked benefits for the patient and improve compliance and acceptance of the therapy.

COX-2 in other forms of cancer

Most of the work, experimental or clinical, relating COX-2 to cancer has involved CRC and, to a lesser extent, gastric and oesophageal cancer. However this correlation has been tested in a wider range of neoplastic cells, generally those derived from epithelial cells in the organs involved, typically lung, prostate or breast (see Table 2). Although there is less work with the cancers in these organs and the conclusions are correspondingly less secure, overall, there is general support for the correlation. Thus, in clinical samples, there is more COX-2 in the tumour tissue than in normal tissue (Soslow *et al*., 2000). In cultures of cells derived from these human tumours, growth was inhibited by inhibitors of COX activity (Hsu *et al*., 2000). Finally, in models of chemically induced carcinogenesis, development of tumours was inhibited by NSAIDs or by C2Is (Alshafie *et al*., 2000).

There is one important practical difference between CRC and other cancers – the epidemiological evidence for protection with NSAID use, which is very clear for colon, rectal, gastric and oesophageal cancers, is much less strong for breast or lung cancer (Thun *et al*., 1993; Egan *et al*., 1996; Harris *et al*., 1996; Thun, 1996; Schreinermachers & Everson, 1999). Nonetheless there are positive findings, particularly in breast cancer, which deserve further attention.

The success of tamoxifen in chemoprevention of breast cancer (Decensi & Costa, 2000) and the links to the BRCA1 and BRCA2 genes (Brown & Lippman, 2000) should encourage further genetic correlations using the large library of samples already typed for BRCA which could now be assessed for COX-2. Further analyses could look for other signs of defects (such as microsatellite instability) in the genes for the MMR enzymes in breast cancer, bearing in mind that the phenotype of HNPCC also includes breast, uterine and ovarian tumours (Emery *et al*., 2001). Another very important feature of breast cancer is the hormonal responsiveness of the tumour which is frequently lost in the more invasive forms. A particular advantage of the C2Is is that they are equally effective in oestrogen-sensitive or -resistant cell lines (Trimboli *et al*., 1999). If this result were to be substantiated clinically, it would give the C2Is a very valuable feature. Further, on the basis of experimental results, the C2Is are likely to inhibit endometrial cancer, and this is one of the major side effects of tamoxifen treatment. These benefits coupled with the inherently low side effect profile of the C2Is will strengthen the case for clinical trial of C2Is as a component of the chemopreventive mixture in breast cancer.

Adjuvant therapy in breast cancer is well established and a number of regimens have been validated (Hortobagyi, 2000). However the agents used have serious, though reversible, side effects and even now recurrence is a real problem. Another useful modality is pre-operative chemotherapy to reduce tumour size and induce a degree of regression. This may be combined with radiotherapy (Kishi *et al*., 2000). In all of these regimens C2Is could contribute positively at best and, at least, add little to the side effect burden of the existing therapies (Vainio & Morgan, 1998; Howe *et al*., 2001).

Concluding remarks

There is now enough strong and unequivocal evidence for a casual link between COX-2 and CRC, which could be extended to a significant proportion of other epithelial derived cancers. The use of C2Is in FAP patients (Steinbach

et al., 2000) is proof of concept in a small sub-group of the susceptible population and the extension of C2I use into the adjuvant chemotherapy mixture for CRC should follow soon. The low level of side effects associated with C2Is relative either to the old NSAIDs or to the usual cytotoxic agents (5-FU, irinotecan, etc) will be a key component of the tolerability of C2I therapy – at least do no harm. More and further analysis of mammary and lung cancers, two major and intractable forms of human disease, by study both of human samples and of experimental models can only enlarge the therapeutic possibilities of the C2Is, even if they turn out to be effective in a small proportion of the cases.

Finally, the experimental approaches and models involving COX-2 that are now available have expanded our knowledge and understanding of neoplasia and their potential has not yet been exhausted nor even fully realized. The roles of COX-2, PGE_2 synthase, 5- and 15-lipoxygenase and other enzymes of fatty acid oxidation, when fully elucidated, will add significantly to our understanding of carcinogenesis. Even the demonstration of non-PG mechanisms exerted by the NSAIDs or C2Is and their congeners will open new possibilities and suggest new approaches to the central question of growth control in neoplasia.

Not all CRC or all epithelial cell-derived cancers will be dependent on COX-2 in their development. The variable phenotypes of the same genotype seen in Min mice (Shoemaker *et al*., 1997; Hong *et al*., 2001) will undoubtedly be reproduced in the genetically much more diverse human population and there may indeed be even more variation (Spirio *et al*., 1996; Tomlinson *et al*., 1996). In this context it would be valuable to screen CRC patients for genetic variants in phospholipases, as well as mutations in the genes for APC and MMR enzymes. This programme would be analogous to the pharmacogenomic analysis of receptors that is presently being undertaken to improve treatment of hypertension and mental illness. The techniques for such genetic screening are available and becoming increasingly routine and the enhancement of outcome resulting from better targeting would clearly justify the effort.

Inhibition of COX-2 will not be a panacea for cancer but it will at least provide a very significant therapy for a very significant proportion of the patient population. At best, that population may actually decrease, as chemoprevention by C2Is becomes a clinical reality.

It is a pleasure to acknowledge the efforts of many of my colleagues in encouraging me to write this review and then in criticising what I had written, thereby greatly increasing its clarity and intelligibility. Nonetheless, the facts, interpretations and mistakes are solely my own responsibility.

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(Received August 3, 2001
Revised August 28, 2001
Accepted September 4, 2001)