The Medicinal Chemistry Implications of the Anticancer Effects of Aspirin and Other NSAIDs

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Abstract: The regular intake of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) has been associated with decreased incidence of certain types of cancer particularly those with an inflammatory component. The protective effects of these drugs in colorectal cancer are particularly marked, with a 40–50% reduction in risk. Research in this area has focussed on understanding and optimising these cytoprotective effects. NSAIDs are believed to operate by inhibiting COX-2, an enzyme that appears to be involved in a number of cancer promoting processes. This hypothesis is consistent with the observation that the COX-2 selective inhibitors dramatically decrease tumour formation in human and animal studies. Surprisingly aspirin, which is selective for COX-1 over COX-2, and sulindac, which is an equipotent inhibitor of the COX isoenzymes, appear to have a similar anticancer profile to the COX-2 selective NSAIDs. A number of mechanisms have been proposed to explain the anomalous effects of aspirin. The first of these relates to the unique mode of action of aspirin, which acetylates the COX-2 enzyme and generates the cancer-suppressing 15R-hydroxyeicosatetraenoic acid at the site of a potential tumour. The alternative rationale relates to the metabolism of aspirin to salicylic acid, which has a cyclooxygenase independent anti-inflammatory mechanism, preventing the inflammatory response at the gene transcription level. A new generation of drugs could evolve from approaches to improving the therapeutic index of aspirin or by modifications to known therapies such as sulindac and celecoxib.

Keywords: Colorectal cancer; aspirin; salicylic acid; cyclooxygenase; NF-κB; chemoprevention; chemotherapy.

1. INTRODUCTION

The regular intake of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) is associated with decreased rates of certain cancers with an inflammatory component, especially colorectal cancer but also including lung, oesophageal, pancreatic, cervical, skin and ovarian cancers [1-3]. The protective effect of these drugs in colorectal cancer is particularly marked with a 40–50% reduction in risk [4] but lesser effects have also been shown for other gastrointestinal (GI) malignancies [5]. Malignancies of the GI tract, especially colorectal cancer, are among the most common diseases worldwide and pose a significant burden of suffering and expense internationally [6]. Sulindac [7] and celecoxib [8] are the only chemotherapies for colorectal cancer that result in significant regression of colorectal tumours. Chemoprevention of cancer involves a careful balance between the likelihood of benefit to and the potential risk of side-effects to healthy individuals who may have remained healthy regardless of the chemoprevention [9]. With the lifetime probability of contracting colorectal cancer only just exceeding 1 in 20, the probability of an individual developing colon cancer in a given year is much lower. Therefore chemopreventative agents for use by healthy individuals require minimal side-effect profiles. The GI side-effect profile of conventional NSAIDs restricts their long-term use. Thus, while epidemiological evidence of an inverse relationship between colon cancer and NSAID use is highly encouraging, it is not yet strong enough to allow a recommendation for NSAID use in unselected patients. The advent of the selective COX-2 inhibitors with improved GI profiles relative to the NSAIDs opens new possibilities in cancer chemoprevention; however it is not clear that all of the anti-cancer effects associated with the conventional NSAIDs is exclusively attributable to their ability to inhibit the cyclooxygenases. This review aims to separate the anticancer effects of aspirin and related NSAIDs and discuss the possibilities and opportunities for improvement.

2. MODE OF ANTI-INFLAMMATORY ACTION OF NSAIDS

Classically, NSAIDs are considered to act by inhibiting the rate limiting enzyme prostaglandin-H synthase (PGHS) which participates in the first committed step in the synthesis of prostaglandins from arachidonic acid. PGHS is a membrane-bound bifunctional enzyme containing cyclooxygenase (COX) and peroxidase active sites. However, due to the prevalence of inhibitors that act at the COX active site [10] the enzyme is commonly referred to as the COX enzyme. Arachidonic acid is released from membrane-bound arachidonate by phospholipase A_2 in response to a diverse range of stimuli. This is oxygenated at the COX active site forming prostaglandin G_2 (PGG_2), which is then reduced to prostaglandin H_2 (PGH_2) at the peroxidase site. PGH_2 is converted to prostacyclin, the prostaglandins and thromboxanes by their respective specific synthases (Fig (**1**)) [2, 11]. The COX enzyme exists in two known isoforms termed COX-1 and COX-2. COX-1 is constitutively expressed and is involved in 'house-keeping functions', for example the maintenance of mucosal integrity in the GI-tract

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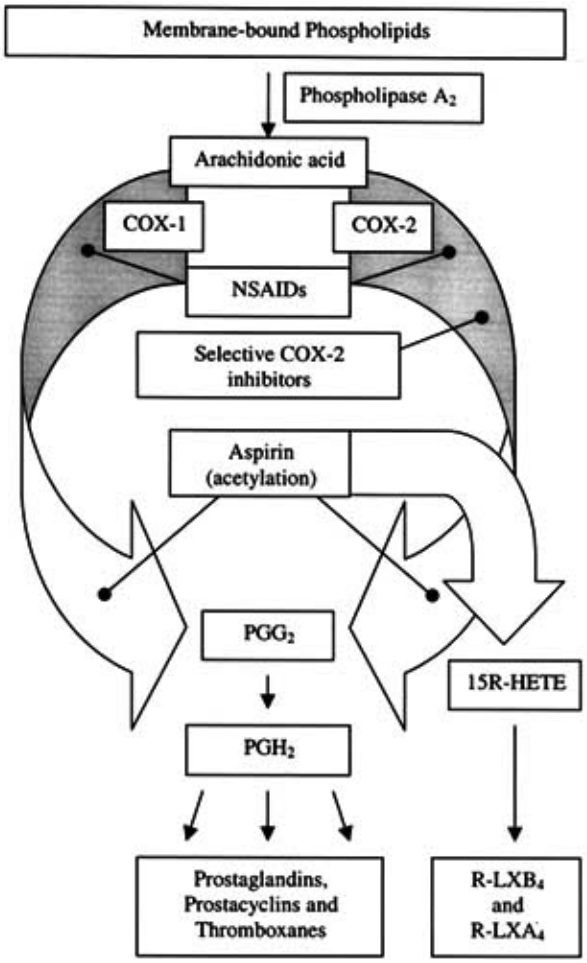

Fig. (1). The arachidonic acid cascade, as catalysed by COX, showing the redirection of the cascade when COX-2 is acetylated.

and modulation of platelet function. The COX-2 enzyme is expressed in response to inflammatory stimuli but is also found constitutively in some tissues [12]. Aspirin and the NSAIDs are established anti-inflammatory and analgesic agents whose therapeutic profiles are frequently explained by reference to their relative inhibition of the two isoforms of the COX enzyme [10]. Elevated levels of COX-2 have been found in many types of cancer and this isoform in particular is now considered to play a key role in cancer pathogenesis [2, 3, 13-16].

3. ROLE OF COX-2 IN MALIGNANCIES OF THE GASTROINTESTINAL (GI) TRACT

COX-2 is over-expressed in patients with both the pre-cancerous and cancerous stages of most GI cancers, including colorectal cancer [2, 15, 16]. Colorectal cancer is the second leading cause of cancer related death [17] with the advanced disease state being incurable [18]. There is a substantial body of evidence linking other localised inflammatory disorders with an increased risk of developing cancer of that region. For example persistent ulcerative colitis correlates with incidences of colon cancer 5 to 7-fold higher than expected but with no corresponding increase in other types of cancer showing the direct link with chronic localised inflammation [19]. This direct link is further demonstrated by the normalisation of the cancer risk by the treatment of colitis with the anti-inflammatory drugs sulfasalazine [20, 21] or 5-aminosalicylic acid [21, 22] (Fig (**2**)).

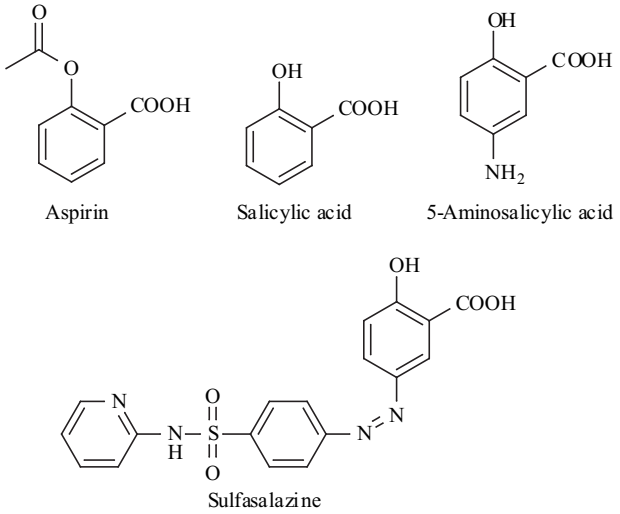

Fig. (2). Structures of salicylate based NSAIDs.

Oesophageal cancer results in the normal squamous epithelium being replaced by a specialized protective columnar epithelium due to persistent chronic acid reflux in a disorder termed 'Barrett's oesophagus' [5]. Bile acids have been shown in cell cultures to increase COX-2 protein expression and this effect is manifest in patients suffering from Barrett's oesophageal, oesophageal adenocarcinoma or squamous cell carcinoma, all of which exhibit elevated levels of COX-2 [5].

Because gastric cancer is incurable at advanced stages, great attention is paid to chemoprevention in this area. COX-2 has been linked with gastric cancer promotion and has been found elevated in 70% of both intestinal and diffuse gastric carcinomas, compared to controls. Levels of COX-2 are also routinely elevated in cases of Helicobacter pylori gastritis [23]. This bacterial infection induces COX-2 as part of the normal immune response resulting in much higher incidences of localised gastric cancer due to the associated inflammation [24]. Epidemiological studies show that NSAIDs have a protective effect against oesophageal and gastric cancer but no prospective studies have been carried out to date [5].

4. OTHER NON-GI TRACT MALIGNANCIES

Patients suffering from asthma have a significantly elevated risk of lung cancer [25, 26]. This observation is associated with the persistent localised inflammation in the lungs due to the asthma [27, 28]. Inflammation has also been linked to a variety of cancers including skin [29, 30], prostate [31], pancreatic [32], liver [33, 34], ovarian [35], breast [36], cervical [37] and other cancers [38].

5. ROLE OF COX-2 IN NEOPLASIA

While nearly all cancers with an inflammatory component over-express COX-2, not all cancers that over-express COX-2 have a clearly defined inflammatory component. COX-2 seems to be significantly linked to the promotion and acceleration of the cancer process. For this reason cancer progress seems to involve the induction of COX-2 at the pre-malignant and early malignant stages [39]. Colorectal cancer proceeds in a histopathologically detectable fashion and consequently treatment of colorectal cancer at an early stage would be a logical approach [18].

A number of multi-step models encompassing COX-2 have been established to explain the molecular basis for colorectal cancer (Fig. (**3**)) [1, 15, 40]. The initiation of colorectal cancer involves an irreversible mutation of the adenomatous polyposis coli (APC) gene (possibly in conjunction with other unknown genes), which results in the generation of a tumour from a single parent cell. The APC mutation causes an elevation in the level of COX-2 along with other cellular events that increase the propensity for subsequent mutations leading to cancer [1, 15, 40]. Small non-malignant polyps can remain dormant for decades before additional mutations occur [1, 40]. These subsequent mutations (p53, K-ras, DCC, DPC4, JV18-1/MADR2 genes and other unknown mutations) increase the sub-population of mutated daughter cells which increases the size and invasiveness of the tumour leading to eventual metastasis [1, 40]. Although colorectal cancer proceeds in a histopathologically detectable fashion, the overall process does not appear to be dependent upon a unique sequence of mutations nor is it likely that all of the known mutations are required. The process might be considerably more dynamic than the models imply [1, 40].

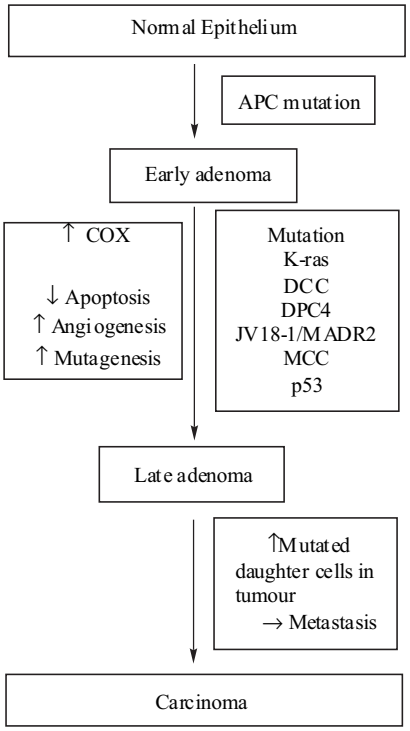

Fig. (3). Principles of multi-stage carcinogensis.

In patient populations with an elevated risk of developing colon cancer, such as those with familial adenomatous polyposis (FAP) disease, the role of inflammation and COX in cancer progression is better defined [41]. FAP is an inherited disorder that carries an absolute probability of colon cancer, due to the inheritance of the APC mutation and patients suffering from this disease are therefore good model subjects for the study of colon cancer development. Both celecoxib [8, 42], a COX-2 selective inhibitor and the non-selective COX inhibitor sulindac [7, 43, 44], significantly reduce the number of colorectal polyps in patients with FAP. On cessation of treatment this effect is reversed [7] illustrating the anticancer effect of NSAIDs for a disease with known elevated levels of COX-2. The roles of COX-2 in cancer progression are reviewed extensively elsewhere [3, 13, 15] so only a brief summary is given below.

5.1 Decreased Apoptosis

A change in the balance between cellular proliferation and programmed cell death (apoptosis) is crucial to the progression of cancer. A decreased rate of apoptosis increases the likelihood of genetic mutations within an individual cell and if a mutation occurs the mutated cell gives rise to elevated levels of transformed daughter cells. Over-expression of COX-2 has been linked with decreased apoptosis in premalignant and malignant neoplasms. COX-2

over-expression also increases the level of the anti-apoptotic Bcl-2 protein thereby increasing the duration of cell survival [45]. This process is reversed by treatment with NSAIDs [46, 47].

5.2 Increased Angiogenesis

Angiogenesis involves the formation of new blood vessels allowing tumours to grow and is a crucial determinant for metastasis. The over-expression of COX-2 in colon cancer cell lines increases the production of pro-angiogenic vascular growth factors, the migration of endothelial cells through collagen matrices and the formation of capillary like networks in-vitro [48]. These properties in conjunction with the anti-apoptotic effect are thought to be the two main features of COX-mediated neoplasia [13]. These effects were reduced by NS-398 a selective COX-2 inhibitor [48]. Reduction in tumour angiogensis by selective COX-2 inhibitors celecoxib [49] and NS-398 [50] has been demonstrated experimentally.

5.3 Increased Mutagenesis

The cancerous process is accelerated by the generation of reactive species capable of interacting with DNA and causing mutations [51]. COX-2, present in precancerous and cancerous tumours at elevated levels, diminishes cellular arachidonate causing the peroxidase site of PGHS to revert to alternative substrates to PGG_2. The peroxidase site reduces known procarcinogens to carcinogens particularly in cases where the cytochrome level is naturally low as in the GI tract [3]. Some procarcinogens such as benzo[a]pyrene, obtained from char-grilled foods or from cigarette smoking can induce COX-2 and thereby promote their own conversion to a carcinogen [52, 53]. Another potentially important COX-related mutagenic process involves the generation of malondialdehyde (MDA), which has been shown to be both carcinogenic and mutagenic in different species, presumably through its ability to form adducts with DNA [54]. MDA is generated spontaneously from PGH_2 and enzymatically during the conversion of PGH_2 to thromboxane. Other potential sources of MDA have been identified recently [55], however the pooling of PGH_2 in COX-2 rich cells seems likely to be a significant source.

5.4 Immune Suppression

The growth of tumours is associated with immune suppression, decreasing the cytotoxicity activity of natural killer cells that would normally act in response to foreign antigens [56]. Colony-stimulating factors released by tumour cells activate monocytes and macrophages to synthesise PGE_2 which inhibits B and T-cell lymphocytes, decreasing the cytotoxic activity of natural killer cells and inhibiting the production of tumour necrosis factor [57]. The NSAID indomethacin and aspirin have been shown to reduce these effects [58].

5.5 Metastasis

The stage of the cancer process that frequently marks the end of our current curative capabilities is the ability of malignant cells to break loose from their own tissue, invade neighbouring tissues and travel via the systemic circulation to sites distant from the original tumour. There is evidence that elevated levels of prostaglandins from COX-2 transfected colorectal cancer cells can activate membrane bound metalloproteinase-2 and increase mRNA expression of metalloproteinase-1 [59]. This leads to reduced intracellular anchorage through the digestion of the collagen matrix of the basement membrane allowing blood vessel penetration. These metastasic processes have been reversed by the action of sulindac [59] in colorectal cell lines and by NS-398 in prostate cell lines [60].

6. THE ASPIRIN PUZZLE

The regular intake of aspirin (Fig. (**2**)) has been shown in a number of retrospective studies to decrease the incidence of colorectal cancer by up to 50% [61-64]. The optimal dose is not known and it may take up to 10 years before the cancer preventative effects are seen [65, 66]. The confounding biases of retrospective epidemiological studies are well known [67] but to-date no primary intervention studies have been carried out with colorectal cancer as the primary endpoint. The Physicians' Health Study [68], which looked into the effect of low dose aspirin on incidences of colorectal cancer, found no protective effect after five years of the randomised study or following a 12-year post trial follow-up [69]. However, the primary end goal of that study was the analysis of the cardio-protective effect of aspirin and not any effect with regard to colorectal cancer. The trial has been criticised over its choice of subject population, the dosing regimes adopted, the short period of randomisation, the post trial follow-up [70, 71] and the lack of systematic screening for adenomatous polyp or cancer at the beginning and end of trial [9]. One phase I, de-escalating dose study on aspirin as a chemopreventative agent, suggested that doses as low as 81 mg/day are sufficient to inhibit preneoplastic biomarkers [72]. However it is not clear whether this can be attributed exclusively to aspirin rather than its metabolites as the study found that colonic prostaglandin levels were suppressed long after aspirin and salicylic acid had cleared from the plasma [72]. This may indicate that metabolites of salicylic acid whose anti-inflammatory properties are unproven can inhibit prostaglandin formation. Curiously, results from a recent prospective study suggest that 80 mg aspirin per day may be more effective than a 325 mg daily dose at inhibiting recurrent adenomas [73]. Phase II clinical results in patients with colon adenomas look promising [74] and phase III trials studies of aspirin are ongoing [70].

These observations are somewhat surprising in the context of the established role of COX-2 in carcinogenesis. Aspirin exhibits about ten-fold selectivity for COX-1 over COX-2 depending on the experimental model used, with significantly higher COX-1 preferences also reported. Furthermore, aspirin is only about 50% bioavailable being rapidly metabolised *in vivo*, partly through interaction with the cyclooxygenases but also by acetylation of a wide range of proteins including the cholinesterases, haemoglobin and serum albumin [75] ($t_{1/2}$ 15–20 min [76]). The acetylation of platelet cyclooxygenase in the portal circulation is of course critical to the cardioprotective effects of aspirin. It is

noteworthy that the product of these processes — salicylic acid — has negligible affinity for either COX isoform. Thun *et al.*, [9] recently proposed that the aspirin anomaly could be explained by the regulation of COX-2 expression by platelet COX-1 [77]. However, the two theories that have gained most widespread acceptance in explaining these anomalous observations are firstly that aspirin, by acting as a covalent modifier profoundly alters the biological role of COX-2, rather than merely blocking it, as with the COX-2 selective inhibitors. The second explanation is that the anticancer effects attributed to aspirin are due instead to its metabolite salicylic acid, which has profound and long lasting effects *in vivo*, but not through direct COX inhibition. These two hypotheses will be considered in turn.

6.1 Aspirin as a Covalent Modifier

Aspirin irreversibly inhibits COX-1 by acetylating a serine residue in the cyclooxygenase channel. Although this serine residue does not appear to play a significant role in the catalytic process, its acetylation renders COX-1 catalytically inactive by sterically blocking substrate access to key residues in the cyclooxygenase active site. Acetylation of the corresponding serine residue in the slightly larger COX-2 active site also prevents PGG_2 production but acetylated COX-2 can still accommodate the arachidonic acid substrate [78, 79]. In approaching the active site of acetylated COX-2, arachidonic acid is forced to adopt an alternative binding conformation leading to the formation of 15R-hydroxyeicosatetraenoic acid (15R-HETE) rather than the normal prostaglandin precursor PGG_2 [79] (Fig. (**1**)). 15R-HETE is undetectable as a product of wild-type COX-2 [79]. The natural occurring 15-HETEs, formed via 15-lipoxygenase action on arachidonic acid, are of the 'S' configuration and are subsequently transformed by the action of the 5-lipoxygenase and hydrolases to LXA_4 and B_4. The principal roles of LXA_4 and B_4 appear to be in leukocyte regulation [80]. The R-series products, R-LXA_4 and R-LXB_4 inhibit neutrophil adhesion and cell proliferation more potently than the corresponding 'S' compounds [80]. Most importantly, 15R-HETE suppresses tumour growth and consequently the benefits of acetylating COX-2 appear to be two-fold; acetylation blocks the formation of prostaglandins that participate in cancer progression while simultaneously generating the tumour suppressor 15R-HETE. This redirection of arachidonic acid may be especially important in the early stages of tumour development when COX-2 upregulation first occurs. Significantly elevated levels of 15R-HETE were observed in a mouse model following treatment of induced inflammation with aspirin [81]. Subsequent work recently reviewed by Serhan [80], showed that aspirin tolerant asthmatics have elevated levels of 15R-HETE in comparison to aspirin intolerant asthmatics [82].

6.1.1 COX-2 Selective Irreversible Inhibitors

Marnett *et al.*, in 1998 reported the first COX-2 selective irreversible inhibitor apparently capable of mimicking the mechanism of action of aspirin [83]. This approach maintains the anti-inflammatory, analgesic, antipyretic and anticancer benefits of aspirin associated with COX-2 inhibition while potentially obviating the GI side-effects associated with COX-1 inhibition. The only caveat to this approach is that drugs in this class would be devoid of the beneficial anti-thrombotic effects of aspirin associated with inhibition of platelet COX-1 [84-87].

6.1.2 Aspirin Pro-drugs and Nitric Oxide Releasing Aspirins and NSAIDs

An interesting approach to reducing the gastric toxicity of aspirin, which also avoids the loss in the COX-1 dependent antithrombotic effect, is the development of highly efficient aspirin prodrugs [88]. As mentioned earlier the anticancer dose of aspirin could be as low as 81 mg/day, which is equivalent to the low dose of aspirin used to treat cardiovascular disease (75−150 mg/day). Therefore these prodrugs offer the prospect of safe dual prevention of both cardiovascular disease and colorectal cancer in patients (50−80 years of age) at high risk of both diseases. The gastric toxicity profile of these compounds has yet to be established. An alternative approach to improving the therapeutic index of aspirin is the design of nitric oxide-releasing aspirin derivatives, termed *nitro-aspirins* or *NO-NSAIDs* in which the aspirin is linked to a carrier bearing a nitrate group [89-91]. These molecules are designed to cross the gastrointestinal barrier and then undergo cleavage by esterases, liberating aspirin and nitric oxide. Nitric oxide release protects the stomach from aspirin and NSAID-induced gastric erosion by promoting blood flow and reducing leucocyte adhesion. The validity of this strategy has been established in a number of animal models of gastric toxicity including a hemorrhagic shock model [92] an ulcerogenic rat model [93] and a diabetic rat model [94].

6.2 The Role of Salicylic Acid

Salicylic acid (Fig. (**2**)) has been a known anti-inflammatory since the earliest written history [95], but its pharmacological mode of action is still not fully elucidated.

6.2.1 Epidemiology of Salicylic Acid in Cancer

The highest rates of colorectal cancer occur in North America, Western Europe and Australasia with rates as high as 25–35 per 100,000 whereas the lowest rates of 1−3 per 100,000 are observed in India [96, 97]. There is strong evidence for an environmental influence, as the incidence of colorectal cancer is increasing, probably due to westernisation, in countries that previously had low rates of colorectal cancer. Within one generation, migrants moving from countries with low occurrences have rates equivalent to their destination country [98-100]. A decrease in fruit and vegetable intake is part of the westernisation of the diet that has been attributed as a cause of colorectal cancer [101]. An inverse relationship between vegetable consumption and colorectal cancer incidence had been reported in a number of case-control and cohort studies; no consistent relationship has been found for fruit consumption [102]. Salicylates are present in vegetables, most fruits and are most concentrated in herbs and spices [103]. Paterson and Lawerence [101] have suggested that the serum salicylate levels obtained from dietary sources in vegetarians can overlap those taking low dose aspirin (75 mg/day) whereas in comparison non-vegetarians had much lower serum salicylate levels [104, 105]. They further suggested that the predominant anti-cancer effect of aspirin is due to the metabolite salicylic acid.

The authors are not aware of any prospective case-controlled studies into the cancer protective effects arising from daily salicylic acid treatment.

Salicylic acid has virtually no inhibitory activity against purified COX, although it inhibits prostaglandin synthesis in intact cells indicating that it exerts its antiinflammatory effects independent of direct COX inhibition. Some controversy surrounds the effects of aspirin and salicylic acid on COX-2 expression with different studies showing a suppressive effect [106], no effect [107] or a potentiation of COX-2 expression [108]. Potential explanations for these observations were reviewed recently by Amann [76]. The most widely accepted COX independent activity of aspirin and salicylic acid involves their actions on NF-κB, which regulates the expression of proinflammatory enzymes, cytokines, chemokines, immunoreceptors and cell adhesion molecules all of which play a key role in inflammation and the immune response [109].

6.2.2 NF-κB and Cancer

NF-κB has diverse roles in cancer promotion. In addition to GI cancers, NF-κB is known in the disease pathology of leukaemia, lymphoid malignancies and breast cancer [110]. NF-κB activity is elevated in gastric and colorectal cancer cell lines and it has been suggested that NF-κB might serve as a "missing link" between inflammatory stimuli and cancer [110].

6.2.3 Role of NF-κB in the Tumour Process

NF-κB activates gene targets that control cellular proliferation by encoding growth factors that stimulate cellular proliferation. NF-κB has also been shown to stimulate the transcription of G1 and D1 cyclins, which are known activators of proliferation. NF-κB decreases apoptosis by activating the transcription of several target genes that are known to block apoptosis. NF-κB increases the production of chemokines, which can promote angiogensis, tumour invasion, extracellular matrix destruction. NF-κB has been shown to upregulate COX-2 [106] and inhibitors of NF-κB activation obtained from chemical and adenoviral vectors, were found to reduce COX-2 expression [111]. Surh [111] recently reviewed the area and proposed that induced nitric oxide synthase (iNOS) was also under the control of NF-κB. iNOS catalyses the oxidative deamination of L-arginine to produce NO, a potent pro-inflammatory mediator with roles in mutagensis and carcinogensis [111].

6.2.4 Salicylates and NF-κB

NF-κB is normally stored within cells as inactive heterodimers complexed with inhibitor κB (IκB) proteins in a so-called inhibitor Iκ-B kinase (IKK) present in the cytoplasm. The decomposition of this complex is mediated by IKK-β and α which phosphorylate IκB, breaking the IKK complexes into free NF-κB dimers that translocate to the nucleus, activating genes involved in inflammation. Aspirin, salicylic acid [112] and sulfasalazine [113] have been shown to inhibit the action of IκB kinase β at high concentrations, maintaining the IKK complexes and preventing the release of NF-κB. Salicylic acid and aspirin were specifically shown to be competitive inhibitors of ATP binding to IKK-β with the binding of aspirin being irreversible or very slowly reversible, but not covalent [112]. Among the major caveats to this model is the observation that in a p105-knockout mouse (essentially NF-κB deficient), aspirin and salicylic acid retained their anti-inflammatory activity suggesting that this pathway is not an important target for these drugs in the mouse. It is notable that dexamethasone, a known NF-κB inhibitor was inactive in this knockout model. Cronstein proposed that by inhibiting oxidative phosphorylation, salicylates promote the release of adenosine, a potent mediator of anti-inflammatory effects [114]. The proposed mechanisms appear to be complementary as a decrease in intracellular ATP would attenuate phosphorylation of IKK complexes and consequently NF-κB release. Questions also surround the clinical relevance of the salicylate concentrations used in these studies which are generally 1−10 mM, whereas salicylate levels *in vivo* following oral administration of normal doses of aspirin or salicylic acid are sub-millimolar [76, 115].

6.3 Sulindac

The anti-tumour effects of sulindac were recently reviewed [115]. Sulindac exists *in vivo* in a dynamic equilibrium with the COX-bioactive sulindac sulphide (formed by reductive metabolism) and the reverse oxidative metabolism (liver and kidneys) oxidizing sulindac sulphide back to sulindac [115]. Further irreversible oxidation to the COX-inactive sulphone also occurs [116] (Fig (**4**)). Sulindac

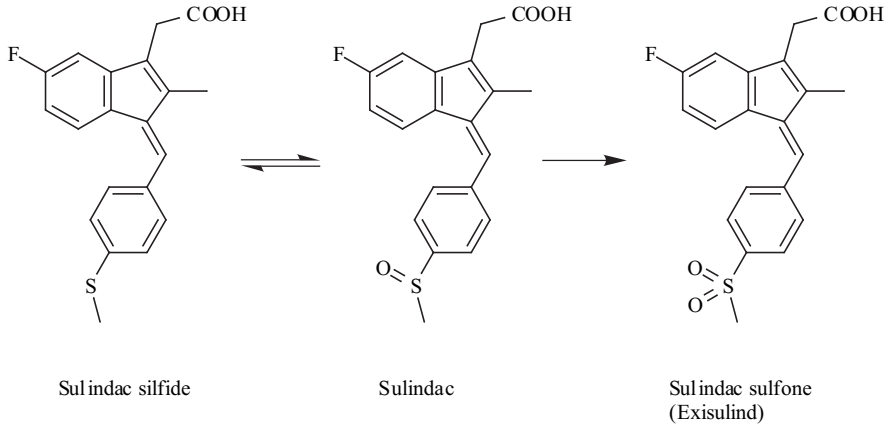

Fig. (4). Structure of sulindac and its metabolites; sulindac sulfide and sulindac sulfone (exisulind).

sulphide is a potent inhibitor of both COX-1 and COX-2 with IC_{50} values of 1 and 2 μM respectively. Sulindac on the other hand is less potent but is selective for COX-2 over COX-1 with IC_{50} values of ~60 and >100 μM respectively [115]. Intriguingly, both sulindac and its COX inactive sulfone metabolite appear to possess marked antineoplasic activity [117]. Sulindac sulfone has only been shown to prevent azoxymethane-induced tumourigenesis in rats when given at the initiation stage, whereas the sulphide metabolite still inhibited the same tumourigenesis when administration only began during late stage tumourigensis [116]. Additionally, sulindac sulfide causes intestinal tumour regression in APC mutant mice [118]. Unfortunately, it is difficult to separate out the mode of action of sulindac from sulindac sulphide because of their interconversion *in vivo*. Clearly, sulindac sulfone (also called exisulind) acts by a mechanism other than direct COX inhibition and possibly by a similar mechanism to salicylic acid. It is not clear whether sulindac in its dynamic equilibrium and sulindac sulfone share the same non-COX mode of action. However it remains possible that they do.

Modifications to sulindac to offer improved chemotherapy have been suggested [119], as it appears to have both COX-dependant and COX-independent modes of action, unlike celecoxib. Numerous modifications discussed in this review would be possible while hopefully maintaining the COX independent modes of action. Successful modifications to non-selective NSAIDs to improve COX-2 selectivity has been achieved before for flurobiprofen [120], ketoprofen [121], indomethacin [122] and meclofenamic acid [123].

6.3.1 PPARδ, Non-Aspirin NSAID Modes of Actions

The PPARs are ligand activated transcription factors that belong to the nuclear hormone superfamily. Three PPAR isoforms have been identified; α, δ, and γ. These receptors appear to be involved in diverse biological processes such as cellular differentiation and lipid metabolism [124]. While specific high affinity endogenous ligands for PPARs have yet to be identified some prostaglandins, particularly PGI_2 and 15-deoxy$^{\Delta 12,14}PGJ_2$ are activators of PPARδ and PPARγ respectively [125]. Concern has been expressed regarding the tissue distribution and relative potencies of these PGs towards their classical G-protein coupled receptor targets and the PPARs (nM v μM) however there is evidence that PGI_2 may be a genuine endogenous ligand in some tissues [126]. Similarly, non-salicylate NSAIDS are capable of activating α and γ receptors albeit at superpharmacological concentrations [127]. PPARδ has attracted much interest as a cancer chemotherapeutic target because of its role in cellular differentiation. PPARδ has been shown to be upregulated by the APC mutation and is co-localised with COX-2 in tumours [126]. The APC gene encodes a large protein that inhibits the production of β-catenin, which has important roles in cellular adhesion and development. The APC mutation causes increased levels of free β-catenin, which migrates to the nucleus where it forms a complex with T-cell factor 4. This complex binds to DNA and induces the expression of genes that promote cellular growth and proliferation, including the expression of the nuclear hormone PPARδ [128]. The NSAIDs indomethacin (100–400 μM) and sulindac sulphide (100–200 μM) were shown by He and colleagues to inhibit the function of PPARδ, thereby counteracting the effects of the initial genetic mutation [128]. The physiological relevance of these concentrations has been questioned. No study has definitely elucidated the role of PPARδ in the cancer process and furthermore it is not clear whether activation or inactivation of PPARδ is beneficial [126]. It is difficult to say at the present time whether PPARδ, in particular, represents a genuine target for cancer chemoprevention.

6.4 Efficacy of Selective COX-2 Inhibitors for Colorectal Cancer in Humans

The COX-2 selective inhibitor celecoxib (Fig. (**5**)) is effective in the treatment of FAP, demonstrating that COX-2 plays a key role in colorectal cancer etiology [8, 42]. Trials are currently ongoing with rofecoxib (Fig. (**5**)) and celecoxib, both selective COX-2 inhibitors into the treatment of less specific forms of colon cancer such as hereditary nonpolyposis colorectal cancer and pre-malignant pathologies such as Barrett's oesophageal and sporadic adenomatous polyps. COX-2 inhibitors are also in clinical trials for the prevention of non-GI malignancies including, oral, skin, bladder, breast, prostate and non-small-cell lung cancers [9]. In addition COX-2 inhibitors are being used in the treatment of cervical, prostate and metastatic breast cancer [129]. While the overall picture for COX-2 inhibitors is encouraging there are on-going concerns about possibility of renal side-effects that may limit the therapeutic utility of these compounds in cancer treatment and prevention [130]. Interestingly, NS-398 (Fig. (**5**)), a COX-2 selective inhibitor does not require COX-2 expression to exhibit an anti-proliferative effect. However, in cell lines expressing COX-2, G1 arrest and apoptosis were observed in addition to the complete inhibition of proliferation [131]. However, Smith [131] does note that others found that the anti-proliferative effects of SC58125, a more COX-2 selective form of celecoxib [47] and meloxicam [132] (Fig. (**5**)) required COX-2 expression.

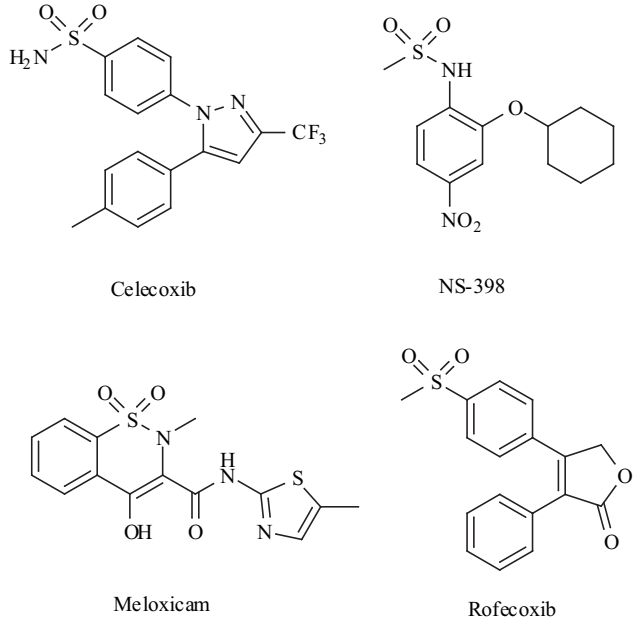

Fig. (5). Structures of selective COX-2 inhibitors.

7. CONCLUSION

The development of COX inhibitors for the treatment and prevention of cancer is a rich field of research. The COX-2 selective irreversible inhibitors offer the lipoxin generating benefit of COX-2 acetylation, but would be devoid of the non-COX dependent anti-inflammatory mechanisms of salicylic acid. They should have lower GI side-effects due to lower acetylation of COX-1, but therefore also lack the anti-thrombotic effects of COX-1 acetylation in platelets. A properly designed aspirin prodrug or nitro-aspirin should be capable of systemic COX inhibition but with depressed GI toxicity, a feature of nitro-NSAIDs and NSAID ester prodrugs. An exciting possibility is that such compounds could simultaneously reduce the risk of colon cancer through lipoxin generation and cardiovascular disease through COX-1 platelet inhibition. NF-κB inhibitors might be developed from a pharmacophore of its currently known inhibitors, even though a specific active site is undefined. In terms of chemotherapy of adenomatous polyps or malignancies of the colon, sulindac modified for improved COX-2 selectivity or other salicylate properties offers great clinical possibilities. The cognate receptor for sulindac sulfone (exisulind), which is COX inactive but tumour suppressing, remains a potentially valuable target for the development of new agents. Finally, colorectal cancer epidemiology strongly supports further investigation into the mechanism of action of salicylic acid, an enduring pharmacological enigma.

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