THE ROLE OF CYCLOOXYGENASE AND LIPOXYGENASE IN CANCER CHEMOPREVENTION

Muriel Cuendet and John M. Pezzuto

Program for Collaborative Research in Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, and University of Illinois Cancer Center, University of Illinois at Chicago, IL 60612, USA

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SUMMARY

The involvement of prostaglandins (PGs) and other eicosanoids in the development of human cancer has been known for over two decades. Importantly, an increase in PG synthesis may influence tumor growth in human beings and experimental animals, and numerous studies have illustrated the effect of PG synthesis on carcinogen metabolism, tumor cell proliferation and metastatic potential. PGs produced by cyclooxygenases (COXs) are represented by a large series of compounds that mainly enhance cancer development and progression, acting as carcinogens or tumor promoters, with profound effects on carcinogenesis. Further investigations suggest that arachidonic acid (AA) metabolites derived from lipoxygenase (LOX) pathways play an important role in growth-related signal transduction, implying that intervention through these pathways should be useful for arresting cancer progression. We discuss here the implications of COX and LOX in colon, pancreatic, breast, prostate, lung, skin, urinary bladder and liver cancers. Select inhibitors of COX and LOX are described, including nonsteroidal antiinflammatory drugs (NSAIDs), selective COX-2 inhibitors, curcumin, tea, silymarin and resveratrol, as well as a method useful for evaluating inhibitors of COX. Although a substantial amount of additional work is required to yield a better understanding of the role of COX and LOX in cancer chemoprevention, it is clear that beneficial therapeutic effects can be realized through drug-mediated modulation of these metabolic pathways.

KEY WORDS

cyclooxygenase, lipoxygenase, cancer chemoprevention

1. INTRODUCTION

The involvement of prostaglandins (PGs) and other eicosanoids in the development of human cancer has been known for over two decades /1/. Importantly, an increase in PG synthesis may influence tumor growth in human beings and experimental animals /2/, and numerous studies have illustrated the effect of PG synthesis on carcinogen metabolism, tumor cell proliferation and metastatic potential /3,4/. As a result, inhibition of PG synthesis has been

examined as a means of preventing tumor development /4,5/. Two major observations demonstrating the role of PGs in the genesis of cancer follow: inhibition of PG synthesis hinders tumor development in animal models and in some human cancers /3/, and there is a direct relationship between the levels of PG synthesized and cancer incidence in both humans and animal models. Moreover, members of the arachidonic acid (AA) metabolizing enzyme family seem to play a significant role in carcinogenesis, since modulation of these pathways results in suppression of tumor growth /6/.

PGs produced by cyclooxygenases (COXs) are represented by a large series of compounds that mainly enhance cancer development and progression, acting as carcinogens or tumor promoters, with profound effects on carcinogenesis /7/. Further investigations suggest that AA metabolites derived from lipoxygenase (LOX) pathways play an important role in growth-related signal transduction, implying that intervention through these pathways should be useful for arresting cancer progression /8,9/. Most malignant human tumors have a prolonged period of pathological development during which they pass through several preneoplastic and premalignant stages. This situation affords the opportunity of interrupting or reversing tumorigenesis at an early stage /10,11/. Thus, the ability of regulating COX and LOX pathways provides an excellent opportunity for cancer chemoprevention.

2. ARACHIDONIC ACID PATHWAY

Metabolites of AA, e.g., PGs, prostacyclins, thromboxanes and various LOX products, collectively known as eicosanoids, are produced in many tissues and facilitate a diverse group of physiological and pathophysiological responses. For example, these bioactive lipids are potent mediators of several signal-transduction pathways that modulate cellular adhesion, growth and differentiation /12/.

AA is cleaved from membrane phospholipids by phospholipases. AA can then be metabolized by the COX pathway to produce PGs, or the LOX pathway to produce hydroxy derivatives and leukotrienes (Fig. 1). COX, also known as PGH-synthase, is the rate-limiting enzyme in the metabolic conversion of AA to PGs and related eicosanoids. AA is converted to PGH_2 through the action of COX which exhibits two distinct catalytic activities, cyclooxygenase and

Fig. 1: Overview of the metabolism of eicosanoids. COX metabolites of arachidonic acid include PGs, prostacyclin, and thromboxanes. COXs catalyzes the sequential formation of PGG_2 and PGH_2. PGH_2 is enzymatically and non-enzymatically converted to various bioactive products, including $PGF_{2\alpha}$, PGE_2, and PGI_2 (prostacyclin). PGH_2 can also be metabolized to thromboxane A_2 and B_2. LOXs catalyse conversion of arachidonic acid to hydroperoxides (HPETE), which can subsequently undergo a number of reactions including reduction to hydroxyeicosatetraenoic acids (HETEs) and leukotrienes.

peroxidase /13/. Subsequently, PGH_2 is converted by cell-specific synthases to products such as PGE_2, $PGF_{2\alpha}$, PGI_2 or thromboxanes /14/.

Two COX isoforms, COX-1 and COX-2, are known. COX-1 is constitutively expressed in many tissues /14,15/, and PGs produced by COX-1 are thought to mediate "housekeeping" functions such as cyto-protection of the gastric mucosa, regulation of renal blood flow and platelet aggregation /16-18/.

In contrast to COX-1, the recently discovered isoform COX-2 is not generally detected in most tissues /19/. However, COX-2 is an inducible enzyme that is expressed in response to pro-inflammatory agents, including cytokines, endotoxins, growth factors, tumor promoters and mitogens /20,21/. COX-2 is expressed in a few specialized tissues such as brain, testes and macula densa of the kidney, in the apparent absence of any induction process.

AA is metabolized via LOXs to hydroxyeicosatetraenoic acids (HETEs) or leukotrienes (5-LOX pathway) /22,23/. Previously, three LOXs were recognized in humans: a 5S-LOX found in leukocytes, a 12S-LOX found in platelets and certain epithelia, and a 15S-LOX in reticulocytes, eosinophils, macrophages, and skin /24/. More recently, in studying LOX expression in human skin, a second 15S-LOX (herein referred to as 15-LOX-2) was discovered /25/. In addition to structural differences, 15-LOX-2 exhibits differences in certain enzymatic characteristics. In contrast to the reticulocyte type of 15S-LOX (15-LOX-1), 15-LOX-2 converts AA exclusively to 15S-hydroperoxy-eicosatetraenoic acid (15-HPETE). Unlike the distribution of 15-LOX-1, 15-LOX-2 was not detected in peripheral blood leukocytes, nor was it detected in liver, kidney, spleen, thymus, testis, ovary, skeletal muscle, heart, brain, or intestinal tissue /25/.

3. COX AND LOX IMPLICATIONS IN TUMORIGENESIS

3.1 Colorectal cancer

Colon cancer is the second leading cause of cancer mortality in developed countries /26/. Although this disease is potentially curable in its early stages of development, the prognosis of metastatic disease is usually poor /27/. However, the developmental path for colorectal cancer is especially well documented. Histopathologically, the first

stage involves hyperproliferation in colon mucosa, followed by formation of adenomas with varying degrees of malignant potential, and finally adenocarcinoma /28,29/. This well-defined histopathology along with the accessibility of all stages of colon carcinogenesis facilitates the evaluation of chemopreventive activity /30/.

Patients with a history of adenomatous polyps provide a feasible cohort for clinical chemoprevention studies, since their risk of developing new adenomas is high. In several studies, new adenomas were seen at rates ranging from 37-60% within 1-4 years following polypectomy /31/.

Anti-inflammatory drugs have shown potent chemopreventive activity in animal colorectal carcinogenesis models. These include the nonsteroidal anti-inflammatory drugs (NSAIDs) sulindac, piroxicam, aspirin, and ibuprofen, as well as curcumin, tea polyphenols, and the selective COX-2 inhibitor, celecoxib (Fig. 2) /32,33/. Use of such COX inhibitors as the NSAIDs, sulindac and aspirin, has a particularly strong association with prevention of gastrointestinal cancers /30/. In case studies and limited intervention studies, sulindac consistently caused regression of existing colonic polyps and prevented formation of new polyps. Furthermore, epidemiological studies have demonstrated up to 50% decrease in the relative risk of colorectal cancer among persons who regularly ingested acetylsalicylic acid or other NSAIDs /34/. Indeed, one complicating factor in estimating the sample size required for adenoma prevention trials is the widespread use of aspirin as a cardioprotective agent in the target population. Current evidence supporting the role of NSAIDs and COX-2 specific inhibitors in the prevention or treatment of intestinal polyposis and colon cancer is derived from both animal and epidemiological studies, but pioneering experimental work in this area was based on a murine model of familial colonic polyposis /35/.

In *min* mice, which are prone to colonic polyps, there is a genetic defect in the *Apc* gene that is similar to that associated with the appearance of adenomatous polyposis of the colon (benign adenomas) in humans; therefore, this model is appropriate for studying human disease. When bred with COX-2 knockout mice, *min* mice did not tend to contract adenomas, and, furthermore, COX-2 specific inhibitors appeared to block the formation of these neoplasms in the *min* mouse. However, the complexity of this model was demonstrated when it was found that breeding of the *min* mouse with the COX-1

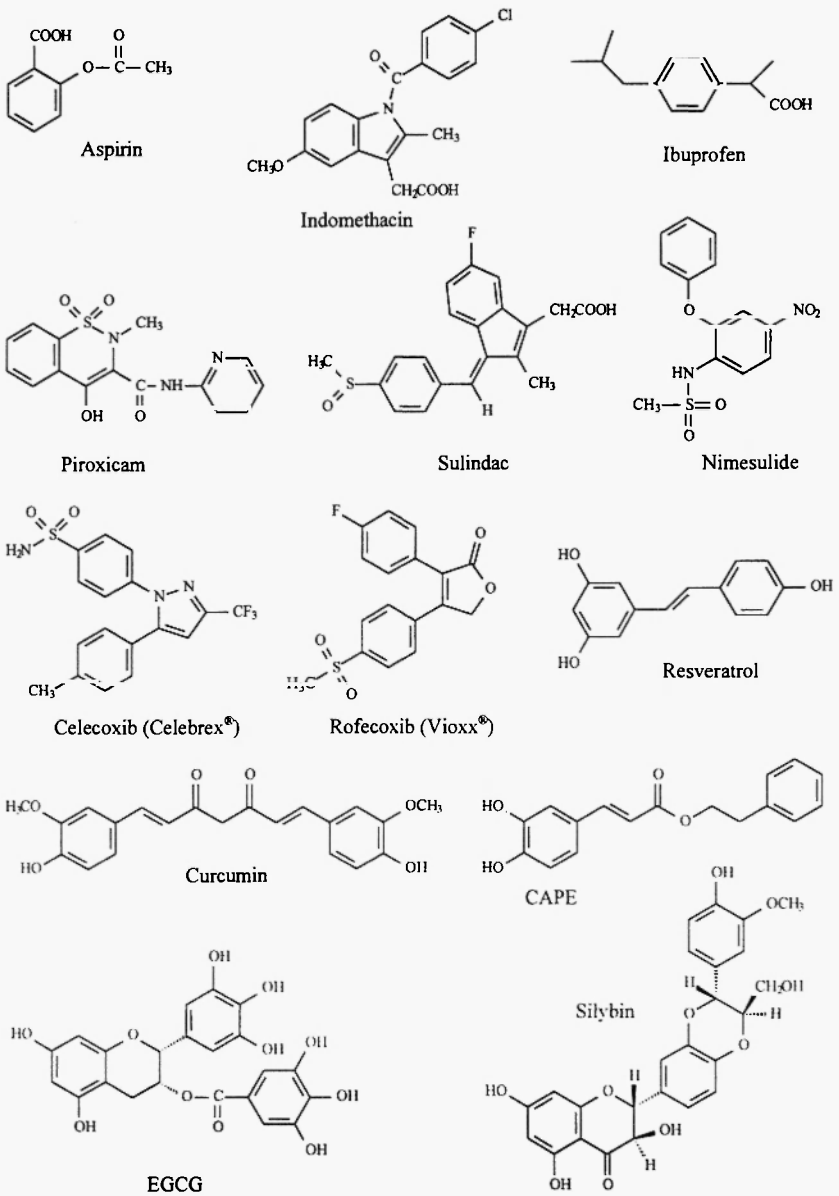

Fig. 2: Structures of aspirin, indomethacin, ibuprofen, piroxicam, sulindac, nimesulide, celecoxib, rofecoxib, curcumin, EGCG, silybin, resveratrol and CAPE.

knockout mouse reduced adenoma formation /36/. These results suggest a role for both COX-1 and COX-2 in adenoma development. Nonetheless, Oshima *et al.* /37/ demonstrated that treatment of $Apc^{\Delta 716}$ knockout mice with the investigational COX-2 inhibitor MF-tricyclic reduced polyp number to a significantly greater extent than sulindac. A COX-2-generated AA metabolite (a prostanoid) was required for the survival of adenomas in mice.

A large body of experimental evidence supports the premise that COX-2 expression may contribute to tumorigenesis and that COX-2 inhibitors might be useful in the prevention or treatment of intestinal polyposis and colon cancer. Work by DuBois *et al.* /38/ showed an increase in COX-2 mRNA (by Northern blotting) in six of six rodent colonic tumors, and an increase in COX-2 protein (by Western blotting) in four of five such tumors. On the other hand, COX-1 mRNA levels were equivalent in both tumor and normal intestinal mucosal cells. Tsujii *et al.* /39/ extended these findings by demonstrating that expression of COX-2 resulted in phenotypic changes that could affect the metastatic potential of colorectal cancer cells. Experimental evidence for the prospective use of COX-2 inhibitors in colon cancer prevention is abundant, and celecoxib was approved by the US Food and Drug Administration for this use in late 1999.

The mechanism by which (over)expression of COX-2 contributes to carcinogenesis is not completely understood. The bifunctional nature of COX appears of importance since the peroxidase activity of COX may catalyze the conversion of procarcinogens to proximate carcinogens /40/. In liver, this type of oxidative reaction is catalyzed principally by cytochrome P-450s; however, extrahepatic tissues, e.g., the colon, frequently have low levels of cytochrome P-450s and other monooxygenases. Therefore, appreciable quantities of xenobiotics could be converted to mutagens by the peroxidase activity of COX /40/, and this could be especially relevant in the colon because of exposure levels /41/. Furthermore, the turnover of AA through COX metabolism is sufficient to produce mutagens. For instance, oxidation by-products, such as malondialdehyde, are highly reactive and form adducts with DNA.

Additional studies have shown a clear causal linkage between the expression of COX-2 and the inhibition of programmed cell death /42,43/. It is possible that up-regulation of COX-2 prolongs the survival of abnormal cells and thereby favors the accumulation of

sequential genetic changes that increase the risk of tumorigenesis. Treatment with the NSAID sulindac sulfide reversed the delay of apoptosis induced by overexpression of COX-2 /42/. Since chronic inflammation with elevated COX-2 is recognized as a risk factor for epithelial carcinogenesis /44/, a cause-and-effect link between chronic inflammation and carcinogenesis via overexpression of COX-2 can be established and understood through a reasonable mechanistic interpretation.

Another physiological response typically associated with the growth of tumors is immune suppression /45/. Colony-stimulating factors released by tumor cells lead to the production of PGE_2 by monocytes and macrophages, which in turn inhibit the production of immune regulatory lymphokines, T- and B-cell proliferation, and the cytotoxic activity of natural killer cells. PGE_2 also inhibits the production of tumor necrosis factor while inducing the production of interleukin-10, which has immunosuppressive effects /46/. Inhibitors of COX such as aspirin, sulindac, and indomethacin (Fig. 2) can attenuate tumor-mediated immune suppression /47/.

COX-2 can also modulate the invasive properties of human colon cancer cells. Thus, when human colon cancer cells were permanently transfected with a COX-2 expression vector, production of PGs increased, and the cells became more invasive relative to parental cells /39/. Increased invasiveness was associated both with activation of membrane metalloproteinase-2 and increased mRNA expression of the membrane-type metalloproteinase-1. These enzymes digest the collagen matrix of the basement membrane stimulating the invasive and motile phenotype of tumor cells. Both the increased production of PGs and invasiveness were reversed by treatment with sulindac sulfide /39/. In further studies with colon cancer cells, overexpression of COX-2 enhanced the production of vascular growth factors, the migration of endothelial cells through a collagen matrix, and the formation of tubular networks /48/. The effect of COX-2 on angiogenesis was blocked by a selective inhibitor. In addition, however, aspirin or treatment of the endothelial cells with antisense oligonucleotides to COX-1, but not COX-2, inhibited angiogenesis /48/, indicating that the activity of COX-1 in endothelial cells was critical for their response to vascular growth factors. Furthermore, some colon cancer cell lines do not express COX-1 or COX-2, but nonetheless stimulate angiogenesis.

In addition to COX-related activities, AA and linoleic acid metabolites formed by the enzymatic activity of LOX need to be considered. Unsaturated fatty acid metabolism and the expression of the enzymes responsible for lipid metabolism during sodium butyrate (NaBT)-induced apoptosis and cell differentiation have been examined in human colorectal carcinoma Caco-2 cells. These cells underwent apoptosis and cell differentiation in response to NaBT, and had a mutated *Apc* gene expressing the truncated *Apc* protein product /49/. AA metabolism was dramatically shifted during the NaBT treatment, from PGs to metabolites of 15-LOX, mainly 15-HETE. Caco-2 cells poorly expressed COX-1, but a high expression of COX-2 was observed. Treatment with NaBT modestly attenuated the expression of COX-2 but significantly increased the expression of 15-LOX, as confirmed by RT-PCR and restriction enzyme analysis. The relative concentrations of COX-2 and 15-LOX observed at different times during apoptosis and cell differentiation suggest these enzymes work in parallel to modulate these processes in Caco-2 cells /50/. The expression of 15-LOX-1 was only observed during apoptosis and cell differentiation. Inhibition of the LOX resulted in an enhancement of apoptosis, which supports the hypothesis that LOX metabolites act as inhibitors of apoptosis in this cell system.

A subsequent report presented evidence for the expression of 15-LOX-1 in human colorectal cells and demonstrated a significantly higher expression of 15-LOX-1 in a tumor sample compared with normal adjacent tissues /51/. As shown by immunohistochemistry, 15-LOX-1 is primarily localized in the epithelium. The physiological importance of 15-LOX-1 expression in human colorectal tissue and increased expression in colorectal tumors is not clear. 15-HETE, the AA metabolite formed by 15-LOX-1, is reported to have both anti- and pro-inflammatory functions. It can promote cell growth, play a role in signal transduction with mucous-secreting cells, and alter integrin expression and cell migration /52/.

Another report studying 5-LOX with Caco-2 cells showed that butyrate-induced differentiation was associated with a significant up-regulation of 5-LOX at the level of mRNA and protein expression without affecting its activity. This is likely to result from enhanced 15-HETE levels, suppressing the 5-lipoxygenation of AA /53/.

3.2 Pancreatic cancer

Pancreatic carcinoma is characterized by a poor prognosis with lack of response to conventional therapy /54-56/. The 5-year survival rate is less than 2%, and median survival after diagnosis is only 4-6 months /56/. The incidence of this disease has increased significantly in recent years /56/. Several factors have been implicated in this rise in frequency, including cigarette smoking, gallstones, a diet high in animal fat, and chronic calcific pancreatitis /57/. Although a few studies have suggested the possible role of K-*ras* oncogenes, tumor suppressor genes (p16, p53, and DPC4), and growth factors (epidermal growth factor, basic fibroblast growth factor, and insulin-like growth factor I) in carcinoma of the pancreas, the exact pathogenic mechanism and progression of this neoplasm remain to be clarified, and no effective strategy for treatment has been established thus far /58-62/.

Very recently, it was reported that human pancreatic tumors and cell lines express increased levels of COX-2 mRNA and protein. Each study demonstrated that at least 50% of pancreatic carcinoma cases overexpressed COX-2 mRNA /63-65/. In contrast, the expression of COX-1 mRNA was similar in cancer and normal tissues. Furthermore, the results of mRNA analysis paralleled those of immunohistochemisty /63/. COX-2 mRNA levels were increased in two of four specimens of normal pancreas adjacent to tumor, relative to pancreas from a healthy individual. The explanation for this observation is unclear, but it may be a consequence of tumor cell contamination or the induction of COX-2 expression in normal tissue by the adjacent tumor /64/.

COX-2 was expressed in the majority of human pancreatic carcinomas, irrespective of histological type. This aspect may be a unique characteristic of pancreatic carcinoma since in carcinomas of the colon, lung and liver /66-68/, COX-2 expression was dependent on histological grade or type. Moreover, COX-2 expression was not affected by several parameters that are associated with tumor progression, such as tumor size, lymph node status or distant metastasis, and clinical stage /63/.

Since mutations in the K-*ras* oncogene /69/ are common in pancreatic cancer, a study was performed to establish whether the expression of oncogenic K-*ras* correlated with increased COX-2

expression. Using primary human pancreatic adenocarcinomas /70/, it was found that Ras activation did not appear sufficient to mediate the induction of COX-2 expression, suggesting activation of signal pathways other than or in addition to Ras might determine the extent of COX-2 expression in cancer cells. Such pathways may include the p38 mitogen-activated protein kinase which has been reported to regulate the induction of COX-2 in lipopolysaccharide-treated human monocytes /71/. In addition, in human vascular endothelial cells, the transcription factor NF-κB p65 was found to mediate induction of COX-2 in response to hypoxia /72/. Thus, the induction of COX-2 expression in pancreatic carcinoma appears to be mediated by multiple signaling pathways. The specific pathway(s) activated may depend on the cell type as well as the stimulus. Further experiments will be required to delineate which signaling pathways are functional in pancreatic tumor cells.

Only a few studies have been performed to examine the role of LOX in pancreatic cancer cell lines. A substantial expression of both 5-LOX and platelet-type 12-LOX has been observed in human pancreatic cancer cell lines but not in normal human pancreatic ductal cells /73,74/. As demonstrated with LOX inhibitors, blocking either 5-LOX or 12-LOX activity substantially inhibited *in vitro* pancreatic cancer cell proliferation, suggesting that both 5-LOX and 12-LOX were required for rapid pancreatic cancer cell proliferation. Furthermore, it has been shown that pancreatic cancer cell growth and apoptosis were regulated by both 5-LOX and 12-LOX. Growth inhibition of pancreatic cancer cells by LOX inhibitors was associated with induction of apoptosis and differentiation /75/.

3.3 Breast cancer

Breast cancer is the most commonly diagnosed malignancy, and despite intensive cancer control efforts, it currently remains the second leading cause of cancer deaths among American women /76/.

PGs appear to play a key role in the growth and progression of rodent and human breast tumors /77-80/. One line of evidence that most strongly supports a role for PGs in breast tumor growth came from recent epidemiological studies which demonstrated a significant inverse association between the intake of NSAIDs and the risk of breast cancer /81,82/. Both COX-1 /83/ and -2 /83,84/ are reported to be elevated in human breast cancer tissues. Of further importance is

the observation by Zhao *et al.* /85/ that the chief PG, PGE_2, effectively and specifically induces the promoter II region of the cytochrome P-450 aromatase gene (CYP-19). This paracrine effect of PGE_2 therefore can potentiate local biosynthesis of estrogen and provided a critical link between the PG cascade and deregulation of estrogen biosynthesis in mammary carcinogenesis. *In vivo* evidence supportive of this effect has recently been reported by Brueggemeier *et al.* /86/, who observed a significant positive correlation between the genetic expression of COX and CYP-19 in human breast cancer.

The expression of leukocyte 12-LOX has been shown to increase in breast cancer cells and tissues, compared with their normal counterparts /87,88/. In contrast, 15-LOX mRNA expression was not regulated in a similar manner. In order to evaluate the potential functional significance of altered 12-LOX expression in the breast cancer cells, the effects of two specific 12-LOX inhibitors were examined. Both LOX inhibitors led to a marked inhibition of serum-induced growth of MCF-7 cells. This study indicated that LOX products can initiate several growth-related signaling events, such as activation of oncogenes, protein kinase C (PKC), and mitogen-activated protein kinases.

Recently, EGF-dependent metabolism of arachidonic and linoleic acids has been examined with the BT-20 cell line /89/. 15-LOX-dependent formation of 13(*S*)-hydroxyoctadecadienoic acid (HODE), a lipid mediator which up-regulates the EGF receptor signaling pathway, was observed. Therefore, it can be suggested that increased formation of 13-HODE as a consequence of high dietary intake of linoleic acid could result in amplification of the EGF receptor signaling pathway, which in turn could lead to enhanced cellular proliferation in breast carcinoma tissue.

3.4 Prostate cancer

Prostate cancer (PCA) is the most common type of cancer in men, and one of the leading causes of cancer-related deaths in the United States /90/. In 1998 alone, it was estimated that about 185,000 new cases (30% of all new cancers in men) were diagnosed and more than 39,000 patients died from PCA in the USA /26/.

Various studies have shown that COX inhibitors induce apoptosis in human prostate cancer cell lines. In prostate carcinoma cell line LNCaP, constitutively expressing COX-2, addition of a selective COX-2 inhibitor, NS398, was found to induce apoptosis by down-

regulation of Bcl-2 expression /91/. Growth inhibitory and apoptotic effects of sulindac derivatives were seen in both the androgen-sensitive LNCaP cells and androgen-insensitive PC3 cells, as well as in derivatives of LNCaP cells that stably express increased levels of the anti-apoptotic protein Bcl-2 /92/.

It is also interesting to consider the possible link between known genetic alterations in PCA and COX-2. Mutations of the *Apc* gene also occur in human PCA /93/. The relationship between COX-2 and *Apc* was highlighted by the finding that COX-2 deficiency protected against tumor formation in $Apc^{\Delta 716}$ knockout mice /37/. Very recently, an *in vivo* study demonstrated overexpression of COX-2 in PCA /94/; COX-2 mRNA in PCA was 3.4-fold higher than in non-cancerous tissue, and COX-2 protein was overexpressed in 83% of the tumor samples.

Most of the work carried out with LOX and PCA has studied the role of 5-LOX. AA was found to effectively stimulate the *in vitro* growth of human prostate cancer cells at micromolar concentrations. Selective blockade of the different metabolic pathways of AA (COX, 12-LOX, 5-LOX) revealed that this growth stimulatory effect was inhibited by 5-LOX specific inhibitors, 2-(12-hydroxydodeca-5,10-dinyl)-3,5,6-trimethyl-1,4-benzoquinone (AA861) and 1-(4-chloro-benzyl-3-*tert*-butylthio-5-isoprop-2-yl)-2,2-dimethylpropanoic acid (MK886), but not by other inhibitors /95/. Blockade of 5-LOX was sufficient to mediate growth arrest and subsequent cell death /96/. Under serum-free conditions, the following observations were made: (1) human prostate cancer cells constitutively produce 5-HETE, which is dramatically increased by exogenous AA; (2) inhibition of 5-LOX activity by MK886 blocks 5-HETE production and induces massive apoptosis in both hormone-responsive and nonresponsive human prostate cancer cells, and (3) exogenous 5-HETE and its derivatives protect these cells from apoptotic cell death induced by MK886. These findings led to the notion that dietary AA might promote the progression of PCA by providing a potent antiapoptotic factor that allows the cells to survive androgen removal and even removal of all exogenous growth factors. Other reports have shown that 12-LOX mRNA is elevated in prostate tumor samples, compared with normal tissue from the same patient /97/, and that a reduction or loss of 15-LOX-2 and 15-HETE formation in prostate carcinoma could be a crucial event in the development or progression of prostate adenocarcimoma /98/.

3.5 Lung cancer

Lung cancer led to approximately 180,000 deaths in the USA in 1999 and is the single greatest cause of cancer mortality /99/. About 75% of lung cancer is classified as non-small cell lung carcinoma (NSCLC). Adenocarcinoma, a secretory tumor, is the most common NSCLC type among smokers, and the only form of lung cancer that non-smokers develop. Adenocarcinoma incidence has increased alarmingly in both smokers and non-smokers in recent decades /100/. The peripheral nature of adenocarcinoma makes early detection by sputum cytology difficult and the disease is seldom diagnosed until advanced stages yield clinical symptoms; unfortunately, advanced adenocarcinoma is therapeutically intractable /101/.

Epidemiological studies have shown that NSAIDs such as aspirin significantly reduced the risk of lung, colon and breast cancers /102/. Wolff *et al.* reported elevated levels of COX-2 mRNA in well-differentiated human lung adenocarcinoma tissues when compared with their paired controls devoid of cancer cells. Immunohistochemical staining of the COX-2 protein showed a positive response in 90% of adenocarcinomas and in all squamous cell carcinomas /103/. Animal studies have also shown an increase of COX-2 in lung tumors. COX-2 was highly expressed in *N*-nitroso*bis*(2-hydroxypropyl)amine (BHP)-induced lung adenomas and adenocarcinomas, but only to a much lesser extend in squamous cell carcinomas and alveolar/bronchiolar hyperplasias /104/. Mouse lung tumors ranging in size from small adenomas to large adenocarcinomas, as well as hyperplastic foci, contained more of both COX-1 and COX-2 than equivalent amounts of protein prepared from whole lung extracts /105/. In contrast, only high levels of COX-2 (not COX-1) have been reported in human lung cancers /67,103/. The potent tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is present in cigarette smoke and most likely involved in lung tumorigenesis in smokers /106,107/. In a rodent model, inhibitory effects of NS-398, aspirin, sulindac, ibuprofen, piroxicam and naproxen on lung carcinogenesis induced by NNK have been reported when the agents were given during and after the NNK-treatment /108-110/. Inhibition of COX-dependent NNK activation by aspirin and NS-398 in lung tissue is a possible mechanism of preventing lung cancer.

Avis *et al.* reported that mRNA for 5-LOX and 5-LOX-activating protein (FLAP) are expressed in lung tumor cell lines /111/. They also demonstrated *in vitro* lung cancer cell growth could be selectively inhibited by 5-LOX inhibitors AA861, MK886, and nordihydroguaiaretic acid (NDGA). The growth inhibition mediated by MK886 in a SCLC cell line could be overcome by the addition of 5-HETE. Although the specificity of the 5-LOX inhibitors was not complete, similar results with three different types of 5-LOX inhibitors is consistent with the LOX pathway playing a significant role in growth factor signaling. A similar study was conducted with NSCLC cells and it was found that NDGA inhibited LOX in both human and mouse lung cells /112/. Previously, it has been shown that MK886 inhibits lung carcinogenesis in A/J mice induced by NNK /113/.

A recent study has shown that LOXs metabolize NNK and that A79175 inhibits this activation. These data further suggest that 5-LOX inhibitors should be efficient preventive agents of lung tumorigenesis. Possible mechanisms of action could involve inhibition of cell proliferation as well as inhibition of LOX-mediated NNK activation /114/. A79175 and MK886 were ~10-times more potent inhibitors of proliferation than aspirin, but there was a synergistic effect with a combination of 5-LOX and COX inhibitors /114/.

3.6 Skin tumors

Chronic sun exposure is a well-recognized etiologic agent for skin cancer. Approximately 90% of the estimated 900,000-1,200,000 new cases of squamous and basal cell carcinomas diagnosed in the US each year are attributable to UV light exposure. The majority of these tumors are basal cell carcinomas which are relatively benign; however, squamous cell carcinoma (the second most common cutaneous malignancy) accounts for approximately 2,000 deaths annually. Extensive documentation has validated the role of ultraviolet B irradiation (UVB) (290-320 nm) as both a tumor initiator and promoter /115-118/. One possible mechanism for the promotional activity of UV light involves its ability to induce PG formation. Increased production of free AA and PGs are characteristic responses of human keratinocytes following acute exposure to UVB irradiation /119,120/. These products may then function as tumor promoters in UV-initiated tissue, or they may enhance initiation as a result of their ability to function as oxidants /121,122/. While COX-2 expression in normal

skin was usually very low and restricted to regions of differentiated epidermis /123,124/, studies dealing with mouse and human skin carcinogenesis revealed that overexpression of COX-2 contributed to the development of skin cancers, and COX-2 expression was constitutively enhanced in skin cancers /123-125/. The chemopreventive effects of NSAIDs have been tested. COX-2 formation was stimulated by treatment with phorbol ester tumor promoters, while NSAIDs such as the non-selective COX inhibitor indomethacin and the selective COX-2 inhibitor SC58125 /126/ were found to be potent inhibitors of skin-tumor promotion /125,127/. Moreover, this animal model was used to demonstrate that the anti-tumor effect of NSAIDs is related to PG formation, since the inhibitory activity of indomethacin could be reversed specifically by $PGF_{2\alpha}$ /128/. In addition, the time-course of COX-2 induction correlated with that of $PGF_{2\alpha}$ formation during tumor promotion.

During the course of mouse skin carcinogenesis, distinct LOX isozymes became constitutively overactivated, at least as strongly as COX-2 /129/, and the corresponding AA metabolites, i.e., 8- and 12-HPETE, caused chromosomal damage in epidermal cells /130/. Moreover, like COX inhibitors, LOX inhibitors suppress mouse skin carcinogenesis /131,132/. Interestingly, 8- and 12-LOX-catalysed AA metabolism was found to be up-regulated in premalignant rather than in malignant mouse epidermis /130/. For clinical application, skin cancer is thought to develop from defined pre-cancerous lesions /133/. Therefore, as precursor lesions for squamous-cell carcinomas, actinic keratoses may be potential targets for preventive measures.

3.7 Urinary bladder tumors

More than 50,000 people are diagnosed with transitional cell carcinoma (TCC) of the urinary bladder each year in the United States alone, and 10,000 of these patients are expected to die from the cancer /134/. Most bladder cancer deaths result from invasive, metastatic TCC that is resistant to chemotherapy. COX-inhibiting drugs have induced remission of chemically-induced bladder tumors in rodents and naturally occurring invasive TCC in dogs (a relevant model of human invasive urinary bladder cancer) /135-137/. Western blot analysis and immunohistochemistry revealed COX-2 protein to be barely expressed in normal urinary bladder epithelium, whereas it was highly and diffusely expressed in all the TCCs examined. On the other

hand, roughly equivalent amounts of COX-1 protein were clearly expressed in normal epithelial cells and tumors /138;139/. In addition, since it is known that post-initiation treatment with the selective COX-2 inhibitor nimesulide (Fig. 2) prevents the development of TCCs induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) in the rat urinary bladder /140/, these results clearly suggest potentially important roles for COX-2 in the development of preneoplastic and neoplastic lesions in the rat urinary bladder. We are not aware of studies investigating the expression of LOX in urinary bladder tumors.

3.8 Liver cancer

Primary hepatocellular carcinoma (HCC) is one of the most common tumors in southeast Asia and Africa, where the incidence of this disease is approximately 30 per 100,000 men/year. The prognosis of HCC is generally poor, with the 5-year survival rate limited to 25-39% after surgery /141/. Many investigators have reported a putative link between infection with hepatitis B or C viruses or hepatic cirrhosis and the development of HCC. Hepatic cirrhosis is observed in up to 90% of patients with HCC /142/.

A significantly increased expression of COX-2 was found in HCC cells in comparison with the expression of COX-1, suggesting COX-2 is involved in hepatocarcinogenesis. Of interest, a profound expression of COX-2 was demonstrated in small-sized and well-differentiated HCC, which was confirmed by Western blotting /143/. Reduced expression of COX-2 with less-differentiated HCC was found. Since previous studies had shown that human HCC can progress from a well-differentiated to a less-differentiated histological grade as time passes /144/, enhanced expression of COX-2 in small-sized and well-differentiated HCC suggests COX-2 involvement in the early stages of hepatocarcinogenesis. Enhanced COX-1 expression in both chronic hepatitis liver and cirrhotic liver, and its reduced expression in advanced HCCs, have been demonstrated as well as COX-2 expression, although the immunoreactivity for COX-1 was significantly weaker than that of COX-2 /68/. Such a similarity raises the possibility of the two COX isoforms sharing a common function in hepatic neoplastic and non-neoplastic parenchymal cells. However, other studies have demonstrated that overexpression of COX-1, but not COX-2, was involved in the tumorigenic transformation of immortalized cells /145/. Thus, critical analysis is required to determine

whether the two COX isoforms play distinct functional roles in hepatic neoplastic and non-neoplastic parenchymal cells.

Non-tumor liver tissues often express high levels of COX-2 protein and mRNA, relative to those of carcinoma tissues /146/. These findings are contrary to those described in organs such as colon, stomach, esophagus and lung. COX-2 levels are reported to be elevated in these tumor types compared with non-tumor tissues /63,65-67,83,103/. In the liver, however, non-tumor tissues were generally pathological because of hepatitis C virus-infected chronic hepatitis or cirrhosis. A low level of COX-2 mRNA was observed in normal liver /15/, and normal liver tissue devoid of viral infection was found to show little or no expression of COX-2. Further, Kondo *et al.* reported that COX-2 expression in non-tumor tissue was closely related to the postoperative relapse of HCC /146/.

A functional role of COX has recently been demonstrated by Denda *et al.*, who found that administration of NSAIDs suppressed cirrhosis and subsequent formation of HCC in a choline-deficient, L-amino acid-defined rat model /147/. We are not aware of studies investigating the expression of LOX in liver cancer.

3.9 Other types of cancer

Various other types of cancer have been associated with increased levels of COX-2 expression, including gastric cancer /148,149/, esophagial cancer /150/, uterine cervical cancer /151/ and retinoblastoma /152/. Further investigations are needed to define the role of COX-2 in the development of these cancers.

4. INHIBITORS OF COX AND LOX

4.1 NSAIDs and COX-2 inhibitors

The extraction of sodium salicylate from white willow bark (*Salix alba vulgaris*) in 1897 represented a major therapeutic advance in providing one of the most commonly used and durable drugs for treatment of inflammation and fever /153/. Since that time, clinical applications of NSAIDs have diversified to include analgesia and anti-platelet activity. In more recent years, NSAIDs have been cited as possible agents for the chemoprevention of colorectal adenomatous

polyps and cancer. The concept of chemoprevention by NSAIDs has been addressed in epidemiological, clinical, animal and basic science studies /4/.

There is good evidence to support the hypothesis that aspirin and other NSAIDs may help to reduce the risk of colorectal cancer. The origins of this hypothesis can be traced to 1975 when Bennett and Del Tacca /154/ noted that human colorectal cancer produced more PGE_2 than the surrounding mucosa. They hypothesized that cancers which produced excessive amounts of PGE_2 might promote their own growth and/or spread. This hypothesis has subsequently gained support from a series of experiments in rodents. Experimental animal models of colorectal carcinogenesis have consistently and reproducibly revealed the ability of NSAIDs such as indomethacin and piroxicam to inhibit chemically-induced pre-cancerous adenomatous polyps and early carcinomas of the colon /155,156/. NSAIDs reduced the incidence, multiplicity and size of tumors. This effect was sustained even when NSAID treatment started weeks after exposure to the chemical initiator.

Beginning in 1983, clinical studies with the NSAID sulindac were conducted in patients with familial adenomas polyposis (FAP) /157/. This condition is characterized by the development of hundreds to thousands of colorectal adenomatous polyps and eventually colorectal cancer. Clinical studies have consistently shown that sulindac was effective in reducing the size and number of colorectal polyps in FAP patients /158/. In addition, over 20 epidemiological studies have consistently shown that regular aspirin use lowered the risk of sporadic adenomatous colorectal polyps and fatal colorectal cancer by about 50%. These studies have differed in design, location, population and motivating hypotheses /102,159-162/. There was substantial evidence from clinical and epidemiological data to suggest regular long-term use of NSAIDs facilitated chemopreventive action against colorectal polyps and cancer, but infrequent or previous use generally was not associated with reduced risk /102,159-161,163/.

The fact that all NSAIDs in clinical use were COX inhibitors provided a putative link between the inhibition of COX activity and the chemopreventive effect of NSAIDs /164,165/. The discovery of the two isoforms of COX led to the suggestion that the therapeutic activity of NSAIDs was primarily the result of inhibition of COX-2, whereas the toxicity of NSAIDs might primarily result from inhibition

of COX-1 /166/. Several lines of evidence suggested a critical role of COX-2 expression in colon cancer and that specific COX-2 inhibitors might represent novel chemopreventive tools: (1) COX-2 but not COX-1 was overexpressed in a high percentage of colorectal polyps and cancers /66/; (2) both gene disruption and pharmacological inhibition of COX-2 dose-dependently reduced the number of intestinal polyps in $Apc^{\Delta 716}$ knock-out mice /37/; (3) administration of the highly selective COX-2 inhibitor SC58635 (celecoxib) to carcinogen-treated rats caused 40% reduction in colonic aberrant crypt foci formation /167/. Moreover, the highly selective COX-2 inhibitor SC58125 reduced tumor formation by 85-90% in nude mice implanted with a transformed human colon cancer cell line expressing COX-2 (HCA-7) but not in mice implanted with HCT-116, a cancer cell line not expressing COX-2 /168/. Furthermore, endoscopically controlled studies showed that a selective inhibitor of COX-2 caused much less injury to the mucosa of the upper gastrointestinal tract than classical NSAIDs /169/. This was confirmed recently in a randomized, double-blind, placebo-controlled trial with rofecoxib (Fig. 2) /170/. Therefore, selective inhibitors of COX-2 appeared to be safe enough to allow large-scale administration, on a chronic basis, to healthy people.

The antiproliferative mechanism(s) of NSAIDs is not completely understood. It is known that NSAIDs can induce apoptosis in several cell lines and tumors /91,171-173/. All of these studies suggested that the overexpression of COX-2 favors the survival of certain cells, thus enhancing tumorigenesis. Considering the molecular basis for COX-2 inhibitor-induced apoptosis, it has been proposed that it might due to the stimulation of ceramide production /174/, the down-regulation of Bcl-2 expression /43,91/, or the blockage of Akt activation, thereby attenuating the activity of a major anti-apoptotic pathway /175/. In contrast, there was also evidence that certain NSAIDs exerted their effects via non-COX-mediated mechanisms. For example the *R*-enantiomer of flurbiprofen (which does not inhibit COX) had chemopreventive activity in the *min* mouse model of intestinal polyposis /176/, and sulindac sulphone (a metabolite of sulindac which does not inhibit either COX isoform) inhibited azoxymethane (AOM)-induced colon carcinogenesis in rats /177/. This evidence suggested that NSAIDs could act via both COX-dependent and COX-independent mechanisms. Recently, the US Food and Drug Administration approved the use of celecoxib as an adjunct to usual care for patients

with FAP /178/ and several trials of celecoxib are already in progress to define the basis of COX-2 action.

4.2 Curcumin

Curcumin (Fig. 2) is a spice used extensively in curries and mustards as a coloring and flavoring agent. It is the major yellow pigment extracted from turmeric, the powdered rhizome of the perennial herb *Curcuma longa* L. /179/. Turmeric is also used as a coloring and flavoring agent in foods such as gelatins and puddings, condiments, soups, meats, and pickles /180/. In India and Southeast Asia, curcumin is used extensively in food and also as a treatment (as turmeric) for inflammation /181-183/, skin wounds /183/ and tumors /181,184/. Curcumin is known to possess both anti-inflammatory /185,186/ and antioxidant properties /187,188/.

Various studies have shown the activity of curcumin in cancer. Topical application of curcumin inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced epidermal DNA synthesis, tumor promotion in mouse skin, and edema of mouse ears /189,190/. Topical application of curcumin also inhibited benzo[*a*]pyrene-induced DNA adducts and skin tumors as well as 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced skin tumors /191/. Administration of 2% turmeric in the diet inhibited DMBA-induced skin, benzo[*a*]pyrene-induced forestomach, and AOM-induced small and large intestinal tumors in mice /192,193/. Moreover, 2000 ppm curcumin in the diet significantly suppressed AOM-induced colonic aberrant crypt foci formation, which are early preneoplastic lesions, in male F344 rats /194/. Feeding curcumin also had a marked inhibitory effect on the size, multiplicity and incidence of adenomas and adenocarcinomas /195,196/. A strong inhibitory effect of curcumin on colon carcinogenesis was observed when this compound was fed either during the initiation or post-initiation phases of AOM-induced colon carcinogenesis.

The mechanism of action of curcumin is not completely understood, although a substantial amount of information is available. Dietary administration of curcumin inhibited AOM-induced ornithine decarboxylase (ODC) and tyrosine protein kinase activities, formation of LOX and COX metabolites in the liver and colon, and aberrant crypt foci formation in the colon /194/. Several of the chemopreventive properties of curcumin could be explained, in part, by inhibition of COX-2. For example, curcumin suppressed the formation

of adducts between metabolites of benzo[*a*]pyrene and DNA /191/. This effect of curcumin might be due to inhibition since COX can convert a broad array of xenobiotics, including benzo[*a*]pyrene, to mutagens /40/. Treatment with curcumin also stimulated apoptosis in the colon /197/, and overexpression of COX-2 in intestinal epithelial cells is known to inhibit apoptosis /42/. Moreover, since PGs are mediators of inflammation and chronic inflammation leads to a predisposition to malignancy /44/, the inhibition of COX-2 by curcumin is likely to contribute to both its anti-inflammatory and chemopreventive activities. Zhang *et al.* showed curcumin as a regulator of *COX-2* gene expression /198/. It thus decreased both the cyclooxygenase and peroxidase activities of the enzyme, whereas NSAIDs do not inhibit the peroxidase function, which contributes to the activation of procarcinogens. Curcumin is being evaluated in clinical trials for its use in chemoprevention of oral cavity, colon and breast cancers /199/.

4.3 Green tea and black tea

Tea is a beverage made from the leaves of *Thea sinensis* L. (Theaceae). This beverage is one of the most ancient and, next to water, the most widely consumed liquid in the world. Tea leaves are primarily manufactured as green, black, or oolong, with black tea representing approximately 80% of tea products consumed. Green tea is the non-oxidized/non-fermented product and contains several polyphenolic components such as epicatechin, epicatechin gallate, epigallocatechin (EPG), and epigallocatechin gallate (EGCG) (Fig. 2). EGCG is the major green tea polyphenol. The major components of black tea (the fermented product) are theaflavins and thearubigins. Theaflavins, which determine the quality and flavor of the tea, are formed by oxidation of quinones derived from the epicatechins. Oolong tea is the partially oxidized/fermented product which retains a considerable amount of the original polyphenolic material /200/. In black tea, the above tea catechins are reduced to about one-tenth to one-third of those in green tea, and theaflavins account for 1-2% of the total dry matter /201/.

Although not conclusive, epidemiological studies have suggested a protective effect of black or green tea consumption against human cancers of the breast, colon and rectum, gall bladder, liver, lung, nasopharynx, pancreas, stomach, and uterus /201-205/. In contrast, a

number of other ecological, cohort, and case-control studies /201/ have associated an increased risk for cancer of the breast, colorectum, esophagus, kidney, lung, pancreas, and stomach with tea intake. These inconsistencies may be attributed to consumption of salted or very hot tea (esophagus), or to geographical location, as observed with stomach cancer (e.g., inhibition of endogenous formation of nitroso compounds which are a major cause of gastric cancer in some areas). Other confounding factors and variables may include the use of tobacco and alcohol and lack of information on the type of tea consumed (e.g., black or green).

Many studies have shown the inhibitory actions of green tea, black tea, and tea polyphenol preparations against carcinogenesis in rodent models /201,206-208/. These included cancers of the skin, lung, esophagus, stomach, liver, duodenum and small intestine, pancreas, and mammary gland. The most extensively studied system is the skin carcinogenesis model caused by chemicals, UV light, and TPA /209-215/. The antipromoting effect of a major green tea constituent, EGCG, has been demonstrated /209,212,213,215/. Tea polyphenols considerably decreased the mutagenicity of different types of carcinogens /216/. Moreover, the induction of ODC and COX enzymes by skin tumour promoters was significantly inhibited by topical application of green tea polyphenols in SENCAR mice /217/. Green tea polyphenols were effective free radical scavengers /218/, chain-breaking antioxidants /219/ and scavengers of reactive nitrogen species /220/. The protective effects of green tea polyphenols have been attributed to both their antioxidant properties as scavengers of reactive oxygen species and the activation of phase II detoxifying enzymes /221-224/. The application of green tea polyphenols prior to that of TPA also resulted in significant inhibition in TPA-induced epidermal COX and LOX activities as observed by decrease in the formation of, respectively, PG and HETE metabolites /213/. Another study showed that the effect of EGC on COX-2 expression differed between UVA-irradiated FEK4 and KB cells. UVA-induced COX-2 expression was up-regulated in FEK4 cells that had been incubated overnight with EGC, but decreased in EGC-treated KB cells /225/. The protective effect observed in KB cells was consistent with the hypothesis proposed by Agarwal *et al.* /217/ that green tea polyphenols prevented *in vivo* UV-induced COX activity by scavenging UV-generated free radicals. The EGC protection of UVA-mediated

COX-2 expression in keratinocytes was particularly relevant to COX involvement in cutaneous tumour promotion /226/. Although protection of UVA-mediated COX-2 expression in KB cells is likely to be associated with an antioxidant effect of EGC, the mechanism by which EGC up-regulated COX-2 expression in UVA-irradiated FEK4 cells remains obscure. Tea and EGCG are in clinical trials to evaluate chemopreventive effects in skin, head and neck and colon cancers /199/.

4.4 Silymarin

Silymarin, a polyphenolic flavonoid isolated from milk thistle [*Silybum marianum* (L.) Gaertn], is composed mainly (~80%, w/w) of silybin (Fig. 2), with smaller amounts of other stereoisomers such as isosilybin, dihydrosilybin, silydianin, and silychristin /227,228/. Several studies showed that silymarin is a very strong antioxidant, capable of scavenging both free radicals and reactive oxygen species, resulting in enhancement of cellular antioxidant defense machinery, thus increasing the antioxidant potential of cells /229-231/. For more than 25 years, silymarin has been used clinically in Europe as an anti-hepatotoxic agent /229,230/; in recent years, silymarin has also been used in Asia as a therapeutic agent for treatment of liver diseases. As a therapeutic agent, silymarin is well tolerated and largely free of adverse effects /231/. More recently, silymarin has been marketed in the United States and Europe as a nutritional supplement.

Several studies have shown that silymarin protects against lipid peroxidation /232/. Others report that silymarin inhibits the formation of transformed rat tracheal epithelial cell colonies induced by exposure to benzo[*a*]pyrene /233/ and inhibits TPA-promoted mammary lesion formation in organ culture /234/.

Some mechanistic studies have shown that silymarin strongly inhibited TPA-induced epidermal ODC activity and mRNA expression and also significantly inhibited epidermal ODC activity induced by structurally distinct skin tumor promoters, including free radical-generating compounds /235/. More recently, it was shown that topical application of silymarin on mouse skin resulted in highly significant protection against UVB radiation-induced tumor initiation, tumor promotion, and complete carcinogenesis in SKH-1 hairless mice, and TPA-, okadaic acid-, and benzoyl peroxide-mediated tumor promotion in DMBA-initiated SENCAR mice /236-238/. Additional studies

dissecting the preventive effect of silymarin on stage-specific tumor promotion in mouse skin showed that its anti-tumor-promoting effect was primarily targeted toward stage I tumor promotion /238/. Topical application of silymarin resulted in highly significant inhibition of TPA-mediated induction of 8-LOX activity in mouse epidermis, based on reduction of 8-HETE formation /239/. Moreover, silymarin significantly inhibited TPA-mediated induction of PGE_2 formation and TPA-mediated expression of COX-2 in mouse epidermis /239/. These effects may be related to the anti-tumor-promoting potential of silymarin recently reported /238/. Treatment of human epidermoid carcinoma cells with silymarin resulted in the inhibition of ligand-induced activation of epidermal growth factor receptor activation as well as inhibition of constitutive autophosphorylation of the tyrosine kinase domain of the receptor /240/. Based on these results, it is possible that silymarin-mediated inhibition of TPA-induced expression of COX-2 in mouse epidermis is due at least in part to effects on upstream receptor tyrosine kinase activation and IL-1α release after TPA treatment.

4.5 Resveratrol

Resveratrol (*trans*-3,4′,5-trihydroxystilbene) (Fig. 2) occurs naturally in grapes and a variety of medicinal plants, where it functions as a phytoalexin that protects against infections and other stress factors /241/. Resveratrol has been reported to have antiplatelet /241/, anti-inflammatory /242,243/ and anticarcinogenic effects /244/. It inhibited the development of preneoplastic lesions in carcinogen-treated mouse mammary glands and blocked tumorigenesis in a two-stage model of skin cancer /244/. Resveratrol suppressed TPA-mediated induction of PG synthesis by inhibiting *COX-2* gene expression and the enzyme activity of COX-2 /245/. Resveratrol has the ability to reverse H_2O_2-induced increases in LTB_4 and PGE_2 concentration. This effect was, in turn, a result of inhibiting 5-LOX and the cyclooxygenase and peroxidase activity of COX in the same cells /246/. In regard to the mechanism by which resveratrol modulated TPA-mediated activation of *COX-2* transcription in human mammary epithelial cells, PKC signal transduction pathways were inhibited at multiple levels. Resveratrol also blocked the induction of *COX-2* promoter activity by ERK1 and c-jun; TPA-mediated induction of c-jun and activator protein-1 (AP-1) activity was suppressed by resveratrol. These inhibi-

tory effects could be explained, in part, by the antioxidant properties of resveratrol, as other phenolic antioxidants inhibited both phorbol ester-mediated activation of PKC /247/ and AP-1 /248/. The inductive effects of TPA and suppressive effects of resveratrol on COX-2 expression are mediated via the cyclic AMP response element (CRE). Xie and Herschman /249/ showed that c-jun, a component of the AP-1 transcription factor complex, activated the murine COX-2 promoter via the CRE /249/. Thus, it seems likely that resveratrol blocked TPA-mediated induction of COX-2 by suppressing AP-1-dependent transactivation via the CRE /250/. The anti-AP-1 effect of resveratrol could potentially be explained if resveratrol induced Fra expression like other phenolic antioxidants /251/. Heterodimers of c-jun and Fra do not activate AP-1-mediated gene expression as effectively as c-jun homodimers or c-jun/c-fos heterodimers /252/. Alternatively, resveratrol could suppress TPA-mediated increases in AP-1 activity by inhibiting the induction or phosphorylation of c-jun /253/. Very recently, resveratrol was found to inhibit the activation pathway of NF-κB /254/.

4.6 Other compounds

Some additional natural compounds showing activity against COX or LOX have been tested for their anticancer properties. Propolis from honey beehives contains various chemical constituents that exhibited a broad spectrum of activities including antibacterial, antifungal, cytostatic, and anti-inflammatory properties /255,256/. Gribel and Pashinskii /257/ have shown that honey possessed moderate antitumor and pronounced antimetastatic effects in tumors in five different stains of rats and mice. Caffeic acid and its esters, which are present in propolis at levels of 20-25% /258/ are agents suspected of having a broad spectrum of biological activities including tumor suppression potential. In particular, caffeic acid phenethyl ester (CAPE) (Fig. 2), a phenolic antioxidant derived from propolis, has anticancer /259-261/ and anti-inflammatory /260/ properties. This agent inhibited the development of AOM-induced aberrant crypts in the colon of rats /259/, and blocked tumorigenesis in the mouse two-stage model of skin cancer /261/. A recent study showed that CAPE inhibited the release of AA from cell membranes, suppressed the enzymatic activities of COX-1 and COX-2, and inhibited the activation of *COX-2* gene expression /262/.

A study of some compounds with a resorcin-type structure (quercetin, kaempferol, genistein, resveratrol and resorcinol) illustrated suppression of TGFα-mediated activation of COX-2 transcription through inhibition of the activation of protein-tyrosine kinases (PTKs) /263/. Therefore, the resorcin moiety may play a critical role in the inhibition of COX-2 promoter activity, although other physiochemical factors may also be involved. On the other hand, EGCG showed no inhibitory effect on COX-2 promoter activity, even though its structure contains a resorcin moiety and it inhibited the activity of PTKs. Elements other than a resorcin moiety in the structure of EGCG might prevent inhibition of COX-2 promoter activity.

Epidemiological studies have suggested that a diet rich in soy products can reduce the risk of several cancers, notably those of the breast and prostate /264/, and in animal experiments, a soy-based diet resulted in a reduced incidence of chemically-induced rat mammary carcinomas /265/. These protective effects have been associated with isoflavonoids formed from soy-based precursors, prominent among which are the aglucones of genistein and daidzein /263,266/.

5. SELECTIVE COX-2 INHIBITOR PLANT EXTRACTS

The use of naturally derived products or their active principles in the prevention and/or treatment of chronic diseases is based on the experience of traditional systems of medicine practiced in various ethnic societies, and on epidemiological observations of the relationship of dietary practices and disease patterns. Isolation, identification, and testing of active substances can not only provide naturally occurring novel agents as inhibitors of cancer development, but also offer unique opportunities to study mechanisms of carcinogenesis.

As COX-2 plays an important role in cancer development, we have tested about 1500 plant extracts for inhibition of COX-1 and COX-2. The assay is based on the measurement of PGE_2 produced in the COX reaction via an enzyme immunoassay. About 60 extracts inhibited COX-2 activity by more than 70% at a concentration of 10 μg/ml, whereas the same extracts showed weak or no activity against COX-1. Active extracts are currently being fractionated using the biological assay to guide the activity /267,268/, with the hope of discovering new selective inhibitors of COX-2.

Materials and methods (Fig. 3)

The effect of test compounds on COX activity was determined by measuring PGE_2 production. Reaction mixtures were prepared in 100 mM Tris-HCl buffer, pH 8.0, containing 1 μM heme, 500 μM phenol, 300 μM epinephrine, sufficient amounts of COX-1 or COX-2 to generate 150 ng of PGE_2/ml, and various concentrations of test samples. The reaction was initiated by the addition of arachidonic acid (final concentration, 10 μM) and incubated for 10 min at room temperature (final volume, 200 μl). The reaction was terminated by adding 20 μl of the reaction mixture to 180 μl of 27.8 μM indomethacin, and PGE_2 was quantified by an ELISA method. Samples were diluted to the desired concentration with 100 mM potassium phosphate buffer (pH 7.4) containing 2.34% NaCl, 0.1% bovine serum albumin, 0.01% sodium azide and 0.9 mM Na_4EDTA. Following transfer to a 96-well plate (Nunc-Immuno Plate Maxisorp, Fisher) coated with a goat anti-mouse IgG (Jackson Immuno Research Laboratories), the tracer (PGE_2-acetylcholinesterase, Cayman Chemical, Ann Arbor, MI) and primary antibody (mouse anti-PGE_2, Monsanto, St. Louis, MO) were added. Plates were then incubated at room temperature overnight, reaction mixtures were removed, and wells were washed with a solution of 10 mM potassium phosphate buffer (pH 7.4) containing 0.01% sodium azide and 0.05% Tween 20. Ellman's reagent (200 μl) was added to each well and the plate was incubated at 37°C for 3-5 hours, until the control wells yielded an OD of 0.5-1.0 at 412 nm. A standard curve with PGE_2 (Cayman Chemical, Ann Arbor, MI) was generated on the same plate, which was used to quantify the PGE_2 levels produced in the presence of test samples. Results were expressed as a percentage relative to control (solvent-treated) samples, and dose-response curves were constructed for the determination of IC_{50} values.

6. CONCLUSIONS

The detection of elevated levels of COX-2 in a variety of human cancers combined with the chemopreventive effect of NSAIDs in colon cancer demonstrated that COX-2 might be an important participant in carcinogenesis. The reported biological consequences of COX-2 up-regulation include inhibition of apoptosis /42/, increased

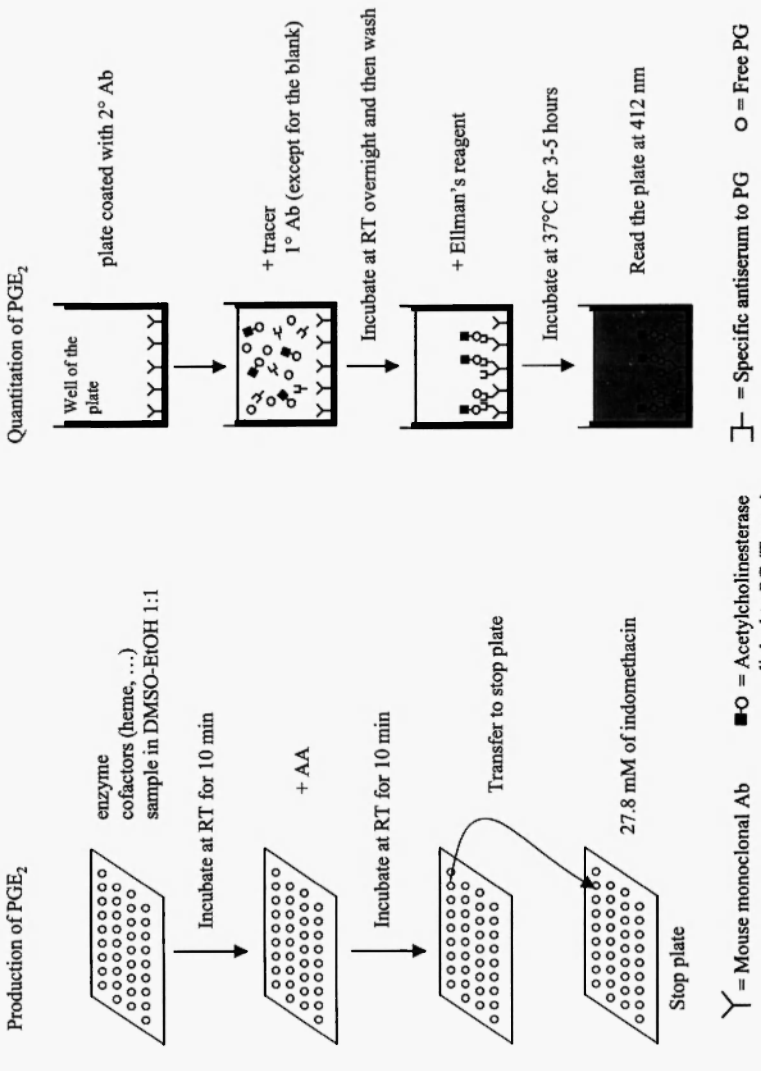

Fig. 3: Determination of COX activity by measuring PGE_2 production.

metastatic potential /39/, and promotion of angiogenesis /48/. It is likely that these events contribute to cell transformation and tumor progression.

LOX products are considered biologically to be at least as important as PGs produced through the COX pathway, due to their involvement in many aspects of inflammation /269/. Compared to COX, however, reports on the regulation of specific LOX in cancer growth and development are limited, although several lines of evidence have shown the importance of LOX in regulating human cancers and rat melanoma cancer cells /9,74,95,269/. Presently, the field of LOX research is somewhat overshadowed by the intense interest in COX.

It has long been a principle of cancer therapy that drug combination treatment, when appropriately selected, is likely to prove more effective than the use of a single agent. The design of combination regimens is governed in part by the demonstrable partial activity of each component and the absence of overlapping toxicity when the agents are administered at their optimal doses /270/. However, the combination of different individual treatment modalities is not always beneficial. For example, a soy-supplemented diet negated the inhibitory effects of indomethacin on MDA-MB-435 breast cancer cell progression in a nude mouse model /271/.

On the other hand, an optimal cancer chemopreventive regimen for heavy smokers, for example, may be different from an optimal cancer chemopreventive regimen for people exposed to aflatoxin B_1, or for heavy smokers who have stopped smoking /272,273/. Greater efforts should be made to understand mechanisms of cancer chemoprevention and to determine whether a potential chemopreventive agent is useful in many experimental settings or whether it is only useful in a limited number of experimental settings. Some compounds may be extremely effective cancer chemopreventive agents in one experimental setting but actually enhance carcinogenesis in another experimental setting. Accordingly, it may be necessary to tailor the cancer chemopreventive agent to individual patients with known carcinogen exposures or to individuals at high risk for cancer with mechanistically defined pathways of carcinogenesis so that chemopreventive agents can be better tailored to the individual and selected on a more rational basis /274/.

Taking all of these factors into consideration, a substantial amount of additional work is clearly required to yield a better understanding of the role of COX and LOX pathways in cancer chemoprevention. Safe and effective specific inhibitors of COX-2 are now available to the public, and their role in cancer chemoprevention will likely expand in the future. It may be anticipated that inhibitors of LOX will also be developed and found to be useful for the chemoprevention of human cancer.

ACKNOWLEDGEMENTS

The authors are grateful to the National Cancer Institute for support provided under the auspices of program project P01 CA48112 entitled "Natural Inhibitors of Carcinogenesis".

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