REVIEW

Non-steroidal Anti-inflammatory Drugs and Cancer, with an Especial Focus on Esophageal Cancer

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) have been extensively used for relief of pain and fever, and prevention of cardiovascular and cerebrovascular diseases for several decades. Recently, the use of these compounds has been reported to be associated with reduction in occurrence of a variety of cancers. In this paper, we reviewed anti-cancer mechanisms of NSAIDs and their potential preventive and even theraputic effects on cancer, focusing on esophageal cancer in particular.

Keywords: NSAIDs - COX-2 - apoptosis - cancer - esophagus

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Introduction

It is not deniable that discovering new chemotherapeutic agents and perfecting the radio- chemotherapeutic regimen may improve survival of cancer patients, however, some agents targeting at molecular biological marker which is related to cancer may bring about breakthrough for the prevention and treatment of cancer. From late 19 centrury, a kind of NSAIDs, salicylic acid has extensively used for relief of pain and fever (Elwood, 2001). Its preventive effects on cardiovascular and cerebrovascular disease has also identified for several decades (Manson et al., 1991; Goodnight, 1996; Elwood et al., 1998). In recent years, the use of these compounds has been reported to be associated with reduction in occurrences of a variety of cancers (Farrow et al., 1998). Extensive studies have reported that NSAIDs exerts their anti-cancer effects via inhibition of prostaglandin synthetase - cyclooxygenase (COX) (Alberts et al., 1995; Lupulescu, 1996; Attiga et al., 2000).

The discovery of new chemotherapeutic agents and improvements in radio-chemotherapeutic regimens may increase survival for cancer patients. Salicylic acid has been extensively used for relief from pain and fever (Elwood, 2001), and its preventative effects on some cardiovascular and cerebrovascular diseases have been known for several decades (Manson et al., 1991; Goodnight, 1996; Elwood et al., 1998). In recent years the use of aspirin and other NSAIDs has been reported to reduce the occurrence of a variety of cancers, including oesophageal cancer (Farrow et al., 1998). Many studies have reported that NSAIDs exert their anti-cancer effects via the inhibition of the cyclooxygenase (COX) enzymes, COX-1 and/or COX-2 (Alberts et al., 1995; Lupulescu, 1996; Attiga et al., 2000).

A number of non-randomised studies have reported that people who regularly use aspirin or other NSAIDs have a lower incidence of adenomatous polyps and lower incidences of or deaths from colorectal cancer compared with non-users (Deutsch, 1992; Gaut, 1993). Sustained use of NSAIDs has been reported to be associated with a 30-50% reduction in adenomatous polyps, incident disease and death from colorectal cancer (Paganini-Hill et al., 1991; Paganini-Hill, 1995; Kauppi et al., 1996; Sandler et al., 1998). Retrospective studies have demonstrated a 40-50% risk reduction of colorectal cancers in NSAID users (Kune et al., 1988; Rosenberg et al., 1991; Suh et al., 1993; Peleg et al., 1994; Reeves et al., 1996). Prospective studies have also shown a reduction in the incidence of and mortality from colorectal cancer in subjects who have used these compounds (Kune et al., 1988; Rosenberg et al., 1991; Thun et al., 1991; Gann et al., 1993; Suh et al., 1993; Giovannucci et al., 1994; Peleg et al., 1994; Schreinemachers and Everson, 1994; Kauppi M, et al., 1996; Reeves et al., 1996; Rosenberg et al., 1998; Sturmer et al., 1998). The results of epidemiological studies have suggested that the duration and continuity of NSAID use may be more critical than the daily dose(Thun and Heath, 1995; Collet et al., 1999; Smalley et al., 1999). NSAIDs have also been reported to reduce the risk of cancers of the oesophagus (Farrow et al., 1998), stomach(Thun et al., 1993), breast (Thun et al., 1993; Schreinemachers and Everson, 1994; Egan et al., 1996; Rosenberg, 1996), lung (Schreinemachers and Everson, 1994), prostate (Bucher et al., 1996), urinary bladder (Thun et al., 1993), and ovary (Thun et al., 1993; Cramer et al., 1998).

In some studies in rodents, aspirin, and other conventional NSAIDs such as piroxican, indomethecin,

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sulindac, ibuprofen, and ketoprofen and selective COX-2 inhibitors such as celecoxib, have inhibited chemically induced carcinogenesis (Craven and DeRubertis, 1992; Reddy et al., 1993; Barnes et al., 1997; Kawamori et al., 1998; Li et al., 1999). Oshima crossed heterozygous APC (delta716) knockout mice (a mouse model of human familial adenomatous polyposis) to COX-2 gene knockout mice. The double knockout mice for both APC (delta716) and COX-2 genes had marked reduction in the size and frequency of intestinal polyps, as did the APC (delta716) knockouts fed a selective COX-2 inhibitor (Oshima et al., 1996). Thus blocking the action of COX-2, either by the introduction of a COX-2 gene mutation, or feeding with the COX-2 selective inhibitor to the APC (delta716) knockout mice, reduced the number and size of intestinal polyps dramatically.

Increased levels of COX-2 have been reported in carcinomas of the colon (Sano et al., 1995; Fujita et al., 1998), as well as stomach, breast, oesophagus, lung, liver and pancreas (Ristimaki et al., 1997; Hida et al., 1998; Hwang et al., 1998; Wolff et al., 1998; Koga et al., 1999; Okami et al., 1999; Tucker et al., 1999; Zimmermann et al., 1999). In contrast, most studies report that the levels of COX-1 are similar between normal and tumour tissues (Sano et al., 1995). These findings suggest that COX-2 may be associated with carcinogenesis and/or progression of certain types of human malignancies.

Anticancer Mechanisms of NSAIDs

COX-2 and tumourigenesis

In the 1970s Vane and associates found that cyclooxygenase (COX) had 2 isoforms, COX-1 and COX-2 (Vane et al., 1971). COX-1 is expressed constitutively within many normal tissues and is thought to be responsible for the maintenance of normal physiological function, such as cytoprotection of the stomach, vasodilation in the kidney (Vane, 2000), and control of platelet aggregation. In contrast, COX-2 is not expressed normally, but is rapidly induced in response to proinflammatory and mitogenic stimuli including cytokines, endotoxins, interleukins and phorbolester (DuBois et al., 1994; Hempel et al., 1994; Prescott and White, 1996), and thus is thought to be responsible for pathological changes. Many more recent studies have further highlighted the relevance of COX-2 to human carcinogenesis.

COX-1 and COX-2 are rate-limiting enzymes for the formation of prostaglandins. The substrate for both COXs, as well as the lipooxygenase (LOX) enzymes, is arachidonic acid, an essential polyunsaturated fatty acid consumed in the diet or derived from elongation and desaturation of dietary linoleic acid (Marnett, 1992). Arachidonic acid can also be formed from the hydrolysis of phospholipid precursors catalysed by the enzyme phospholipase A_2. The COX enzymes introduce 2 molecules of O_2 into the arachidonic acid to form prostaglandin (PG) endoperoxides, from which PGH2 is formed. PGH_2 is an intermediate product that may be converted to biologically active prostaglandins (PGE_2, PGD_2, PGF_2, PGI_2, etc.) and thromboxanes (e.g., TXA_2) by various specific synthases or reductases. PGH2 may also be converted to malondialdehyde (MAD) and 12-hydroxyhepetadec (HHT), spontaneously or via catalysis by thromboxyane synthases or specific cytochrome p450s (Sharma, 2002). MAD, a by-product of prostaglandin biosynthesis, is a mutagen and carcinogen (Sharma, 2002). Under physiological conditions MAD reacts with DNA to form adducts, predominantly with deoxyguanosine to generate pyrimidopurinone-deoxyguabosine adducts (M1G) (Marnett et al., 1986). M1G, thought to be mutagenic, has been detected in a variety of human tissue at a concentration of 3-150 adducts/108 nucleotides (Marnett, 1999).

COX-2 has been reported to contribute to tumourigenesis and the malignant phenotype of tumour cells through one or more of the following several mechanisms: (i) increasing the production of prostaglandins, (ii) converting procarcinogens to carcinogens, (iii) inhibiting apoptosis, (iv) promoting angiogenesis, (v) increasing the invasiveness of cancer cells, or (vi) modulating inflammation and immunoresponsiveness (Dempke et al., 2001; Xu, 2002).

COX-2 derived PGE_2 is a pro-inflammatory agent, and is the major prostaglandin produced in many human solid tumours, including cancer of the colon (Rigas et al., 1993), stomach (Uefuji et al., 2000), and breast (Rolland et al., 1980). There is much evidence that PGE_2 promotes tumour growth. Thus treatment with prostaglandin receptor agonists reversed NSAID induced adenoma regression in Apc (Min/+) mice (Hansen-Petrik et al., 2002), and PGE_2 significantly enhanced carcinogen induced colonic tumour incidence and multiplicity in rats (Kawamori et al., 2003) and increased intestinal adenoma burden in Apc (Min/+) mice (Wang et al., 2004).

PGE_2 has been shown to increase synthesis by macrophages of vascular endothelial growth factor (VEGF), which promotes tumour vascularisation. The increase in VEGF production can be mediated by both specific PGE receptor and PPAR-gamma mediated mechanisms. PGE_2 can transactivate epidermal growth factor receptor (EGFR), which results in stimulation of cell migration through increased PI3K-Akt signaling in colorectal cancer cells (Sheng et al., 2001; Pai et al., 2002; Buchanan et al., 2003). PGE_2 can inhibit apoptosis by inducing the expression of anti-apoptotic proteins such as BCL2 (Sheng et al., 1998), and increasing NF-KappaB transcriptional activity (Poligone and Baldwin, 2001), which is a key regulator of antiapoptotic pathways. PGE2 also can transactivate peroxisome proliferator-activated receptor-delta (PPARD) which in turn promotes tumour cell survival (Wang et al., 2004). Ras is an oncogene and its activation is found in a wide variety of human malignancies. The Ras-MAP kinase cascade is one of the major intracellular signalling pathways responsible for cell proliferation. PGE_2 has been found to activate a Ras-MAPK pathway which in turn upregulates COX-2 expression in a self-amplifying loop, and stimulates colorectal cancer cell proliferation (Wang et al., 2005). PGE_2 also can downregulate Th1 cytokines (tumour necrosis factor α, interferon γ, and interleukin (IL)-2) (Harris et al., 2002) and upregulate Th2 cytokines such

as IL-4, IL-10, and IL-6 (Della et al., 1997; Huang et al., 1998; Shreedhar et al., 1998). Moreover, PGE_2 can modulate immune function through inhibiting dendritic cell differentiation and T cell proliferation, and suppressing the antitumour activity of natural killer cells and macrophages (Goodwin et al., 1983; Yang et al., 2003). PGE_2 has also been demonstrated to upregulate complement regulatory protein decay accelerating factor (Holla et al., 2005). Thus the combined effects of PGE_2 on different components of the immune system may play a role in assisting neoplastic cells to evade immune attack.

Effects of NSAIDs on cancer

COX-2 dependent pathways

Because COX-2 has the potential to play a very important role in tumourigenesis and tumour progression in a variety of cancers, the ability of NSAIDs to inhibit the COX-2 could explain their anti-cancer effects. A summary of the reported effects of NSAIDs in cancer includes the inhibition of cellular proliferation and tumour growth, induction of apoptosis, reduction of angiogenesis, prevention of procarcinogen activation and augmentation of immune response, (Lupulescu, 1996; Shiff et al., 1996). Sheng demonstrated that treatment with COX-2-selective inhibitors induced apoptosis in colorectal cancer cells expressing COX-2 but not COX-1 (Sheng et al., 1998). Studies in vitro with non-selective NSAIDs including salicylic acid, sulindac, sulindac sulfide, aspirin, indomethacin, naproxen, and piroxicam indicated that they have pro-apoptotic properties (Shiff et al., 1995; Shiff et al., 1996; Elder et al., 1997; Sheng et al., 1998). The antiproliferative effects of NSAIDs are controversial, and the data is largely limited to in vitro studies. Shiff et al. (1995) demonstrated the in vitro antiproliferative properties of NSAIDs, including sulindac and sulindac sulfide, on a human colon cancer cell line, HT-29. Subsequent studies using other nonselective NSAIDs such as aspirin, indomethacin, naproxen, and piroxicam, as well as selective COX-2 inhibitors, confirmed the antiproliferative effects of these drugs on colon cancer cell lines in vitro (Shiff et al., 1996; Goldberg et al., 1996; Qiao et al., 1997). However, Craven and DeRuberitis found an increase in proliferation in an induced colonic tumour in rats treated with aspirin (Craven and DeRubertis, 1992). Solid tumours larger than 2 mm in diameter must stimulate the formation of new capillary blood vessels to support further growth by the mass (Masferrer et al., 1996; Holash et al., 1999; Jones et al., 1999). COX-2 expression is widely induced in the angiogenic vasculature of colorectal adenomatous polyps and in carcinomas of the colon, lung, breast, oesophagus, and prostate (Holash et al., 1999; Masferrer et al., 2000). PGE_2 stimulates angiogenesis, and recent studies have demonstrated that tumour growth is dependent on PGE_2 (Form and Auerbach, 1983; Hanahan and Folkman, 1992). Tsuji and colleagues demonstrated that cyclooxygenase affects colon carcinoma-induced angiogenesis by two mechanisms: COX-2 modulates the production of angiogenic factors, which stimulate endothelial tube formation, while COX-1 regulates angiogenesis in endothelial cells (Tsujii et al., 1999), Masferrer (Masferrer et al., 2000), reported that COX-2, but not COX-1, derived prostaglandins regulate tumour-induced angiogenesis in mice implanted with human tumours. Celecoxib blocked the angiogenesis and suppressed tumour growth, consistent with the use of this anti-inflammatory drug in the treatment of human cancer (Masferrer et al., 2000). In model system in which tumour cells were implanted into mice, Williams noted that COX-2 in the host cells (i.e., the tumour stromal cells) appeared to be the most important influence on tumour growth. Therapeutic concentrations of COX inhibitors also suppressed the release of angiogenic growth factor by human or rodent colorectal cancer cells that were cocultured with vascular endothelial cells (Tsujii et al., 1998), and inhibited the growth of several human tumours transplanted into mice (Masferrer et al., 2000; Williams et al., 2000).

Reduced expression of HLA class I and II antigens has been reported in colonic tumours and in the adjacent normal mucosa (McDougall et al., 1990; Tsioulias et al., 1992; Tsioulias et al., 1993). PGE_2 can reduce the expression of these antigens, as well as suppressing T-cell proliferation, lymphokine production, macrophage activation, and T cell-mediated cytoxicity (Levy, 1997; Shiff and Rigas, 1992; Ahnen, 1998).These actions may assist the tumour to escape normal immune surveillance. By inhibiting prostaglandin synthesis, NSAIDs may up-regulate the expression of major histocompatibility complex antigens, which has been demonstrated in animal models such as the azoxymethane-induced rat colonic tumour (Rigas et al., 1994), and by this and other effects may indirectly enhance the immune response to a tumour (Husain et al., 2002).

Cyclo-oxygenase enzymes may promote cancer by means other than the synthesis of prostaglandins. These enzymes can metabolize procarcinogens such as polycylic hydrocarbons, aflatoxins, halogenated pesticides, amines, and phenols, and convert them to active carcinogens (Levy, 1997). NSAIDs could protect against cancer by blocking this conversion of a procarcinogen to a carcinogen.

COX-2 independent pathways

In addition to COX-dependent pathways, some studies have shown that NSAIDs, including COX-2 inhibitors, may exert anti-tumour effects by pathways which are unrelated to the inhibition of COX activity (Grosch et al., 2001; Tegeder et al., 2001). The following two observations support the concept of COX-independent effects of NSAIDs: (i) the dose of NSAIDs used is usually much higher than that needed to inhibit COX-2 enzymatic activity and, (ii) NSAIDs are effective against cancer cells that do not express COX-2 (Marx, 2001). For example, both sulindac sulfide and piroxicam induced apoptosis in COX-2 expressing HT-29 human colon cancer cells as well as the COX-2 deficient HCT-15 human colon cancer cells. Treatment of HCT-15 cells with various prostaglandins did not reverse the apoptotic effects of the drugs in the HCT -15 cells, suggesting a COX-independent effect (Hanif et al., 1996).

NSAIDs can antagonise the anti-apoptotic activity of peroxisome proliferative activated receptor, delta (PPARD) (Marx, 2001), possibly as a result of inhibition

of eicosanoid metabolism. PGI_2 is an activator of PPARD (Gupta et al., 2000; Lim et al., 2001), and the inhibition of carbaprostacyclin ($cPGI_2$)-stimulated DNA binding activity of the PPARD/RXR heterodimer is associated with induction of apoptosis in colorectal cancer cell (He et al., 1999). However, Piazza and colleagues reported a similar results with the sulindac sulfide related compound sulindac sulfone, which is devoid of COX inhibitory activity (Piazza et al., 1997), suggesting that inhibition of PPARD was in part mediated by a direct, prostaglandin-independent effect (He et al., 1999).

Indomethacin induces nuclear receptor subfamily 4, group A, member 1 (NR4A1) (Kang et al., 2000), which induces apoptosis in a number of cell lines exposed to proapoptotic stimuli (Kuang et al., 1999; Youn et al., 1999; Wilson et al., 2003). The induction of NR4A1 by indomethacin is associated with induction of apoptosis in HCT-15 colon cancer cells (Kang et al., 2000). Since these cells do not express COX-2, NR4A1 induction appears to be independent of COX-2.

NF-KappaB is a transcription factor which regulates the expression of a number of genes, including some that protect against cell death, such as the BCL and LAP family (Wang et al., 1998; Jones et al., 2000; Lee and Collins, 2001). NF-KappaB is held in the cytoplasm by I-KappaB until an appropriate signal results in its release. A variety of stimuli including cytokines such as TNF-alpha, IL-1, phorbol esters, LPS, viral infection, the human T-cell leukemia virus type 1-transforming protein Tax, ultraviolet radiation, and free radicals can result in the activation of I-KappaB kinases (IKKs). The IKKs add a phosphate group to I-KappaB which results in its degradation, freeing NF-KappaB. The NF-KappaB can then translocate to the nucleus to activate gene expression and stimulate cell apoptosis. Aspirin and other NSAIDs may exert their antiapoptosis effects by inhibiting the activation of the NF-KappaB pathway (Tegeder et al., 2001; Marx, 2001; Frantz and O'Neill, 1995; Yamamoto and Gaynor, 2001). The mechanisms by which NF-KappaB promotes cell survival are due in part to the up-regulation of anti-apoptotic genes such as members of BCL and LAP families (Wang et al., 1998; Jones et al., 2000; Lee and Collins, 2001). Also both aspirin and sodium salicylate can inhibit NF-KappaB by preventing I-KappaB phosphorylation and degradation (Pierce et al., 1996).

AP-1 is a group of related proteins consisting of products of the JUN, FOS, MAF and ATF subfamilies, which are activated in response to a number of stimulants including UV irradiation, growth factor, TNF-alpha and IL-1. Some of the genes regulated by AP-1 are involved in the immune and inflammatory response to tumour formation and progression, and promote proliferation and suppress apoptosis of tumour cells. AP-1 and NF-KappaB targeted genes partially overlap and most of these genes are activated by both AP-1 and NF-KappaB. Aspirin and COX-2 inhibitors have been shown to inhibit AP-1 activation, which would have an anti-tumour effect (Murono et al., 2000; Ding et al., 2003; Wong et al., 2004).

The WNT pathway is associated with carcinogenesis. WNT binds to membrane receptors encoded by Frizzled genes (FZD1-10). The canonical pathway involves WNT binding to FZD receptors, which leads to phosphorylation of the cytoplasmic protein Dishevelled (DSH), which then binds to axin and causes dissociation of the APC/axin/GSK complex, accumulation of beta-catenin and its subsequent translocation to the nucleus. There, beta-catenin inactivates gene transcription, some of it (e.g., c-Myc, cyclin DI) relevant to cancer. Aspirin and NSAIDs decrease the activity of WNT/beta-catenin pathway, although the precise mechanisms remain unclear (Dihlmann et al., 2003; Boon et al., 2004; Lu et al., 2005; Bos et al., 2006).

There is evidence that some carbonic anhydrase isozymes play a role in carcinogenic processes such as uncontrolled cell proliferation and malignant cell invasion (Kivela et al., 2005), and may be associated with a poor prognosis (Driessen et al., 2006), NSAIDs have been demonstrated to activate CA I and CA II isozymes in a dose-dependent manner (Puscas et al., 2006).

More recently, some studies have demonstrated that certain NSAIDs can inhibit cell cycle progression through inhibition of several kinases. The p70S6 kinase is a mitogen-activated kinase that is important for protein synthesis and G1 cell cycle progression (Hashemolhosseini et al., 1998). Salicylate has been shown to inhibit the activation of p70S6 kinase, which results in a down-regulation of c-myc, cyclin D1, cyclin A, and proliferating cell nuclear antigen (Law et al., 2000), which play an important role in cell proliferation. Their down-regulation might contribute to salicylate-induced growth arrest. In human pancreatic cancer cells, it has been demonstrated that salicylate inhibits the progression from G1 to S and reduce cyclin D1 level (Law et al., 2000). The expression and activity of cyclin and cyclin-dependent kinases (Cdks) are also important in the progression of cell cycle, and aspirin or NSAIDs have been reported to inhibit them, blocking cell division.

NSAIDs have been reported to affect a number of other genes or pathways which could play a role in inhibiting tumour growth. The activity of ribosomal S6 kinase-2, involved in the activation of the mitogen-activated kinase cascade and the stimulation of cell proliferation and differentiation, is suppressed by NSAIDs (Stevenson et al., 1999). The COX-2 selective inhibitor, NS-398, has been reported to induce apoptosis in a number of colon cancer cell lines, including HT-29 (COX positive), HCT 15 (COX negative) and SW 480 (COX-negative) by releasing cytochrome c from mitochondria, leading to the activation of caspase-9 and caspase-3 (Li et al., 2002). Similar findings were reported by Ding (Ding et al., 2005). Exisulid, a derivative of the NSAID sulindac, which does not inhibit COX-2, has anticancer activity, inhibits cancer growth by inhibiting an enzyme that breaks down the intracellular messanger, cyclic GMP. Celecoxib can also induce apoptosis by blocking AKT activation independently of BCL-2 in human prostate cancer cells (Hsu et al., 2000), and by inhibiting (3-phosphoinositid-dependent kinase-1) PDK-1 activity in the HT-29 human colon cancer cell line (Arico et al., 2002). Growth differentiation factor 15 (GDF15), a member of the TGF-beta family of genes (Baek et al., 2001), has antitumourigenic and pro-apoptotic properties (Baek et

al., 2001), and is up-regulated in human colorectal cancer cells by NSAIDs (Kashfi et al., 2005).

This brief overview shows that the mechanisms by which NSAIDs exert their actions against cancers are potentially very complex. Although the detailed anticancer mechanisms of NSAIDs have not been fully elucidated, the effects of these compounds on cancer can be summarized as the inhibition of cell cycle progression (Shiff et al., 1995; Goldberg et al., 1996), the induction of apoptosis (Barnes et al., 1998; Giardina et al., 1999; Shao et al., 2000) and the inhibition of angiogenesis (Jones et al., 1999; Tsujii et al., 1998). The mechanisms by which NSAIDs inhibit tumourigenesis and progression are likely to be through a combination of COX-dependent and COX-independent pathways.

Evidence for COX-2 Expression and Effects of NSAIDs in Oesophageal Cancer

The link between the use of NSAIDs and a decrease in oesophageal cancer incidence has been demonstrated in both epidemiological and experimental studies. People who regularly used NSAIDs have a 40-50% decrease in death rate from oesophageal cancer in comparison with non-users (Thun et al., 1993; Funkhouser and Sharp, 1995; Farrow et al., 1998). Thun (Thun et al., 1993) found that subjects who used aspirin 16 times/month or more for at least 1 year had an approximately 40% lower risk of oesophageal cancer (p=0.054). The data from Funkhouser's study showed a 90% (95% CI=0.01-0.76) decrease in the risk of developing oesophageal cancer in subjects who were occasional aspirin users (Funkhouser and Sharp, 1995). The results of a large population-based case-control study (n=1144) showed a decreased risk of oesophageal adenocarcinoma (OR=0.37, 95%CI=0.24-0.58) and squamous cell carcinoma (OR=0.49, 95% CI=0.28-0.58) in users of aspirin relative to nonusers (Thun et al., 1993; Funkhouser and Sharp, 1995; Farrow et al., 1998). Apart from epidemiological evidence, experimental and clinical data suggest a possible preventative or therapeutic benefit of NSAIDs in oesophageal cancer. Li et al reported a significant inhibition of growth in 10 oesophageal cancer cell lines by ASA, which was time and dose dependent and was associated with induction of apoptosis (Li et al., 2000). Langman showed the protective effects of NSAIDs against oesophageal cancer (Langman et al., 2000). In animal models of oesophageal carcinogenesis NSAIDs have reduced the frequency and number of premalignant and malignant lesions (Thun et al., 1993; Rubio et al., 1984; Rubio et al., 1986). In patients who underwent esophagectomy for suqamous cell cancinoma, the use of aspirin significantly improved the long-term survival for patients with IIa stage esophageal cancer (Liu et al., 2009). Thus, there is significant evidence that NSAIDs can act as chemopreventive or even therapeutic agents in oesophageal cancer.

As in other cancers, COX-2 mRNA, protein, or both, are up-regulated in oesophageal SCC and adenocarcinoma tissue or cell lines (Wilson et al., 1998; Li et al., 2000; Kandil et al., 2001; Morris et al., 2001; Liu et al., 2005; Liu et al., 2006; Zimmermann et al., 2009). Using an immunohistochemical staining technique, Ratnasinghe et al demonstrated strong positive staining for COX-2 in the well differentiated regions of squamous cell carcinoma of the oesophagus, and that smooth muscle cells, some stromal and inflammatory cells were also positive for COX-2 (Ratnasinghe et al., 1999). The same results were demonstrated in SCC of the oesophagus by reverse transcription-polymerase chain reaction (RT-PCR), western blotting, and immunohistochemistry and immunofluorescence (Jiang et al., 2004; Liu et al., 2006). However, COX-2 expression has been shown to vary between SCC of the oesophagus from high-risk compared to low-risk areas, for reasons that are not known (Zhang et al., 2003). Increased expression of COX-2 in oesophageal adenocarcinoma has been found not only in the cancer cells themselves but also in the cells of the tumor stroma (Zimmermann et al., 1999; Liu et al., 2006; Wilson et al., 1998). In addition, increased expression of COX-2 mRNA and protein has been observed in premalignant conditions of the oesophagus, such as squamous dysplasia and Barrett's oesophagus (Wilson et al., 1998; Shamma et al., 2000; Kaur et al., 2002). The expression of COX-2 is progressively up-regulated through each of the stages of oesophageal carcinogenesis from Barrett's metaplasia through dysplasia to adenocarcinoma (Shirvani et al., 2000). Recent studies have also shown that COX-2 overexpression is related to cell proliferation in oesophageal squamous dysplasia and squamous cell carcinoma (Yu et al., 2003). This is similar to oesophageal adenocarcinoma where France has suggested that COX-2 expression might be a better prognostic indicator than traditional histopathological staging (France et al., 2004). In a recent study conducted by Heeren et al, upregulation of COX-2 in adenocarcinoma of the oesophagus was associated with a poor outcome (Heeren et al., 2005).

The effects of NSAIDs have been studied in oesophageal cancer cell lines. The synthesis of PGE_2 is increased in cells expressing COX-2 compared to those cells expressing COX-1 only (Zimmermann et al., 1999). Li reported that the inhibition of growth by aspirin in 10 oesophageal cancer cell lines cancer cells was dose- and time-dependent, and was associated with induction of apoptosis (Li et al., 2000). Recently Cheong demonstrated that synthetic and naturally occurring COX-2 inhibitors suppressed proliferation, and induced apoptosis and cell cycle block, in human oesophageal adenocarcinoma cells (OE33) *in vitro* (Cheong et al., 2004). Liu et al demonstrated that NSAIDs induced apoptosis of esophageal cancer not only in cell line but also in cancer tissue by inhibiting the pathway of NF-KappaB downstream regulation of COX-2 (Liu et al., 2005; Liu et al., 2008).

COX-2 expression can be induced in oesophageal tissues by the tobacco carcinogen benzo[a]pyrene diol epoxide (BPDE) and by tumour promoting bile acids (Li et al., 2000; Zhang et al., 1998; Song et al., 2001). These data demonstrated the relationship between COX-2 and oesophageal cancer, but its potential role in cancer development and progression needs further investigation.

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