REVIEW

NSAIDs as Cancer Preventive Agents

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Abstract

Introduction: Targeting of inflammatory states with non-steroidal anti-inflammatory drugs (NSAID'S) is an attractive proposition for cancer prevention. There is abundant epidemiological and experimental evidence that NSAID'S can inhibit tumour development in a number of organs and such drugs have given positive results in human intervention studies. It is hoped that problems with side effects can be overcome by development of specific inhibitors of cancer- and inflammation-associated enzymes. NSAID Pharmacology: Starting with the production of aspirin, NSAIDs have received an enormous amount of clinical attention. Inhibitors of the two isoforms of cyclooxygenase (COX) or prostaglanding G/H synthase, they have found widespread application with increasing interest focused on their employment for cancer prevention. Particular attention is now being drawn to specific inhibitors of the inducible C0X-2 rather than the constitutive COX-1 isoform. Mechanisms of Action: NSAIDs appear to act via depression of prostaglandin synthesis through inhibiting COX-2, often overexpressed in cancers, and the resultant suppression of proliferation, possibly through enhancement of apoptosis. Future Prospectives: The possibility of applying NSAIDs in conjunction with other chemopreventive agents and developing specific inhibitors of different stages in the pathways leading to prostaglandin production and functions hold hope for improved drugs/ protocols with reduced detrimental side effects for employment in both primary and secondary cancer prevention in the future.

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Introduction

a) General

Use of chemical supplements to interfere with the processes that are involved in neoplasia and thereby prevent cancer development is a very attractive possibility, now generally accepted to be worthy of serious scientific attention. Since inflammatory states and associated chronically elevated levels of proliferation appear to predispose to cancer development in any site of the body (Ames et al., 1995; Preston-Martin et al., 1995; Sugimura, 1996; Moore and Tsuda, 1999) the metabolic pathways that are switched on under such conditions are natural targets. Therefore a great deal of interest has been concentrated on non-steroidal anti-inflammatory drugs (NSAID'S) which act by inhibition of prostaglandin endoperoxidase/cycooxygenase (COX) (see reviews by Weitzman and Gordon, 1990; Subbaramaiah et al ., 1997). The present paper aims to provide an update of publications in this field of research with attention to the possible mechanisms of action and potential application of NSAID'S for practical cancer prevention.

b) Epidemiological Evidence of NSAIDs as Chemopreventive Agents

The first report of a protective role for the NSAID aspirin (see Fig 1 for structural formulae of representative examples of NSAID's) against cancer development appeared in 1988 (Kune et al., 1988), documenting a negative association with colorectal cancer. Subsequently a very large number of studies have been carried out demonstrating similar effects in the colon and/or rectum (see Table 1), in studies of either case control (Rosenberg et al., 1991; Suh et al., 1993; Muscat et al., 1994; Peleg et al., 1994; Reeves et al., 1996) or cohort type (Thun et al., 1993; Giovannucci et al., 1994; 1995). Beneficial effects have also been described for other organs, like the oesophagus and stomach,the gastric cancers in the latter case being generally of non-cardia type (Table 1). In one recent study this was shown to only be the case for

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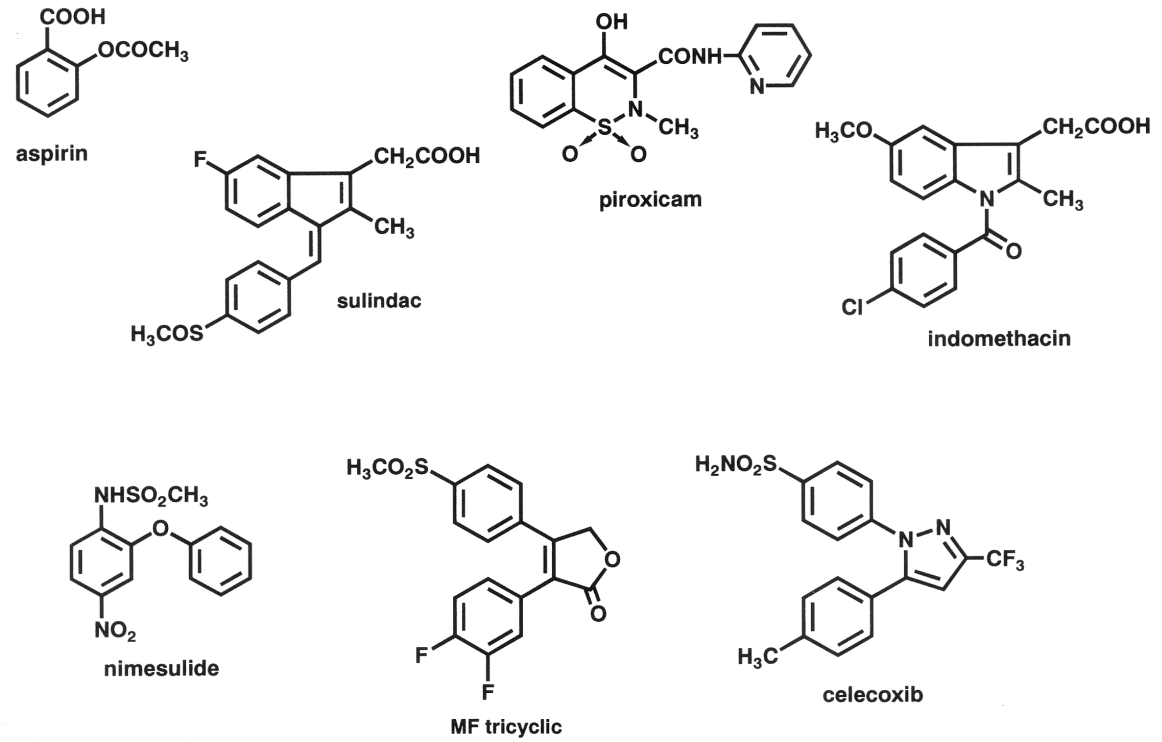


Fig 1 Chemical Structures of NSAIDs

Table 1. Aspirin Prevention of Cancer-epidemiological Studies

Organ	Condition	Effect	Study Type	Reference
Oesophagus		Cancer (OR 0.78)	Cohort	Isomäki et al., 1978
		Cancer (OR 0.78)	Cohort	Thun et al., 1993
Gastric	Rheumatoid arthritis	(OR 0.63)	Cohort	Gridley et al., 1993
	non-Cardia	(OR 0.49)	Cohort	Thun et al., 1993
		(OR 0.46)	Case-control	Farrow et al., 1998
	H. Pylori+	n-C (OR 0.49)	Case-control	Zaridze et al., 1999
	H. Pylori-	None		
Colorectal		Cancer I (OR 0.58-1.1)	Cohort	Isomäki et al., 1978
		Cancer I (OR 0.63-0.72)	Cohort	Gridley et al., 1993
		Cancer M (OR 0.58-0.66)	Cohort	Thun et al., 1993
		Cancer I (OR 1.5)	Cohort	Schreinemachers and Everson, 1994
	Health Professionals	Cancer I (OR 0.68)	Cohort	Giovannucci et al., 1994
	Nurses	Cancer I (OR 0.56)	Cohort	Giovannucci et al., 1995
	Retirees	Cancer I (OR 1.5)	Cohort	Paganini-Hill et al., 1995
		Cancer I (OR 0.6)	Case-control	Kune et al., 1988
		Cancer I (OR 0.5)	Case-control	Rosenberg et al., 1991
		Cancer I (OR 0.24-0.54)	Case-control	Suh et al., 1993
			Case-control	Muscat et al., 1994
		Cancer I (OR 0.32-0.64)	Case-control	Peleg et al., 1994

Cancer I, incidence, M, mortality

Table 2. Peroxicam Prevention of Cancer - Experimental Studies

Organ/Animal	Study Type	Comment	Reference
Tongue-Rat	Post-Initiation	Cancer Incidence	Tanaka et al., 1989
Oesophagus-Rat	Post-Initiation	Tumour Multiplicity	Bespalov et al., 1989
-Mouse	Pre/Post-Initiation	Tumour Multiplicity	Rubio, 1984
	Post-Initiation	Tumour Multiplicity	Rubio, 1986
Colon-Rat	Pre/Post-Initiation	Carc Incidence/Multiplicity	Reddy et al., 1992
	Post-Initiation	Tumour Incidence/Multiplicity	Reddy et al., 1987
Liver-Rat	Pre/Post-Initiation	Tumour Incidence/Multiplicity	Tanaka et al., 1993

Table 3. Indomethacin Prevention of Cancer- Experimental Studies

Organ/Animal	Study Type	Comment	Reference
Buccal Cavity-Hamster			Perkins and Shklar, 1982
Tongue-Rat	Post-Initiation	Cancer Incidence	Tanaka et al., 1989
Oesophagus-Rat	Post-Initiation	Tumour Multiplicity	Bespalov et al., 1989
-Mouse	Pre/Post-Initiation	Tumour Multiplicity	Rubio, 1984
	Post-Initiation	Tumour Multiplicity	Rubio, 1986
Forestomach-Rat	Post-Initiation	Tumour Incidence/Multiplicity	Bespalov et al., 1989
Colon-Rat	Pre/Post-Initiation	Tumour Incidence	Narisawa et al., 1983
	Pre/Post-Initiation	Tumour Incidence	Metzger et al., 1984
	Pre/Post-Initiation	Tumour Incidence	Tanaka et al., 1991
	Post-Initiation	Tumour Incidence/Multiplicity	Kudo et al., 1980
	Post-Initiation	Tumour Multiplicity	Pollard & Luckert, 1981
	Post-Initiation	Tumour Multiplicity	Shibata et al., 1995
Mammary-Rat	Pre/Post-Initiation	Carcinoma Multiplicity	McCormick et al., 1985
	Post-Initiation	Tumour Incidence	Bespalov et al., 1992
Liver-Rat	Pre/Post-Initiation	Cancer Incidence/Multiplicity	Tanaka et al., 1993
Kidney-Rat	Post-Initiation	Tumour Incidence	Alexandrov et al., 1996
Pancreas-Hamster	Post-Initiation	Tumour Multiplicity	Takahashi et al., 1990
Urinary Bladder- Rat	Post-Initiation	Lesion Multiplicity	Shibata et al., 1993
	Post-Initiation	No influence Progression	Ozaki et al., 1997
Mouse	Pre/Post-Initiation	Tumour Incidence	Grubbs et al., 1993
Cervix-Mouse	Pre/Post-Initiation	Tumour Incidence	Rao and Hussain, 1988
Vagina/Cervix-Mouse	Post-Initiation	Carcinoma Incidence	Bespalov et al., 1992
Skin-Mouse	Post-Initiation	Tumor Multiplicity	Fischer et al., 1999
Brain-Rat	Post-Initiation	Tumour Incidence	Alexandrov et al., 1996

Table 4. Selective COX-2 Inhibitor Prevention of Cancer- Experimental Studies

Organ/Animal	Study Type	Comment	Reference
Nimesulide			
Colon-Mouse	Pre/Post-Initiation	ACF Multiplicity	Takahashi et al., 1996
Colon-Mouse	Pre/Post-Initiation	Carcinoma Multiplicity	Fukutake et al., 1998
Intestines-Min Mouse	Pre/Post-Initiation?	Tumour Incidence and Size	Nakatsugi et al 1997
Urinary Bladder-Rat	Post-Initiation	Carcinoma Incidence/Multiplicity	Okajima et al 1998
Celecoxib			
Skin-Mouse	Post-Initiation	Tumour Multiplicity	Pentland et al., 1999
	Post-Initiation	Tumor Multiplicity	Fischer et al., 1999
Colon-Rat			Kawamori et al., 1998
MF Tricyclic			
Min Mouse			Oshima et al., 1996
SC58635			
Colon Rat			Reddy et al., 1996

individuals positive for antibodies against Helicobacter pylori (Zaridze et al., 1999), in line with the finding that the bacteria exerts greatest effects in the non-cardiac region (IARC, 1994). The results indicate a general reduction of neoplasia in the order of 35-50% for both aspirin and other NSAIDs (Peleg et al., 1994; Reeves et al., 1996). Dose dependence was observed for a very large study of nurses (Giovannucci et al., 1995).

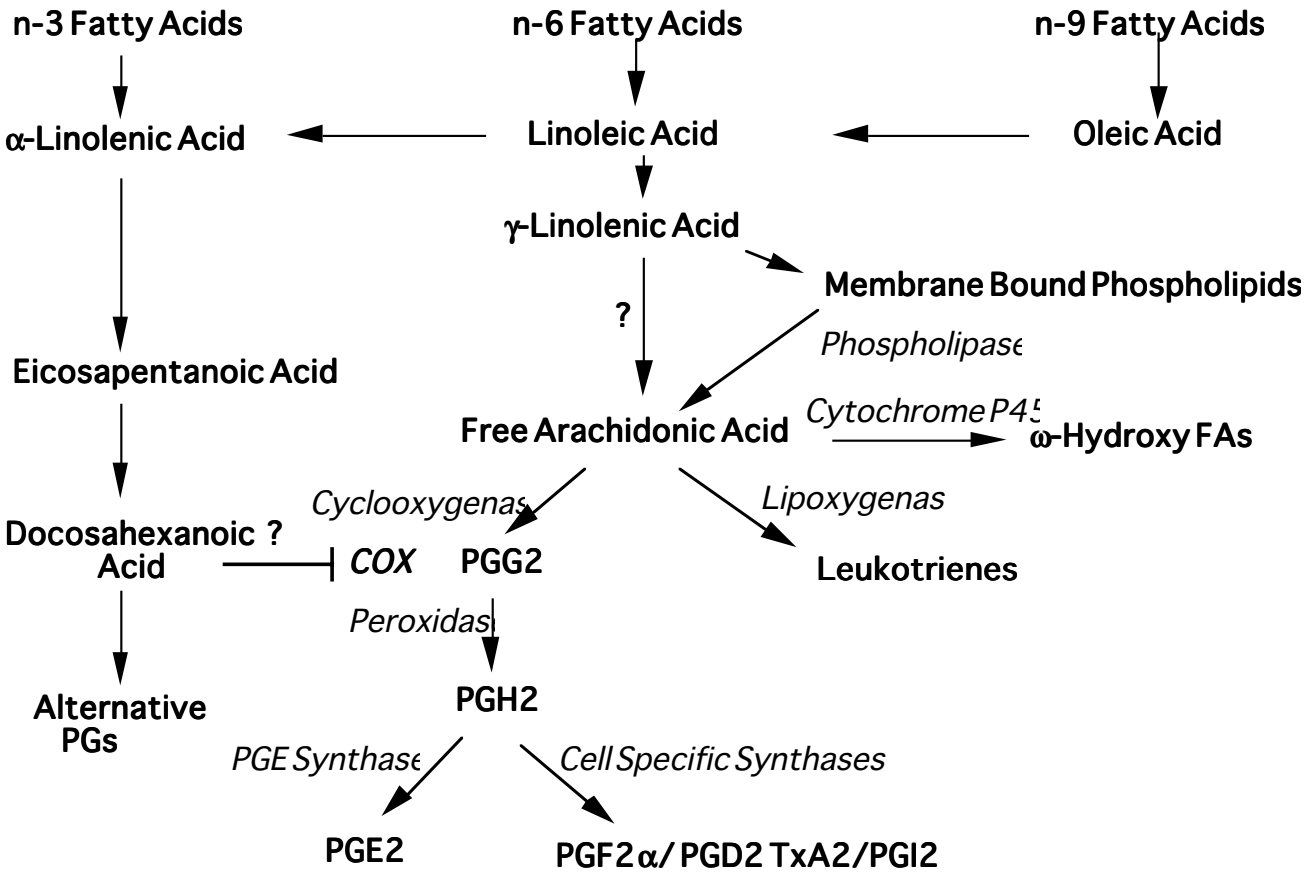
c) Experimental Evidence of NSAIDs as Chemopreventive Agents

Aspirin given experimentally at high doses in the diet was relatively early found to inhibit development of preneoplastic lesions and tumours in the rat colon (Reddy et al., 1993), rat liver (Denda et al., 1994) and urinary bladder (Murasaki et al., 1984; Klän et al., 1993), although enhancement of forestomach carcinogenesis occurred in one of these cases (Murasaki et al., 1984). A number of other general cyclooxygenase inhibitors have now been tested in experimental systems. For example, sulindac has been extensively investigated, generally protecting in the colon of rats and mice (Moorghen et al., 1988; Skinner et al., 1991; Alberts et al., 1995; Rao et al., 1995a), the mammary glands of rats (Thompson et al., 1997), the urinary bladder of mice (Rao et al., 1996) and the lungs of mice (Jalbert and Castonguay, 1992; Pepin et al., 1992). Other non-specific NSAIDs known to inhibit include piroxicam in the colon of rats (Pereira et al., 1996; Wargovich et al., 1995; Pollard and Luckert, 1984: Nigro et al., 1986; Reddy et al., 1987; Rao et al., 1991; Reddy et al., 1992), the liver of rats (Tanaka et al., 1993), the urinary bladder of mice (Moon et al., 1993) and the lung of mice (Jalbert and Castongauy, 1992) (see Table 2). Indomethacin has attracted even more attention as evidenced by a very large literature documenting positive influence (see Table 3), although it may not always significantly modify the incidence or other characteristics of intestinal tumours (Caignard et al., 1984).

One mouse model which has generated particular interest is that featuring a truncation mutation in the *Apc* gene, analogous to the genetic alteration in the human APC gene responsible for not only familial polyposis but also sporadic cancers of the entire digestive tract. This is known to be associated with increased tissue prostaglandin levels (Nugent et al., 1996) and in the so-called min-mouse model loss of heterozygosity results in development of intestinal polyps (Oshima et al., 1995a). Inhibition of COX activity exerts protective effects (Oshima et al., 1996). Recently specific inhibitors of COX 2 have been shown to inhibit in the intestinal tract, including in the Min mouse, and the urinary bladder as well as the skin (see Table 4).

d) NSAIDs as Chemopreventive Agents in Human Intervention Studies

Treatment of humans, has been found to reduce cancer incidence in the colon (Giardiello et al., 1993, Giovannucci et al., 1995) and breast (Schreinemachers and Everson, 1994; Harris et al., 1996). Regression of rectal polyps by indomethacin suppositories or sulindac has been reported in familial adenomatous polyposis (Labayle et al., 1991; Giardellino et al 1993; 1996; Hirata et al., 1994), with reduction of prostaglandin levels by sulindac (Nugent et al., 1996; Giardellino et al., 1998). However such treatment is associated with toxic effects which may preclude long-term application (Akasu et al., 2000, Ishikawa et al., 1997).



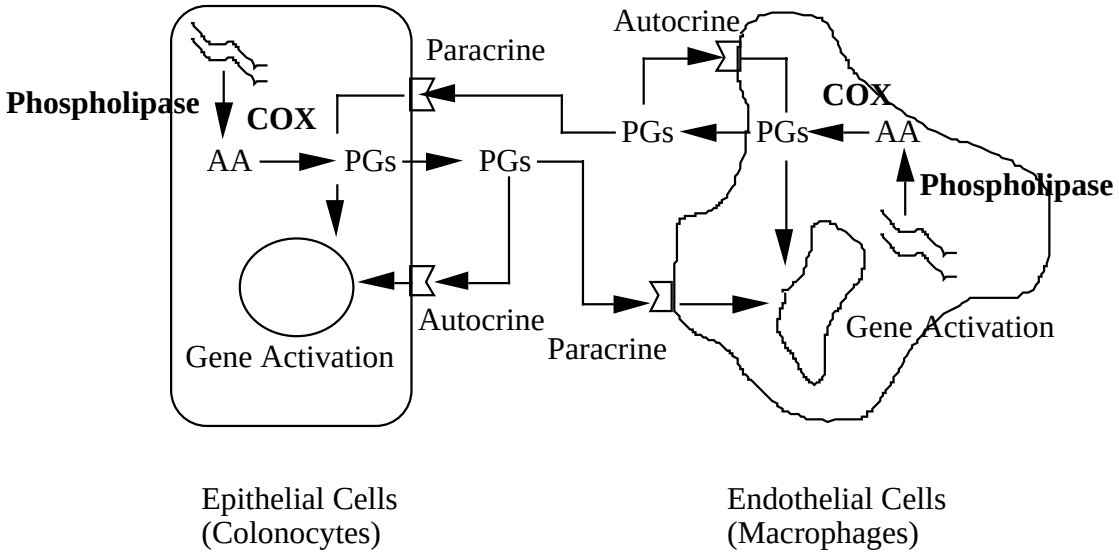


Fig 3. Routes of Action of Prostaglandins (PGs)

Table 5. Comparison of COX-1 and COX-2 (after Subbaramaiah et al., 1997)

Parameter	COX-1	COX-2	References
Gene Location	Human chromosome 9	Human chromosome 1	Funk et al., 1991 Kosaka et al., 1994
Gene Size	22kb, 11 exons/10 introns	8 kb 10 exons/9 introns	Williams and DuBois, 1996
Gene Promotor		TATA box, cis-acting elements	Inoue et al., 1995
mRNA Size	2.7 kb (stable)	4.5 kb (instable due to Shaw-Kamen sequences)	Kosaka et al., 1994
Protein Size	72 kDa (SDS-PAGE peak)	72/74 kDa (SDS-PAGE two peaks)	Otto et al., 1993
Protein Distinctive Feature		18-aminoacid region in carboxy terminus	Habib et al., 1993
Expression	Constitutive (differentiation)	Inducible (up to 80-fold, wide range of chemical stimuli)	Williams and DuBois, 1996
Cellular Location	Endoplamic reticulum (ER)	ER and the nuclear envelope	Morita et al., 1995
Tissue Sites	Essentially ubiquitous	Regions of inflammation	Crofford, 1997

NSAID Pharmacology

a) General

The history of NSAIDs starts in the 1890s with the production of an analgesic by Bayer Company in Germany, due to the efforts of the chemist Felix Hoffman, who wanted a drug to control his fathers severe rheumatoid arthritis (cited in Dubois et al., 1998). This acetylsalicylic acid, or aspirin, was the first of an increasingly large class of drugs, one of most widely used throughout the world for prophylaxis of cardiovascular disease, pain relief from minor injuries and headaches, and symptoms due to inflammatory and degenerative joint diseases.

Their means of action as prostaglandin synthesis inhibitors was first elucidated by Vane (1971), specifically by acting on cyclooxygenase. As shown in Fig 2, the first step in the process is the liberation of arachadonic acid from membrane phospholipid by phosholipases. Prostaglandins are formed by oxidative cyclization of the central carbons within polyunsaturated fatty acids, the key enzyme being COX or PGH synthase, which converts arachadonic acid or other 20 carbon fatty acids into prostaglandin G2 (PGG2) and on to PGH2, which is then converted into a range of different products under the influence of downstream enzymatic machinery which varies with the cell type. The products include thromboxanes and 15-hydroxyeicosatraenic acids (HETEs), these together with prostaglandins being collectively termed eicosanoids. Found throughout animal phyla, PGs play a central role in inflammation but also other physiological processes, like blood clotting, ovulation, nerve growth and organ development, wound healing, kidney function and immune responses (Dubois et al., 1998). Synthesized in various cell types they can act in either autocrine or paracrine fashion (see Fig 3), binding to specific receptors on the cell surface or else directly. This then leads to nuclear events, binding to promoter sequences and transcription of target genes.

b) Cyclooxygenase Isoforms

The action of prostaglandin G/H synthase-1 ((PGHS-1) 8,11,14-eicosatrienoate, hydrogen donor:oxidoreductase, EC 1.14.99.1) or COX 1, was first identified in 1976 (Miyamoto et al., 1976). It is found in many tissues at relatively stable levels, although prostanoid production varies considerably, this initially leading to the conclusion that regulation must involve other factors or some other step. However, in 1989 a second form was isolated (Simmons et al., 1989) and termed prostaglandin endoperoxide synthase-2 (COX-2). In contrast to COX-1 , which is found constitutively expressed in nearly all normal tissues (Crofford, 1997), especially in brain and kidney of rodents, the COX-2 isoform is inducible by a wide range of intracellular stimuli, as reviewed by Williams and DuBois (1996). This is due to the presence of NFkB, NF-IL6 and CRE binding sites in the promoter region (Inoue et

al., 1995). Prostaglandins increase from undetectable resting levels in rat intestinal cells after stimulation with mitogens (DuBois et al., 1994), production of COX-2 being stimulated by a large number of different factors, both extracellular and intracellular. These include lipopolysaccharide (Lee et al., 1992), forskolin (Kujubu and Herschman, 1992), interleukin-1, tumor necrosis factor (TNF) (Jones et al., 1993), serum (DeWitt and Meade, 1993), epidermal growth factor (EGF) (Hamasaki et al., 1993), platelet activating factor and retinoic acid (Bazan et al., 1994), as well as endothelin (Kestler et al., 1994). Less is known about induction of COX-1 although it has been seen under some circumstances in association with changed differentiation (Hoff et al., 1993; Ueda et al., 1997).

The reason for the existence of two isoforms may be linked to differences in subcellular location, COX-1 being found preferentially in the endoplasmic reticulum while COX-2 may also be present in the perinuclear envelope (Morita et al., 1995). They have a number of additional differences at the gene and mRNA level as summarized in Table 5. Computerized motif searches have pointed to the presence of various transcription factor concensus sequences in the COX-2 promoter (Kosaka et al., 1994). The 3'-untranslated region of COX-2 has three polyadenalyation signals and 17 copies of the Shaw-Kamens sequence (ATTA), present in many immediate early genes and thought to be involved in modulating the speed of mRNA degradation (Kosaka et al., 1994). The encoded proteins are about 75% homologous, with conserved cyclooxygenase and peroxidase regions, but COX-2 possesses an 18 residue insert at the carboxy terminus which is absent in COX-1 (Habib et al., 1993). Structural variation could be important in terms of inhibitor interaction, for example with aspirin (Williams and DuBois, 1996). There is apparently variation in the ability to utilize substrate pools, COX-2 being able to use endogenous arachidonic acid while COX-1 may need an exogenous supply (Reddy and Herschman, 1996) Thus its release due to sPLA2, supplied by neighbouring cells, might be regulatory (Herschman, 1996).

The differences between isoforms in functions in different tissues is the background to the adverse side effects of prolonged administration of non-specific but not COX-2 targeting NSAIDs (). Thus COX-1 functions in platelets to provide PG precursors for thromboxane (Schafer, 1995), important for control of blood flow in the kidney and stomach and leading to vasodilation under contractile conditions (DuBois et al., 1998). In the presence of NSAIDs, ischemia and damage can therefore occur (Zambraski, 1995; Trevethick et al., 1995). COX-2 functions under specific physiological conditions have been reviewed by DuBois et al., (1998), for example in the kidney, gastrointestinal tract, ovary and bone. However, inhibition does not appear to lead to major problems (Bjarnason et al., 1997), although this has very recently been called into question (Wallace, 1999).

Mechanisms of Action

a) COX-2 Inhibition

Use of specific inhibitors of COX-2, designed on a structural basis (Kurumbail et al., 1996), suggests that it is this enzyme which is indeed responsible for the effects of NSAID'S. Direct evidence is provided by the finding that crossing COX-2 knockout mice with *Apc* knockout mice is marked reduction in number and size of intestinal polyps (Oshima et al., 1996). COX-2 is known to be upregulated in human cancers of the colon (Eberhart et al., 1994; Kargman et al., 1995; Sano et al., 1995), stomach (Ristimaki et al., 1997), especially in the corpus (Ratnasinghe et al., 1999a), pancreas (Molina et al., 1999; Tucker et al., 1999), lung (Achiwa et al., 1999) well-differentiated liver (Koga et al., 1999; Shiota et al., 1999) and both squamous cell carcinomas and adenocarcinomas of the oesophagus (Zimmermann et al., 1999; Ratnasinghe et al., 1999b), head and neck tumous (Chan et al., 1999), as well as cancers of the breast (Parrett et al., 1997). Gliomas also demonstrate upregulation (Deininger et al., 1999). Furthermore, increased levels have also been reported in premalignant intestinal tumors in man (Eberhart et al., 1994; Hao et al., 1999) and Min mouse experimental animals (Williams et al., 1996) and in experimental lung tumors (El-Bayoumi et al., 1999). Increase has further been described for conditions predisposing to neoplasia, like Barrett's oesophagus (Lord et al., 1999) and Helicobacter pylori-associated gastritis (Fu et al., 1999; McCarthy et al., 1999).

One way in which inhibition of COX could be important for carcinogenesis is through effects on carcinogen metabolism. Heterocyclic amines which may be present in the diet (Sugimura, 1997; Wakabayashi et al., 1997) and other agents can be metabolized to mutagens via COX-mediated metabolism of arachadonic acid (Eling and Curtis, 1992). Interestingly, chemical carcinogens themselves have long been known to be capable of inducing prostaglandins (Levine, 1977). However, other possibilities are that inhibition of COX and therefore prostaglandin synthesis acts by other mechanisms. One is via effects on heat shock protein induction (Etheridge et al.,1998).

b) Downstream Influence

Prostaglandins in general appear to be increased in many tumor types (Bennett 1986). One form which may be particularly important is PGE2, increased in human colon cancers (Rigas et al., 1993). In this context, very compelling evidence that the influence of COX2 inhibitors is through suppression of generation and action of prostaglandins is provided by work with specific inhibitors of prostaglandin receptors. The prostanoids PGD2, PGE2, PGF2a, PGI2 and TXA2 exert their biological actions through binding to receptors with seven transmembrane domains (Coleman et al., 1994; Ushikubi et al., 1995). Of the four subtypes, EP1

to EP4, known to bind PGE2, mice lacking genes for EP1 and EP3 (Ushikubi et al., 1998) have been generated in sufficient numbers to allow investigation of their susceptibility to azoxymethane carcinogenesis. Formation of ACFs was only reduced, to 60% of the control value, in the EP1 knockout mice (Watanabe et al., 1999). Furthermore, application of a selective antagonists of EP1, ONO-8711 or ONO-8713, in the diet also resulted in decrease preneoplastic lesions in the colon of C57BL/6J mice (Watanabe et al., 1999; 2000).

PGE2 is known to be involved in bile acid promotion of colon carcinogenesis and its inhibition by indomethacin (1987). In the colon, its production is associated with increase in the proliferation-associated enzyme ornithine decarboxylase (Giardiello et al., 1997).

One effect of PGE2 is to modulate apoptosis (Sheng et al., 1998). Although induction of this progammed form of single cell death in colon cancer cells by a prostaglandin-independent pathway has been reported (Hanif et al., 1996), overexpression of COX-2 in epithelial cells of the rat intestine is known to be associated with increase in expression of Bcl-2 and reduction in TGFB2 and b-cadherin so that they are resistant to apoptosis (Tsujii and DuBois, 1995). In Min mice, sulindac exposure is associated with reduction of nuclear ß-catenin and Bcl-2 expression in small intestinal tumors, colonic tumors being resistant to inhibitory effects and having higher levels of these (McEntee et al., 1999). Similarly, colon tumors are resistant to piroxicam despite very rapid reduction in the small intestine (Ritland and Gendler, 1999).In fact, a large number of reports have documented the ability of NSAID's to induce apoptosis in normal or cancer cells for example of the stomach (Kusuhara et al., 1998; Wong et al., 1999) and colon (Shiff et al., 1996; Hara et al., 1997). There is a rough correlation between the relative potency of these compounds in inducing apoptosis and their effectiveness in retarding cell proliferation (Shiff et al., 1996). Aspirin may suppress tumour incidence via salicylate by enhancing apoptosis in carcinogen-initiated cells (Barnes et al., 1998; 1999) . Cyclooxygenase-2 inhibitors have also been reported to suppress the growth of gastric cancer xenografts via induction of apoptosis in nude mice (Sawaoka et al., 1998a). One mechanisms might involve downregulation of protein kinase c since its activation and inhibition of myc results in block of steroidal anti-inflammatory drug-induced apoptosis in gastric cancer cells (Zhu et al., 1999b).

Although cyclooxygenase (COX)-2 immunoreactivity has been reported to bear no relationship to p53 and Ki-67 expression in colorectal cancer, and therefore not be directly linked to proliferation (Sakuma K et al., 1999), .Sawaoka et al (1998b) have suggested that this is important on the basis of in vitro data Inhibition of proliferation in intestinal epithelial cells in vitro has been documented but this does not necessarily correlate with depression of PGE2 (Erickson et al., 1999). Reduced proliferation has also been reported in buccal SCC by leukotriene but not PG synthesis inhibitors cells (el-Attar and Virji, 1987; Ondrey et al., 1996), and in cultured bladder cancer cells by indomethacin (Shimamura and Takahashi, 1994)

Promoters in the rat liver, lindane and phenobarbital, induce COX-2 expression in Kupffer cells (Kroll et al., 1999). In colorectal carcinoma cells, overexpression of COX 1 and 2 is associated with proliferation and induction of EGF receptor (Kinoshita et al., 1999) and antiproliferative effect of nonsteroidal antiinflammatory drugs have been documented .(Hixson et al., 1994). Non-small cell lung cancer cycloxygenase activity and proliferation are inhibited by non-steroidal antiinflammatory drugs (Hida et al., 1998). Indomethacin interferes with EGF-induced activation of ornithine decarboxylase in gastric cancer cells (Ishikawa et al., 1998) Data indicate that indomethacin has significant protective activity when administered either during the "early" stage (comprising the carcinogen-target cell interaction) or the "late" stage (postcarcinogen tumordevelopment) of mammary carcinogenesis in rats (McCormick et al., 1985).

Feeding a corn oil diet with indomethacin significantly reduced prostaglandin synthesis and ODC activity and increased LTB4 synthesis of mammary tumors but did not inhibit mammary tumorigenesis (Abou-el-Ela et al 1989). ODC activation by EGF depressed by indomethacin (Ishikawa et al., 1998). Experiments with piroxicam, nordihydroguaiaretic acid, and esculetin, other inhibitors of eicosanoid biosynthesis with varying selectivity for enzymes of the prostaglandin and leukotriene pathways, indicated that breast cancer cell growth was dependent on leukotriene rather than prostaglandin production. (Rose and Connolly 1990)

NSAIDs could also be effective at inhibiting progression of neoplasia, metastasis of gastric cancers being enhanced by COX-2 increase (Murata et al 1999).

Future Prospectives

a) Combination Effects

In future, use of NSAIDs in combination with other chemopreventive agents might find clinical application. It has already been shown that sulindac administered together with S-methylmethane thiosulfonate having antiproliferative activity from cauliflower gives greater protection against promotion/progression of colon cancer in the rat than either agent alone (Reddy et al., 1999a). Also aspirin given together with α-difluoromethylornthine was found to reduced ACF tumors, depressing mitosis and increasing apoptosis in adenomas (Li et al., 1999). Diet is important, for example with a negative relation of colon neoplasia to starch intake (Cassidy et al., 1994). This might possibly be through the action of resistant starch, giving rise to generation of butyrate in the large intestine. Together with aspirin this is known to reduce intestinal tumorigenesis in the Apc 1638 mouse (Williamson et al., 1999). Whether combination with lipoxygenase inhibitors (Steele et al., 1999) might increase beneficial effects of both also warrants attention in this regard.

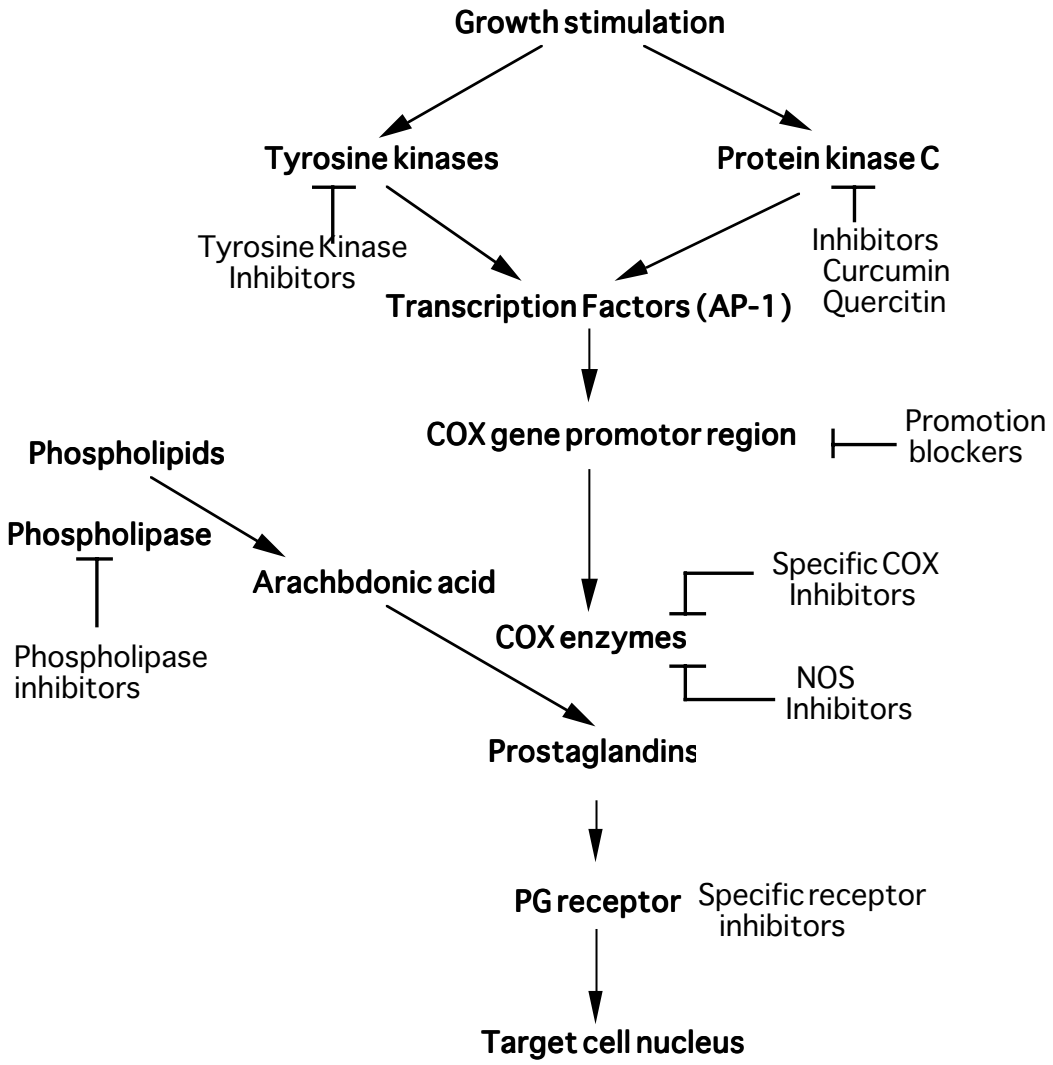


Fig 4. Potential Sites for Intervention in the COX/Prostaglandin Pathway

b) Other Types of Inhibitors

Other agents which can suppress inducible cyclooxygenase, as well as inducible nitric oxide are apigenin and related flavonoids (Liang et al., 1999) and the phenolic antioxidant curcumin (Zhang et al., 1999). This compound may act by downregulation of the protein kinase C pathway and AP-1 mediated gene expression (Huang et al., 1991) and is known to protect against carcinogenesis in the skin (Huang et al., 1988), buccal cavity (Tanaka et al., 1994), duodenum and forestomach (Huang et al., 1994) and colon (Rao et al., 1995). AP-1 heterodimers may play a role in induction of COX-2 (Xie and Herschman, 1995). In addition to inhibiting arachidonic acid metabolism, curcumin also reduces tyrosine protein kinase and ornithine decarboxylase (Rao et al., 1993). Stimulation of kinases by activation of ras results in induction of COX-2 (Sheng et al., 1998b). At the same time, NSAIDs like sulindac may inhibit expression of ras^p21 (Singh and Reddy, 1995). p53 may inhibit COX-2 gene expression (Subbaramaiah et al., 1999).Recent work by Mutoh et al (2000) looking at structure relationships of other potential inhibitors pointed to strong action of quercetin. Signal transduction pathways and tyrosine kinase activity may be enhanced in many cancers. This raises the possibility of specifically targeting COX-2 induction by inflammatory conditions or other specific tumor-related pathways (Subbaramaiah et al., 1997). Retinoids can suppress carcinogenesis in many tissues by stimulating differentiation and apoptosis and may also impact on prostaglandin production (Williasms and Dubois, 1996). .

An alternative means is via docosahexaenoic acid (DHA) which has been found to inhibit lesion development in the Min mouse (Oshima et al., 1995), as well as rat colon ACFs and tumors (Takahashi et al., 1997a; 1997b). Fish oil is in

fact preventive in the liver (Sugie et al., 1995) and could influence both phospholipase and COX activity (Rao et al., 1996). Depression of PGE2 release has also been reported (Bartram et al., 1993). Dietary menhaden oil also suppresses COX activity in rat mammary tumours (Hamid et al., 1999). Since nitric oxide (NO) radicals may contribute to COX functions (Goodwin et al., 1999), inhibitors of the inducible nitric oxide synthase could also exert beneficial effects. In this context it is of interest that colon tumors induced by azoxymethane have increased expression of this enzyme (Takahashi et al., 1997a).

In conclusion, there are a large number of potential sites in the COX/prostaglandin pathway which could be targeted for chemoprevention purposes, as summarized in Fig 4. Clearly, this remains an area of prime importance in terms of future development of chemopreventive agents.

References

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