Pergamon

European Journal of Cancer, Vol. 35, No. 6, pp. 892–901, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(99)00054-4

Review

Non Steroidal Anti-inflammatory Drugs and Colorectal Cancer: is There a Way Forward?

A.K. Kubba

University Department of Surgery, University of Newcastle upon Tyne, U.K.

Non steroidal anti-inflammatory drugs (NSAIDs) have diverse clinical applications through modulation of oxidative processes and cell signalling. Observations that these agents may inhibit human colorectal carcinogenesis have produced great excitement. However, comparative data relating to their chemopreventative effectiveness or to relevant mechanisms of action remains unclear. This review considers the clinical and epidemiological evidence for colorectal tumour prevention by NSAIDs against current concepts of drug mechanisms. We also propose areas of further research for potential therapeutic advancement. © **1999 Elsevier Science Ltd. All rights reserved.**

Key words: NSAIDs, colorectal cancer, chemoprevention
Eur J Cancer, Vol. 35, No. 6, pp. 892–901, 1999

BACKGROUND

THE EXTRACTION of sodium salicylate from white willow bark (*Salix alba vulgaris*)in 1897, represented a major therapeutic advance in providing one of the most commonly used and durable drugs for treatment of inflammation and fever [1]. Since that time, clinical applications of non steroidal anti-inflammatory drugs (NSAIDS) have diversified to include analgesia and anti-platelet activity.

In more recent years, NSAIDs have been cited as possible agents for the chemoprevention of colorectal adenomatous polyps and cancer. Since then, the concept of chemoprevention by NSAIDs has been addressed in epidemiological, clinical, animal and basic science studies [2]. This area of research shows great promise for reduction of colorectal cancer mortality [3, 4] but current comprehension is incomplete and certain reports contradictory.

In this review, available clinical and experimental evidence pertaining to chemoprotective efficacy and mechanisms of action of NSAIDs are summarised with a view to evaluating current scientific principles which might guide further research or provide a rational strategy for clinical use.

Clinical reports

Interest in the relationship between colon cancer and NSAIDs was initiated in 1983 by Waddell and colleagues [5] who reported the treatment of 4 patients with Gardner syndrome (Table 1). One subject had pericarditis secondary to mediastinal desmoid tumour and was treated with indomethicin which resulted in the resolution of pericarditis and the reduction of the size of the mediastinal tumour. Another patient with a similar problem who had Gardner syndrome was treated with sulindac which caused the shrinkage of his colonic polyps as well as a desmoid tumour.

Giardiello and colleagues [6] conducted a randomised double-blind controlled trial of 22 patients with Familial Adenomatous Polyposis (FAP) (18 of whom received no surgery) to receive placebo or sulindac (150 mg bd) for 9 months. They evaluated the number and size of the polyps on a 3-monthly basis for 12 months and found a reduction in number (to 44% of baseline) and size (to 35% of baseline) of colonic polyps. However, 3 months after stopping the treatment, there was a small increase in the number and size of the polyps in the study group. This suggests that any protective effect of NSAIDs is not permanent and indeed only lasts while the patient is taking the drugs.

Keller and colleagues [7] randomised 21 patients with FAP to sulindac (300 mg/day) or placebo for 3 months. They found that sulindac significantly decreased the number of polyps ($P < 0.01$) and reduced the apoptotic ratio in the flat mucosa of those patients ($P < 0.01$). They suggested that apoptosis may play an important role in the mechanism of action of these drugs in macroscopic growth inhibition.

Di Sario and colleagues [8] conducted a randomised controlled trial of 40 patients with 4–12 mm recto-sigmoid polyps

Correspondence to A.K. Kubba, 32 Falkland St, Glasgow G12 9QY, U.K.
Received 21 Oct. 1998; revised 8 Feb. 1999; accepted 8 Feb. 1999.

Table 1. *Summary of the clinical data on the use of NSAIDs in colorectal cancer*

Authors [ref.]	Patients (*n*)	NSAID	Response rate
Waddell [5]	4 (Gardner's)	Sulindac	100%
Giardiello [6]	22 (FAP)	Sulindac	44% of baseline ($P<0.014$)
Keller [7]	10	Sulindac	50% ($P<0.01$)
Di Sario [8]	40 (24 versus 16 controls)	Sulindac	Decrease in size ($P<0.032$)
	Adenomas		Prevention ($P<0.0006$)
Von Stolk [9, 10]	17	Sulindac sulfone	11/17 decreased number of polyps
Takayama [11]	11	Sulindac	64% disappeared
Winde [77]	38	Rectal sulindac	78%

but no FAP to placebo, sulindac 150 mg/day and sulindac 300 mg/day. The subjects were treated for 12 months, and demonstrated that sulindac (both doses) decreased the size of the polyps ($P<0.032$) and the number of new polyps ($P<0.0006$) in treated patients. The latter two reports are only available in abstract form and we await the full manuscript with interest. More recently sulindac sulphone (a metabolite of sulindac) was given to 9 patients with FAP (in a randomised controlled trial involving 18 patients) who have had subtotal colectomy. Three doses of the drug were used (400 mg, 600 mg and 800 mg per day) for 6 months. They found that sulphone did not effect proliferation but caused a significant increase in apoptosis in adenomatous polyps ($P<0.09$) [9]. The same group examined the effect of two of the doses (600 mg and 800 mg) on 9 of the 18 randomised patients and followed them for 24 months. They reported that 600 mg of sulphone was very well tolerated and caused the arrest of the expected development of further adenomas [10].

Takayama and colleagues [11] randomised 20 patients with aberrant crypt foci of colon to receive Sulindac (300 mg/day) for 8–12 months ($n=11$) or control (no treatment, $n=9$). They found that the number of foci was significantly decreased in the treated group ($P<0.001$).

Epidemiological studies (Table 2)

In 1988 Kune and colleagues [12] questioned (by interview and completed questionnaire) 715 patients with colorectal cancer and 727 age/sex matched controls in Melbourne, Australia. They reported that the relative risk for the development of colorectal cancer in individuals using regular aspirin was 0.53 ($P<0.001$) and for those using other NSAIDs was 0.77 ($P=0.06$). This was only true when the individuals had used NSAIDs for more than 5 years.

A Californian hospital based case–control study [13] compared 1326 patients who had colorectal cancer with 4891 controls with respect to NSAIDs use. Regular use of NSAIDs (4 or more times a week) was associated with 50% reduction in risk of colon cancer. For those who stopped using aspirin for more than 2 years, the drug afforded them no beneficial protective effect.

Thun and colleagues [3] followed up 662 424 patients from a cancer screening register in 1982 (average age at enrolment was 57 years). Six years later, 6.7% of patients had

Table 2. *Summary of the epidemiological data on the use of NSAIDs in colorectal cancer risk*

Authors [ref.]	No. of patients	NSAID	Relative risk
Kune [12]	715 Ca	Aspirin	0.53 ($P<0.001$)
	727 control		
Rosenberg [13]	1326 Ca	NSAID (> 4/wk)	0.5
	4891 control		
Thun [3, 20]	662 424 cohort	Aspirin (other NSAIDS)	0.58 (0.6)
	598 Ca		
	3058 control		
Giovannucci [4]	331 Ca	Aspirin (> twice per wk	0.56
	89 446 (cohort)	only after 10–20 years)	
Peleg [14]	97 Ca	Aspirin	0.25–0.41 (DD)
	97 control	Nonaspirin	0.38–0.8 (DD)
Schreinemachers [15]	1257 (all cancers)	Aspirin	0.17–0.73
	12 668 cohort		(Dose–response)
Muscat [16]	511 Ca		
	500 control	All NSAIDs (> 3 wk)	0.36–0.68
Logan [17]	147 Ca	All NSAIDs	0.49
	153 controls		
Suh [18]	340 Ca	All NSAIDs	0.44
	212 polyps		0.33
	1662 control		
Greenberg [19]	793 adenoma		
Paganini-Hill [21]	13 987 (cohort)	Aspirin	1.5
Gann and colleagues [22] and	331 Ca	Aspirin (3.5/wk)	1.03–1.07
Sturmer and colleagues [23]	89 446 (cohort)		

DD, dose dependent; Ca, Cancer case.

died of various causes. They found that the relative risk of colorectal cancer among those who used aspirin (16 times or more per month) for at least 1 year was 0.6 and 0.58 for men and women respectively. Adjustments for family history, obesity, physical activities and dietary factors did not significantly alter the findings but there was a positive dose response relationship. Giovannucci and colleagues [4] studied 89 446 women participants in health questionnaires and 331 new cases of colorectal cancer between 1984–1992. They found that only women who took aspirin (more than twice weekly) for 10–20 years had a statistically significant decrease in cancer risk (RR = 0.56, $P = 0.008$). The reduction in risk is most pronounced when subjects took more than 4–6 tablets per week. Other risk factors for colorectal cancer and the early removal of colonic polyps did not influence the results.

Peleg and colleagues [14] studied 97/216 patients with colorectal cancer in one centre from whom they could establish the use of NSAIDs for 4 years prior to cancer presentation. They compared these with 97 sex and age matched controls out of a pool of 388 subjects. They demonstrated a negative statistical relationship between aspirin ($P < 0.001$) and other NSAIDs ($P < 0.019$) use and subsequent development of colorectal cancer. This relationship was dose dependent but only occurred after a certain threshold in the dosage of NSAIDs.

A prospective study [15] examined aspirin consumption in 1257 patients with cancers of the lung, breast and colon. These patients were detected from a 12 year follow up of a population of 12 668 subjects attending a health screening programme. They found a lower risk of, and mortality from, colorectal cancer in subjects taking aspirin (OR = 0.17–0.73).

Muscat and colleagues [16] reported a case–control study conducted in five hospitals involving 511 patients with colorectal cancer and 500 sex and age matched controls (subjects were sent a questionnaire and interviewed). NSAIDs use was defined as usage of three or more times per week for 1 or more years prior to hospital admission. The prevalence of drug use was 15% (male) and 8% (female) of patients in the cancer group versus 20% in the controls. This resulted in a significant estimated risk reduction of 36 and 68% in men and women, respectively. NSAIDs use did not effect the site, side or stage of the tumours. Furthermore no dose or duration response relationship was found. Logan and colleagues [17] examined NSAIDs use in 147 cases of colonic adenomas and 153 age and sex matched controls and followed up these patients for 7 years. They found that NSAIDs offer significant protection from the development of adenomas (OR = 0.49).

Suh and colleagues [18] reported a case–control study, conducted by questionnaires, review of case notes and telephone enquiries. The study group included 340 patients with cancer and 212 patients with polyps only. They used 2 control groups (hospital attender, $n = 524$ and healthy volunteers, $n = 1138$). Users of aspirin for more than 12 months before the diagnosis of colorectal cancer or polyp were included in the study. They found that aspirin use significantly reduced the risk of colorectal cancer (by 60%) and colonic polyps (by 70%) in a dose dependent manner.

Similar results were reported by Greenberg and colleagues [19] who examined 793 patients with colonic adenomas and found regular NSAIDs use to lower the risk of new adenoma formation at a 12 months follow-up.

The consensus is that this decreased risk of cancer is due to reduction in the genesis of colonic polyps [20]. One prospective study from the U.S.A. failed to confirm this assertion [21]. This involved 13 987 cohort of subjects from a Californian retirement community taking daily NSAIDs. They found the relative risk of colorectal cancer to be 1.5 suggesting no protective benefit from using NSAIDs.

An American interventional study [22] giving a group of 22 071 healthy male physicians between 40 and 84 years of age aspirin on alternate days and following them up for 6 years showed no beneficial effects. Recently, this group reported a follow up study: they offered those who were randomised to aspirin in the original study, the choice of continuing aspirin intake. Those who self selected continuing aspirin intake and were followed up for 12 years did not have a decreased incidence of colorectal cancer when compared to those who did not receive aspirin [23].

This must be viewed with Giovannucci and colleagues findings in mind that cancer prevention may only become evident after 20 years of usage.

To summarise, there is substantial evidence from clinical and epidemiological data to suggest that the regular long-term use of NSAIDs has a chemopreventive action against colorectal polyps and cancer. However, the type of NSAIDs which is most useful and the dose and duration of usage required to achieve such effect is not fully established. These questions are difficult to answer but one can only attempt to answer them by large multicentre clinical and epidemiological studies. A challenge yet to be tackled.

Animal studies

The Min mouse model is an important development in colorectal cancer research. It is an experimental model of FAP. This model has an autosomal dominant heterozygous for nonsense mutation (converting colon 850 from leucine to a stop codon) of the mouse Apc tumour suppressor gene whose inactivation is implicated in the early development of sporadic colorectal cancer [24, 25]. The Min mouse develops small intestinal tumours and normally dies of anaemia or bowel obstruction at approximately 120 days [26]. It is postulated that this model is closer to cancer in man than chemically induced cancer. This is because in man cancer develops from foci of dysplasia and tumour development is not secondary to high-doses of genotoxic chemical carcinogens and because mutations in the APC gene is encountered in FAP and some sporadic colorectal cancer [27]. However, the Min mouse's main defect is that tumours develop mainly in the small intestine.

Barnes and colleagues [28] examined the effect of Aspirin (250 and 500 ppm) upon the Apc min/+ (Min) mouse model. All Min mice but none of the control group (normal mice) developed gastrointestinal tumours. They found that aspirin significantly reduced the number and the size of tumours per mouse. The effect seen with low-dose of aspirin was similar to that noted after a high-dose suggesting that low-dose Aspirin may be adequate to achieve chemoprevention. This is supported by previous carcinogen induced rodent colon cancer studies [29] and epidemiological studies of colon cancer risk in aspirin users [30].

Jacoby and colleagues [31] treated 8 Min mice with piroxicam (50–200 ppm). At 6 weeks, the number of tumours decreased in a dose dependent manner (from 17.3 to 2.1) in the high-dose of piroxicam ($P < 0.001$) compared with

Table 3. List of possible mechanisms

(1) The cyclo-oxygenase pathway
Blockage of cyclooxygenase 1, 2 or both
Inhibition of prostaglandin synthesis
(2) Alteration of cell cycle equilibrium
Inhibition of proliferation
Alterations in differentiation
Induction of apoptosis
Arrest of cell cycle transition
(3) Others
Upregulation of tumour suppressor genes and proteins
Down-regulation of tumour promoting genes and proteins
Immune surveillance
Increased production of ceramide
Inhibition of NFkB transcriptional factors

control Min mice. Plasma thromboxane B2 (TXB2) levels decreased in a parallel manner to tumour multiplicity. It must be noted that this observation does not prove a cause–effect relationship and further studies must examine whether NSAIDs prevent adenomas primarily through cyclo-oxygenase inhibition.

Boolbol and colleagues [32] conducted a controlled experiment using 20 Min mice, half of whom were fed sulindac (0.5 mg/day) for 110 days when they were sacrified. Control Min mice had 11.9 tumours per mouse compared with 0.1 tumour per mouse in the sulindac fed group. Min mice had greater COX-2 and Prostaglandin E2 (PGE2) expression and a lower apoptotic ratio in non tumour intestinal mucosa compared with APC normal mice suggesting that *APC* mutation causes alterations in pre-dysplastic tissue. Treatment with Sulindac returned COX-2 and PGE2 levels and the apoptotic ratio to baseline. Similar observation of the effect of Sulindac upon tumour development in the Min mouse model have been reported by others [33].

Recently a model of colonic tumorigenesis has been created [34] which could facilitate studies of NSAIDs effects upon colonic carcinogenesis.

In vitro *mechanistic studies*

NSAIDs may exert effects through a number of potential mechanisms, though none is yet regarded as predominant. Potential mechanisms are listed in Table 3. (see Figure 1).

Inhibition of cyclo-oxygenase 1, 2 or both

Sulindac has been the most widely researched drug in studies examining the relationship between NSAIDs and colorectal cancer, partly because of its effective cyclo-oxygenase inhibition. A large fraction of sulindac is converted into sulindac sulphide (SUS, 15%) and sulindac sulphone (SOS, 75%) by colonic bacteria (a reduction process which occurs mostly in the caecum) [35, 36] the rest remains unaltered. Sulindac is a pro-drug that is metabolised to a pharmacologically active sulphide derivative which patently inhibits prostaglandin synthesis and sulindac sulphone which does not. Cyclo-oxygenase (COX) is prostaglandin H synthase which is the principal enzyme that mediates the formation of prostanoids (a collective term for prostacyclins, prostaglandins (PG) and thromboxanes (TX)) [37]. Cyclo-oxygenase's and prostaglandins' role in carcinogenesis has been of much interest [2, 38]. PG have been shown to increase cell proliferation [39] and tumour growth [40] and affect the immune response [41]. Cyclo-oxygenase has two isoforms, COX-1 and COX-2. The former is expressed in a variety of cells and tissues [42], whereas the latter is induced by cytokines, growth factors and tumour promoters [37].

In 21 pairs of surgically excised human colon cancers and surrounding normal colonic mucosa, Rigas and colleagues [43] found that the levels of PGE2 were elevated in cancer. However, the levels of PGF2-alpha, TXA2 and leukotrien LTB4 were not significantly different between the two

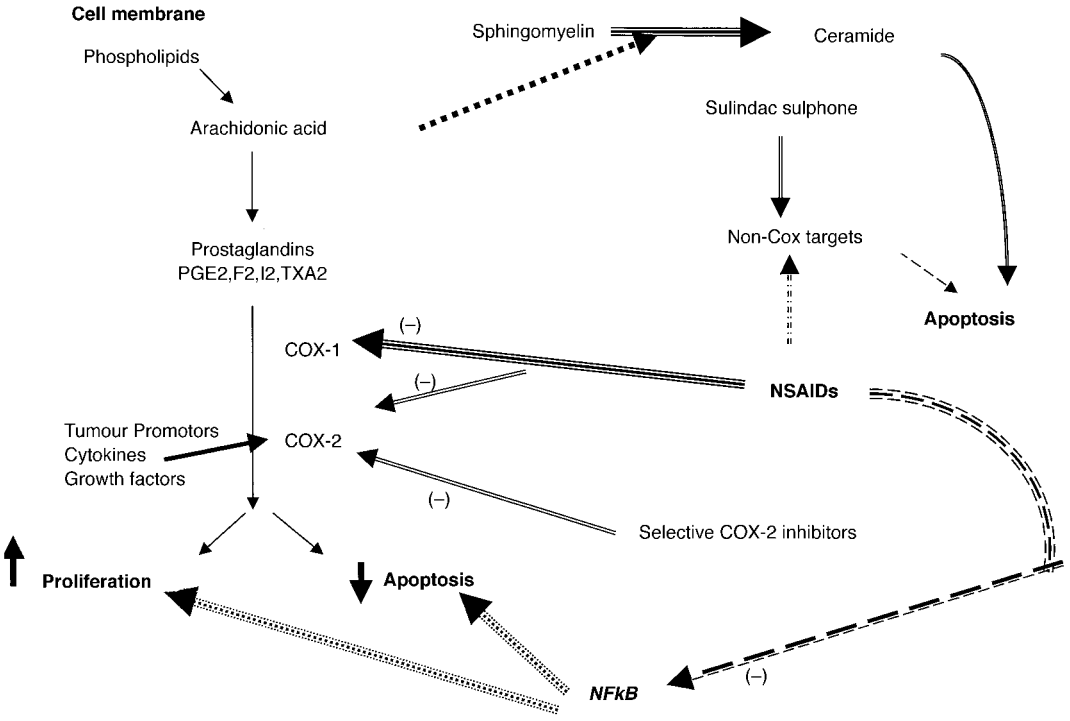

Figure 1. Possible mechanisms by which NSAIDs exert their chemopreventive effects on tumours.

groups. Qiao and colleagues [44, 45] found that PGs especially LTB4 increase the proliferation rate of colon cancer cell lines (HT-29 and HCT-15) without affecting cell cycle or apoptosis.

Yang and colleagues [46] demonstrated that the levels of PGE2, PGD2 and 6-keto-PGF 1 alpha of adenomas is significantly elevated in a tumour size-dependent manner in patients with FAP. COX-2 is elevated in adenomas and adenocarcinomas in animal models [47, 48] and is increased in 90% of cancers and 40% of adenomas in man [49, 50].

In carcinogen-treated rats, levels of PGE2 in normal appearing colonic mucosa in cancer-bearing animals are significantly higher than controls [51].

In 4 patients with FAP, mucosal PGE2 and thromboxane B2 levels were higher in non-tumour colonic mucosa compared with 5 normal patients [52] (other prostaglandins measured did not show a significant difference compared with control). Treatment of these patients with NSAIDs resulted in an interpatient heterogeneous response (with regard to Prostanoids levels) ranging between + 19% and − 89% from baseline but the results were most marked for PGE2 and thromboxane (TXB2). In 1 patient whose PG level was markedly reduced by sulindac treatment, the patient developed cancer 3 years after starting sulindac despite the absence of polypoid adenomatous lesions. This confirms the *in vitro* findings of others [53] that in colorectal cancer cell lines, growth inhibition is not associated with prostaglandin inhibition. These reports add to the debate on the complex mechanism of cancer chemoprevention and the role COX inhibition plays in this process.

THE ROLE OF COX-2, ITS INHIBITION AND SELECTIVE INHIBITORS

Increased COX-2 expression is thought to be associated with carcinogenesis. Upregulation of COX-2 leads to resistance to sodium butyrate-induced apoptosis, upregulation of *bcl-2* gene expression and down regulation of E-cadherin (indicators of potential carcinogenesis) [54]. Cross breeding of COX-2 gene knockout mice with Apc716 knockout mice (which normally develop hundreds of intestinal polyps) results in reduced size and numbers of polyps, thus providing genetic evidence of the role COX-2 may play in carcinogenesis [55].

Sheng and colleagues [56] examined the role of COX-2 in *ras*-induced transformation in rat intestinal epithelial cells (RIE). The H-*ras* transfected cells developed loss of contact inhibition, rapid proliferation, decreased growth factor requirements, resistance to apoptosis and tumour formation in nude mice. A 9–12-fold increase of COX-2 expression and 2.15-fold increase in PGI2 expression in RIE-ras cells was found. Treatment with SC-58125 (a selective COX-2 antagonist) significantly inhibited cell proliferation (growth inhibition of 50–70%) and induced apoptosis both in cell culture and RIE-Ras tumours in mice.

Ras mutations are found in 38–50% of colonic cancers [57, 58] and the Ras-MAP kinase pathway which is activated by various growth factors such as TGF-alpha are important mediators of carcinogenesis making these authors findings important and useful in indicating a link between *ras* mediated transformation and COX-2 overexpression. Traditional NSAIDs have gastrointestinal side-effects that are serious enough to restrict their use as chemopreventive agents. Side-effects are thought to be secondary to COX-1 inhibition, hence prostaglandin biosynthesis [59], whereas COX-2 is not detectable under normal physiological circumstances [37] but is overexpressed in colorectal cancer. Therefore, selective COX-2 inhibitors may have very low side-effects [60].

Selective COX-2 inhibitors (MF-tricyclic and SC-58635) have been shown to inhibit tumour formation in carcinogen-treated rodents (by 40–49%) [61]. Nimesulide (an analogue of NS-398) and SC-58635 inhibit the formation of chemically induced aberrant crypt foci in the rat colon and MF tricyclic suppresses polyp formation in Apc716 mice [62]. Elder and colleagues [62] found NS-398 had a dose dependent anti-proliferative effect upon HT-29 and S/KS cell lines. It induced apoptosis even in cells that do not express COX-2 protein, though cell cycle parameters were unaffected by NS-398 treatment.

Recently Karamori and colleagues [63] showed that feeding the COX-2 selective NSAID celecoxib (SC-58635) to rats completely prevented the development of chemically-induced colon tumours in 93% of treated animals; this inhibition was superior to that encountered with aspirin or sulindac. It would be interesting to determine the COX-2 selective NSAIDs' effect upon tumours in the Apc716/COX-2 deficient mice which might clarify the role of COX-2 more clearly. Apc and COX-2 may be functionally related especially in the modulation of cell adhesion, tumour progression and apoptosis [64].

Curiously, recently McCartney and colleagues [65] compared the effect of a conventional NSAID, diclofenic (COX-1&2 blocker) with selective COX-2 inhibitors (L745337 and SC58125) and reported that HT29 cells' viability was reduced to a greater extent when using diclofenic, therefore, shedding doubts about the efficacy of COX-2 blockers in cancer chemoprevention.

When considering these selective inhibitors in chemoprevention, it is important to remember that 15% of human colorectal cancer and 60% of adenomas do not express the COX-2 protein [66] and that COX-2 selective inhibitors induce apoptosis in cancer cell lines lacking detectable COX-2 protein expression [67].

The future looks promising for COX-2 selective inhibitors but our clinical experience with these drugs is limited and more research need to focus on establishing whether these agents represent an effective and safe option for use in man.

THE NON-COX-2 THEORY

Views on the role of COX-2 in the chemopreventive action of NSAID differ, and there is evidence that their effects are independent of COX-2.

Prostaglandin (PGE2) formation (a COX-2 dependent process) is not decreased by NSAIDs, suggesting an independent pathway of action [32, 68].

Thompson and colleagues [69] found that both sulindac and SOS, which does not inhibit COX 1 or 2 and has no anti-inflammatory action [70] inhibited the development of carcinogen-induced mammary cancer (in mice) in a dose dependent manner, suggesting that the prostaglandin pathway is not essential in this process.

Piazza and colleagues [71, 72] found that both SUS and SOS inhibited the growth of HT-29 cancer cells, although SUS was four times more potent than SOS. Neither drug had a significant effect upon proliferation or cell cycle distribution of non-proliferating cells, but the fraction of proliferating cells in the G1 phase increased. Both drugs induced apoptosis in

approximately 50% of cell population though SUS was 5 times more potent. COX inhibition did not appear to be necessary or sufficient for the growth inhibitory or apoptotic effects of these drugs. Although SUS is 500 times stronger in COX inhibition than SOS, its potency seems to be 4–6-fold greater than SOS. This may be attributed to the fact that SUS is more lipophilic which may enhance its membrane penetration, thereby lowering the effective dose range. The same group found that the prostaglandin analogue dimethyl-PGE2 failed to reverse the apoptosis induced by SUS. Although p53 levels tend to be elevated in apoptotic cells, they reported that SUS did not induce p53 expression. p53 deficient cells (Saos-2) responded to sulindac metabolites. These results suggest that apoptosis is important in sulindac metabolites' tumour inhibitory effects which seems to occur in a p53 independent manner.

Chiu and colleagues [73] found that, although sulindac caused a 95% inhibition in tumour development in Min Mice, it did not alter PGE2 or leukotriensB4 (LTB4) levels in the intestinal mucosa of these animals. Increasing arachidonic acid dietary supply to these animals did not increase the number of developing tumours. Adding sulindac to the arachidonic acid rich diet inhibited the numbers of tumours which developed without significantly effecting PGE2 or LTB4.

Recently, salicylate [74] and piroxicam [53] induced apoptosis in a Cox independent manner in colorectal carcinoma cell lines. In the former study, cancer cells were more sensitive than adenomatous cells to salicylate and this seems to be independent of ras status and COX-2 inhibition [74]. This may have an important clinical significance as the aim of any therapeutic strategy is to destroy abnormal tissue (cancer) while sparing the normal.

ALTERATION OF CELL CYCLE EQUILIBRIUM

Alterations in proliferation and differentiation

Cancer develops when the balance between proliferation and apoptosis is irrevocably disturbed. Shiff and colleagues [75] demonstrated that both sulindac and sulindac sulphide reduced the proliferation rate of HT29 cells, caused changes in their morphology, induced them to accumulate in the G0/G1 phase of the cell cycle and induced apoptosis. These responses are time dependent, reversible and are prominent when the higher concentrations of sulindac (1200 mcMol) and sulindac sulphide (150–200 mcMol) were used but sulfide was 6 times more potent than sulindac itself. Ricchi and colleagues [76] found that aspirin induced profound inhibition of cell replication (in human colorectal cancer Caco-2 cell line), this was associated with increased expression of differentiation markers and a decrease in the growth factor (insulin like growth factor II mRNA). Apoptosis was only induced in these cells when using a higher concentration (> 2 mM) of aspirin.

Alterations of tumour suppressor/promotor genes

Winde and colleagues [77] showed that sulindac reduces p53 protein levels and upregulates bcl-2 levels hence promoting apoptosis in rectal specimens taken from patients with FAP. This is in contradiction to the findings of Piazza and colleagues. Goldberg and colleagues [78] found Sulindac and SUS prevented HT29 cells from progressing from the G0/G1 into the S phase. This was associated with an initial rise then an abrupt decline in the level $p34^{cdc2}$ protein. These drugs also decreased mitotic cyclins (A and B1) increased $p21^{waf1/cip1}$, a tumour suppressor protein which has a regulatory role by inhibiting kinase activities of various cyclins, prevented phosphorylation, inhibited DNA synthesis, decreased the total level of pRB (retinoblastoma proto-oncogene), with a relative increase in pRB (unphosphorylated) and reduced the levels of mutant p53 [79, 80]. SUS has also been shown to: decrease the expression of cyclin B1 and E, increase the expression of cyclins D1,2 and 3, particularly, in the G1 phase of the cell cycle. SUS induced apoptotic cells expressed D and E-type cyclins but not cyclin B1 [81]. These changes are in keeping with the anti-proliferative action of SUS.

Promotion of apoptosis

Apoptosis is a genetically regulated form of cell death. It is thought to be progressively inhibited in colorectal cancer [82]. In terms of the relationship between NSAIDs and cancer, apoptosis may be involved with many of the possible mechanisms discussed in this review. Many of the available NSAIDs have been shown to induce apoptosis in colon cancer cell lines in a p53 and APC independent manner [71, 75, 78, 83].

Arrest of cell cycle

A major way by which NSAIDs may inhibit cell proliferation is to induce cell cycle quiescence in colonocytes; this could be achieved by diminished expression of several cyclins and cyclin dependent kinases and by increased expression of D-cyclins and the cyclin dependent kinase inhibitor $p21^{WAF1-16}$.

Arber and colleagues [84] treated parental rat intestinal cell line (IEC-18) and c-K-*ras* transformed derivative with sulindac sulphide (*ras* oncogenes are thought to regulate apoptosis [85], H-*ras* transformed cells are known to have suppressed apoptosis [86]). Although sulphide at a concentration of 100 mcMol/l caused inhibition of cell growth (5-fold greater than its precursor, sulindac), curiously they found that sulphide (50 mcMol/l) actually stimulated growth in two out of the three clones of transformed cells used. They found that sulphide inhibited cell growth and induced apoptosis in a time and dose-dependent manner at a lower concentrations in IEC-18 cells compared with c-K-*ras* cells ($P < 0.02$–0.05). Treated cells were arrested in the G0/G1 phase (79 versus 56% in control) but cells entered apoptosis throughout the cell cycle. The induction of apoptosis by sulphide seems to be independent of the p53 status of the cells. K-*ras* transformed cells expressed a higher basal level of cyclin-Dl than normal cells. Cyclins and cyclin-dependent kinases play an important role at transition points within the cell cycle [87, 88]. Transformed cells displayed a less marked decrease in cyclin D1 and cyclin D1 & E1 kinases than control. These findings point to the view that NSAIDs' chemopreventive action would be more useful before K-*ras* mutation occurs (i.e. early in colon carcinogenesis). Furthermore *ras* transformation increases the cyclo-oxygenase level and therefore sulindac action upon prostaglandin inhibition would be more effective before *ras* transformation.

Qiao and colleagues [89] found that sulphide reduced cell number, altered cell cycle distribution (increased the proportion of cells in G1 phase) and induced apoptosis in HT-29 and HCT-15 colon cancer cells. At 72 h, SUS reduced the expression of Ki67 (by 61%) and PCNA (by 45%) (an auxiliary protein to DNA polymerase delta and is a co-factor for DNA synthesis and is one of the most reliable markers of proliferation of colonic tissue).

OTHERS

Immune surveillance

The expression of antigens such as HLA class I and II is reduced in colonic adenomas, carcinomas and normal mucosa in cancer patients [90–92]. This process is thought to occur early in carcinogenesis leaving an avenue for intervention. PGE2 reduces the expression of HLA class II molecules at the level of gene transcription in colon cancer cell lines [93]. Furthermore NSAIDs can enhance, by transcriptional effect, the expression of HLA class II in the HT29 cancer cell line [94]. NSAIDs may aid the induction of the suppressed HLA genes in colorectal cancer hence restoring the ability to eliminate DNA damaged cells. Further research is needed.

Increased production of ceramide

Chan and colleagues [95] recently reported that colon cancer cells (HTC116 and SW 480) treated with NSAIDs had a dramatic increase in arachidonic acid concentration (2–5-fold). Arachidonic acid (AA) was found to be a potent inducer of apoptosis. AA stimulated sphingomylinase which catalyses the conversion of sphingomylin to ceramide (a potent mediator of apoptosis). They tested medium (125 mcMol/l) and high (200 mcMol/l) doses of SUS upon apoptosis using cyclohexeimide (CHX) as a protein synthesis inhibitor and found that CHX was able to inhibit apoptosis at the lower-dose of SUS but unable to inhibit apoptosis at the higher-dose, suggesting that SUS can kill cells by apoptosis at low concentration and by a non-specific toxic effect at higher-doses. They demonstrated that SUS dramatically stimulated the production of ceramide (however, it is unknown whether selective COX-2 inhibitors have the same effect). This opens a new avenue for research into the mechanisms under consideration.

Inhibition of nuclear factor kappa B(NF-kB)

NF-kB is a transcriptional eukaryotic factor family which plays a central role in various responses leading to host defence and rapid activation of gene expression [96]. The deregulation of NF-kB and its dependent genes have been associated with a spectrum of pathologies including cancer [97, 98]. Therefore, the activation of NF-kB is seen as a cancer key event in the signalling pathway leading to changes of expression of such genes [99]. These findings make NF-kB a potential candidate for the modulation of the pathological processes dependent on it. TNF and some chemotherapeutic drugs have been found to induce apopotosis more effectively if NF-kB is inhibited [100], suggesting that NF-kB suppresses apoptosis. Therefore, effective therapies dependent upon apopotosis induction may logically include NF-kB inhibition. Recently Mayo and colleagues [101] reported that NF-kB activation is required for preventing apoptosis induced by overexpression of oncogenic *ras* suggesting that NF-kB is important to the activities of certain oncogenes. Chen and colleagues [102] demonstrated that both aspirin (a COX inhibitor) and prostaglandin E2 (PGE2) (a downstream product of a reaction catalysed by COX) suppressed silica-induced NF-kB activation in rat alveolar macrophages, possibly by inhibiting NF-kB degrading enzyme IkB alpha. Similar findings on aspirin were reported by others [103, 104].

Kazmi and colleagues studied the effects of Tepoxalin (a dual inhibitor of 5-lipoxyoxygenase (5LO) and cyclooxygenase). This drug is an anti-inflammatory agent. It is thought to have tissue selective PGE2 inhibition properties, and hence no gastrointestinal ulcerogenic effect upon experimental animals [105]. This agent inhibited NF-kB activities in Jurkat and HeLa cells activated with PMA + ionomycin, H2O2 or TNF alpha (5 ng/ml). The observed activities were not related to PGE2 or leukotrienes production because 5LO and COX inhibitors did not interfere with NF-kB activation.

Kopp and colleagues [106] demonstrated that aspirin and sodium salicylate but not acetaminophen (not a PG inhibitor) nor indomethacin (a PG inhibitor) inhibited the transcription of cells (after transfection of cells with plasmid containing two kB sites) and luciferase activity in a dose dependent manner but a significant effect was only seen in higher-doses.

There is now a renewed interest in the role NF-kB plays in colon cancer and two early reports [107, 108], suggest that NF-kB inhibition is associated with increased apoptosis and decreased proliferation in colon cancer cell lines. We await with interest results from ongoing work on the role NF-kB plays in colon cancers in animal and man.

DISCUSSION

Although chemopreventive agents comprise a diverse group of compounds with different mechanisms of action, their ultimate ability to induce apoptosis may represent a unifying concept for their mechanism of action.

Although there is plentiful evidence that NSAIDs can act independently of COX inhibition, the role the latter play is far from being fully understood. If COX inhibition is unimportant, we are yet to establish why sulindac sulphone is less powerful than sulphide in causing cell death. If COX inhibition is essential for cell death, sulphone should have no effects which is obviously not the case. Further confusion exists as to whether COX1 does or does not have a role to play in NSAIDs chemopreventive effects.

The role *TP53* and other tumour modulating genes may play in NSAIDs' induced chemoprevention is unclear. Contradictory reports exist on whether the observed cellular changes is dependent or not on *ras* or *TP53* status.

The role NF-kB may play in mediating these changes is yet to be clarified, but it may represents an important target for manipulation aimed at modifying the course of disease. Early *in vitro* results are encouraging.

There are three potential uses of NSAIDs in colorectal cancer: chemoprevention, tumour shrinkage and as an adjuvant to cancer chemotherapy. The first has been the subject of this review, the second is supported by some reports from patients with FAP and the third is yet to be explored. Identifying the biochemical pathways by which NSAIDs exert their chemopreventive effects may reveal new targets for the design of antineoplastic and chemopreventive agents.

It is worth noting that our current knowledge does not suggest that NSAIDs can prevent cancer. In a follow up of 10 patients with adenomatous polyps treated with sulindac, cessation of treatment (or prolonged treatment) resulted in recurrence of polyps. One patient in this group developed invasive cancer despite being on NSAID [109]. If non-COX-2 biochemical pathways are involved, then the identification of these pathways should reveal promising new targets for the design of antineoplastic agents. Alternatively understanding how COX-2 inhibition leads to chemoprevention would provide the rational for the pursuit of new and even more potent anticancer drugs. Early results [110] indicate that 80 mg of aspirin inhibits the proliferation of colonocytes in man which may suggest that the beneficial effects of aspirin used by patients for its anticoagulant activities may include

chemoprevention which may in the long-term reduce the incidence of colorectal cancer.

The side-effects of NSAIDs unfortunately limit their value in colorectal chemoprevention and necessitate the development of safer agents.

The role of NSAIDs in the treatment of patients with mutated adenomatous polyposis coli gene who do not have colorectal polyposis, those with hereditary nonpolyposis colorectal cancer, and those with sporadic colorectal adenomas is yet to be clarified.

For cancer prevention, NSAIDs must be effective, have a reasonable lag phase to produce their effect, have acceptable or no side-effects and be cost effective. The research into the effects of NSAIDs is exciting but critical pieces of the jigsaw are still missing and we need more evidence in man before the wider use of NSAIDs in chemoprevention of colorectal cancer can be recommended.

In conclusion, the way forward in colorectal cancer chemoprevention is 4-fold; firstly to clarify and understand the mechanisms of action of chemopreventive agents in man. This may be explored by intralesional injection of chemopreventive agents into polyps or cancers and examining the results. Secondly to find a safe chemoprotective agent. Thirdly to examine whether NSAIDs have a beneficial effect upon patients with treated colorectal cancer (i.e. could a non steroidal be to colon cancer patients what aspirin had become to many stroke patients). Fourthly to conduct multicentre large randomised controlled trials with long follow-up of patients.

1. Shiff SJ, Rigas B. NSAIDs and colorectal cancer: evolving concepts of their chemopreventive actions. *Gastroenterology* 1997, **113**, 1992–1998.
2. Levy GN. Prostaglandin H synthase, NSAID and colon cancer. *FASEB J* 1997, **11**, 234–247.
3. Thun MJ, Namboodiri MM, Heath CW. Aspirin use and reduced risk of fatal colon cancer. *N Engl J Med* 1991, **325**, 1593–1596.
4. Giovannucci E, Egan KM, Hunter DJ, *et al*. Aspirin and the risk of colorectal cancer in women. *N Engl J Med* 1995, **333**, 609–614.
5. Waddell WR, Loughry RW. Sulindac for polyposis of the colon. *J Surg Oncol* 1983, **24**, 83–87.
6. Giardiello FMSR, Hamilton AJ, Krush S, *et al*. Treatment of colonic and rectal adenomas with sulindac in FAP. *New Engl J Med* 1993, **328**, 1313–1316.
7. Keller JJ, Offerhaus GJA, Goodman SN, *et al*. Change in apoptosis in patients treated with sulindac. *Gastroenterology* 1998, **114**(4), G2550.
8. Di Sario JA, Alberts DS, Tietze CC, *et al*. Sulindac induces regression and prevent progression of sporadic colorectal adenomas. *Gastroenterology* 1997, **112**, A555.
9. Van Stolk R, Budd GT, Kresty R, *et al*. Effect of sulindac sulfone on proliferation, apoptosis and polyps in a clinical trial in FAP with rectal polyps. *Gastroenterology* 1998, **114**, G2881.
10. Van Stolk R, Provencher K, Burke C, *et al*. Sulindac sulfone in patients with FAP. Poylp effect and tolerability from an open label extension trial. *Gastroenterology* 1998, **114**(4), G2882.
11. Takayama T, Katsuki S, Takahashi Y, *et al*. Aberrant crypt foci of the colon as precursors of adenoma and cancer. *N Engl J Med* 1998, **339**, 1277–1284.
12. Kune G, Kune S, Watson L. Colorectal cancer risk chronic illness, operations and medications. Case control results from the Melbourne colorectal cancer study. *Cancer Res* 1988, **48**, 4399–4404.
13. Rosenberg L, Palmer JR, Zauber AG, *et al*. A hypothesis: NSAIDs reduce the incidence of large bowel cancer. *J Natl Cancer Inst* 1991, **83**, 355–358.
14. Peleg II, Maibach HT, Brown SH, *et al*. Aspirin and NSAIDs use and the risk of subsequent colorectal cancer. *Arch Intern Med* 1994, **154**, 394–399.
15. Schreinemachers DM, Everson RB. Aspirin use, lung colon and breast cancer incidence in a prospective study. *Epidemiology* 1994, **5**, 138–146.
16. Muscat JE, Stellman SD, Wynder EL. NSAIDs and colorectal cancer. *Cancer* 1994, **74**, 1847–1854.
17. Logan RFA, Little J, Hawtin PG, Hardcastle JD. Effect of aspirin and NSAIDs on colorectal adenomas. Case control study of subjects participating in the Nottingham faecal occult blood screening programme. *Br Med J* 1993, **307**, 285–289.
18. Suh O, Mettlin C, Petrelli NJ. Aspirin use, cancer polyps and large bowel cancer. *Cancer* 1993, **72**, 1171–1177.
19. Greenberg ER, Baron JA, Freeman DH, *et al*. Reduced risk of large bowel adenomas among aspirin users. *Natl Cancer Inst* 1993, **85**, 1220–1224.
20. Thun MJ. NSAID use and decreased risk of gastrointestinal cancers. *Gastroenterol Clin North Am* 1996, **25**, 333–348.
21. Paganini-Hill A, Hsu G, Ross RK, *et al*. Aspirin use and incidence of large bowel cancer in a Californian retirement community. *J Natl Cancer Inst* 1991, **83**, 1182–1183.
22. Gann PH, Manson JE, Glynn RJ, *et al*. Low-dose aspirin and incidence of colorectal tumours in a randomised trial. *J Natl Cancer Inst* 1993, **85**, 1220–1224.
23. Sturmer T, Glynn RJ, Lee IM, *et al*. Aspirin use and colorectal cancer: post trial follow up data from the physicians' health study. *Ann Int Med* 1998, **128**, 713–720.
24. Powell SM, Zilz N, Beaser-Barclay Y, *et al*. Apc mutations occur early during colorectal tumourgenesis. *Nature* 1992, **359**, 235–237.
25. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996, **87**, 159–170.
26. Moser AR, Pitot HC, Dove WF. A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. *Science* 1990, **247**, 668–670.
27. Jacoby RF, Marshall DJ, Schlack S, *et al*. Genetic instability associated with adenoma to carcinoma progression in heridatory non polyposis colon cancer. *Gastroenterology* 1994, **109**, 73–82.
28. Barnes CJ, Lee M. Chemoprevention of spontaneous intestinal adenomas in the adenomatous polyposis coli Min mouse model with Aspirin? *Gastroenterology* 1998, **114**, 873–877.
29. Reddy BS, Rao CV, Rivenson A, *et al*. Inhibitory effect of Aspirin on azoxymethane-induced colon carcinogenesis in F 344 rats. *Carcinogenesis* 1993, **14**, 1493–1497.
30. Ahmen DJ. The genetic basis of colorectal cancer risk. *Adv Intern Med* 1996, **41**, 531–552.
31. Jacoby RF, Marshall DJ, Newton MA, *et al*. Chemoprevention of spontaneous intestinal adenomas in the Apc Min mouse model by the NSAID piroxicam. *Cancer Res* 1996, **56**, 710–714.
32. Boolbol SK, Dannenberg AJ, Chadburn A, *et al*. COX-2 overexpression and tumour formation are blocked by sulindac in a murine model of familial adenomatous polyposis. *Cancer Res* 1996, **56**, 2556–2560.
33. Beazer-Barclay Y, Levy DB, Moser AR, *et al*. Sulindac suppresses tumourgenesis in the Min mouse. *Carcinogenesis* 1996, **17**, 1757–1760.
34. Shibata H, Toyama K, Shiova H, *et al*. Rapid colorectal adenoma formation initiated by conditional targeting of the Apc gene. *Science* 1997, **278**, 120–123.
35. Rao CV, Rivenson A, Simi B, *et al*. Chemoprevention of colon carcinogenesis by sulindac, a NSAI agent. *Cancer Res* 1995, **55**, 1464–1472.
36. Strong HA, Warner NJ, Renwick AG, *et al*. Sulindac metabolism: The importance of an intact colon. *Clin Pharmacol Ther* 1985, **38**, 387–393.
37. Williams CS, DuBois RN. Prostaglandin endoperoxide synthase. Why two isoforms. *Am J Physiol* 1996, **270**, G393.
38. Nariswa T, Kusaka H, Yamazaki Y, *et al*. Relationship between blood plasma prostaglandin E2 and liver and lung metastases of colorectal cancer. *Dis Colon Rectum* 1990, **33**, 840–845.
39. Craven PA, Saito R, DeRubertis FR. Role of local prostaglandin synthesis in the modulation of proliferative activity of rat colonic epithelium. *J Clin Invest* 1983, **72**, 1365–1376.
40. Lupulescu A. Enhancement of carcinogenesis by prostaglandins. *Nature* 1978, **272**, 634–636.
41. Plescia OJ, Smith AH, Grinwich K. Subversion of immune system by tumour cells and the role of prostaglandins. *Proc Natl Acad Sci* 1975, **72**(5), 1848–1851.

42. O'Neill G, Hutchinson AF. Expression of mRNA for COX-1 and COX-2 in human tissues. *FEBS Lett* 1993, **330**, 156–160.
43. Rigas B, Goldman IS, Levine L. Altered eicosanoid levels in human colon cancer. *J Lab Clin Med* 1993, **122**, 518–523.
44. Qiao L, Kozoni V, Tsioulias GJ, *et al.* Selected eicosanoids increase the proliferation rate of human colon carcinoma cell lines and mouse colonocytes *in vivo*. *Biochim Biophys Acta* 1995, **1258**, 215–223.
45. Bortuzzo C, Hanif R, Kashfi K, Staiano-Coico L, Shiff SJ, Rigas B. The effect of leukotrienes B and selected HETEs on the proliferation of colon cancer cells.
46. Yang VW, Shields JM, Hamilton SR, *et al.* Size dependent increase in prostanoid levels in adenomas of patients with familial adenomatous polyposis. *Cancer Res* 1998, **58**, 1750–1753.
47. DuBois RN, Radhika A, Reddy BS, *et al.* Increased COX-2 levels in carcinogen induced colonic tumours. *Gastroenterology* 1996, **110**, 1259–1262.
48. Eberhart CE, Coffey RJ, Radhika A, *et al.* Upregulation of COX-2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 1994, **107**, 1183–1188.
49. Sano H, Kawahito Y, Wilder RL, *et al.* Expression of COX-1 and 2 in human colorectal cancer. *Cancer Res* 1995, **55**, 3785–3789.
50. Sano H, Kawahito Y, Wilder R, *et al.* Expression of COX-1 and COX-2 in human colorectal cancer. *Cancer Res* 1995, **55**, 3785–3789.
51. Tanka M, Nazkazawa S, Kiike M. Investigation of endogenous prostaglandin in DMH-induced colonic cancer in rats. *J Gastroenterol* 1985, **82**, 592–598.
52. Giardiediello F, Spannhake E, DuBois RN, *et al.* Prostaglandin levels in human colorectal mucosa: effects of sulindac in patients with FAP. *Dig Dis Sci* 1998, **43**, 311–316.
53. Hanif R, Pittas A, Feng Y, *et al.* NSAIDs inhibit the growth of colon cancer cell lines by a prostaglandin independent pathway. *Gastroenterology* 1995, **108**, A478.
54. Sheng H, Shao J, Morrow JD, *et al.* Modulation of apoptosis and bcl-2 expression by PGE-2 in human colon cancer cells. *Cancer Res* 1998, **58**, 362–366.
55. Oshima M, Dinchuk JE, Kargman SL, *et al.* Suppression of intestinal polyposis in Apc716 knockout mice by inhibition of COX-2. *Cell* 1996, **87**, 803–809.
56. Sheng GG, Shao J, Sheng H, *et al.* A selective COX-2 inhibitor suppresses the growth of H-*ras*-transformed rat intestinal epithelial cells. *Gastroenterology* 1997, **113**, 1883–1891.
57. Bos JL. Ras oncogenes in human cancer: a review. *Cancer Res* 1989, **49**, 4682–4689.
58. Andreyev HJ, Norman AR, Cunningham D, Oates JR, Clarke PA. K-*ras* mutations in patients with colorectal cancer: the multicentre RASCAL study. *J Natl Cancer Inst* 1998, **90**(9), 675–684.
59. Trujillo MA, Grewal HS, Sampliner RE, *et al.* NSAIDs in chemoprevention of colorectal cancer. At what cost? *Dig Dis Sci* 1994, **39**, 2260–2266.
60. Masferrer JL, Isakson PC, Seibert K. COX-2 inhibitors: a new class of anti-inflammatory agents that spare the gastrointestinal tract. In DuBois RN, Giardiello FM, eds. *NSAIDs, Eicosanoids and the Gastroenteric Tract*. Gastroenterology Clinics of North America 1996, **25**, 363–372.
61. Reddy BS, Rao CV, Seibert K. Evaluation of COX-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. *Cancer Res* 1996, **56**, 4566–4569.
62. Elder DJE, Halton DE, Hague A, Paraskeva C. Cellular and molecular effects of COX-2 selective NSAID on colorectal adenoma and carcinoma cell lines. *Gastroenterology* 1998, **114**(4), G2416.
63. Kawamori T, Rao CV, Seibert K, Reddy BS. Chemopreventive activity of celecoxib a specific cyclooxygenase-2 inhibitor, against colon carcinogenesis. *Cancer Res* 1998, **58**, 409–412.
64. Williams CS, Smalley W, DuBois RN. Aspirin use and potential mechanisms for colorectal cancer prevention. *J Clin Invest* 1997, **100**, 1325–1329.
65. McCartney SA, Vojnovic I, Farthing MJG, *et al.* NSAID induced fall in H29 cell viability correlates with potency to inhibit COX1: use of selective COX-2 inhibitors. *Gastroenterology* 1998, **114**(4), G2642.
66. Elder DJE, Paraskeva C. COX-2 inhibitors for colorectal cancer. *Nature Medicine* 1998, **4**(4), 392–393.
67. Elder DJE, Halton DE, Hague A, Paraskeva C. Induction of apoptotic cell death in human colorectal carcinoma cell lines by a COX-2 selective NSAID: independence from COX-2 protein expression. *Clin Can Res* 1997, **3**, 1679–1683.
68. Samaha HS, Kelloff GJ, Steele V, *et al.* Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate and 6-phenylhexyle isothiocyannate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. *Cancer Res* 1997, **57**, 1301–1305.
69. Thompson HJ, Briggs S, Paranka NS, *et al.* Inhibition of mammary carcinogenesis in rats by sulfone metabolite of sulindac. *J Natl Cancer Inst* 1995, **87**, 1259–1260.
70. Shen TY, Winter CA. Chemical and biological studies of indomethicin, sulindac and their analogues. *Adv Drug Res* 1977, **12**, 90–245.
71. Piazza GA, Rahm AL, Krutzsch M, *et al.* Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. *Cancer Res* 1995, **55**, 3110–3116.
72. Piazza GA, Alberts DS, Hixson LJ, *et al.* Sulindac sulfone inhibits azoxymethane-induced colon carcinogenesis in rats without reducing prostoglandin levels. *Cancer Res* 1997, **57**, 2909–2915.
73. Chiu CC, McEntee MF, Whelan J. Sulindac causes rapid regression of pre-existing tumours in Min/+ mice independent of prostaglandin biosynthesis. *Cancer Res* 1997, **57**, 4267–4273.
74. Elder DJE, Hague A, Hicks DJ, *et al.* Differential growth inhibition by the aspirin metabolite salicylate in human colorectal tumour cell lines: enhanced apoptosis in carcinoma and *in vitro* transformed adenoma relative to adenoma cell lines. *Cancer Res* 1996, **56**, 2273–2276.
75. Shiff SJ, Qiao L, Tsai LL, Rigas B. Sulindac sulfide, an aspirin like compound inhibits proliferation, causes cell cycle quiescence and induces apoptosis in HT-29 colon adenocarcinoma cells. *J Clin Invest* 1995, **96**, 491–503.
76. Ricchi P, Pignata S, Di-Popolo A, *et al.* Effect of aspirin on cell proliferation and differentiation of colon adenocarcinoma Caco-2 cells. *Int J Cancer* 1997, **73**, 880–884.
77. Winde G, Schmid KW, Muller O, *et al.* Influence of low dose sulindac on p53 protein, on bcl-2 protein and on TGF alpha in rectal mucosal biopsies of cancer prone patients (FAP) in chemopreventive trial. *Eur J Cancer* 1997, **33**(Suppl.), S161–S717.
78. Goldberg Y, Nassif II, Pittas A, *et al.* The antiproliferative effect of sulindac and sulindac sulfide on HT29 colon cancer cells: alterations in tumour suppressor and cell cycle regulatory proteins. *Oncogene* 1996, **12**, 893–901.
79. El-Dieiry WS, Harper JW, O'Connor PM, *et al.* WAF1/CIP1 is induced in p53 mediated G1 arrest and apoptosis. *Cancer Res* 1994, **54**, 1169–1174.
80. El-Dieiry WS, Tokino T, Velculescu VE, *et al.* WAF1: a potential mediator of p53 tumour suppression. *Cell* 1993, **75**, 817–825.
81. Qiao L, Shiff SJ, Rigas B. Sulindac sulfide alters the expression of cyclin proteins in HT-29 colon adenocarcinoma cells. *Int J Cancer* 1998, **76**, 99–104.
82. Bedi A, Pasricha PJ, Akhtar AJ, *et al.* Inhibition of apoptosis during development of colorectal cancer. *Cancer Res* 1995, **55**, 1811–1816.
83. Shiff SJ, Koutsos MI, Qiao L, *et al.* NSAIDs inhibits the proliferation of colon adenocarcinoma cells: effects on cell cycle and apoptosis. *Exp Cell Res* 1996, **222**, 179–188.
84. Arber N, Han EK, Sgambato A, *et al.* A K-*ras* oncogene increases resistance to sulindac-induced apoptosis in rat enterocytes. *Gastroenterology* 1997, **113**, 1892–1900.
85. Arends MJ, McGregor AH, Toft NJ, *et al.* Susceptibility to apoptosis is differentially regulated by c-myc and mutated Ha-ras oncogenes and is associated with endonuclease availability. *Br J Cancer* 1993, **68**, 1127–1133.
86. Rak J, Mitsuhashi Y, Erdos V, *et al.* Massive programmed cell death in intestinal epithelial cells induced by three dimensional growth conditions suppression by mutant c-H-*ras* oncogene expression. *J Cell Biol* 1995, **131**, 1587–1598.
87. Van den Heuvel S, Harlow E. Distinct roles for cyclin dependent kinases in cell cycle control. *Science* 1993, **262**, 2050–2054.

88. Pines J, Hunter T. Isolation of human cyclic cDNA:Evidence for cyclin mRNA and protein regulation in the cell cycle and for interaction with p34cdc2. *Cell* 1989, **58**, 833–846.
89. Qiao L, Shiff SJ, Rigas B. Sulindac sulfide inhibits the proliferation of colon cancer cells: diminished expression of the proliferation markers PCNA and Ki-67. *Cancer Letters* 1997, **115**, 229–234.
90. McDougall CJ, Ngoi SS, Goldman IS, *et al*. Reduced expression of HLA class I and II antigens in colon cancer. *Cancer Res* 1990, **50**, 8023–8027.
91. Tsioulias G, Triadafilopoulos G, Godwin TA, *et al*. Loss of colonic HLA antigens in FAP. *Cancer Res* 1992, **52**, 3449–3452.
92. Tsioulias G, Godwin TA, Goldstein MF, *et al*. Expression of colonic HLA class I antigens in sporadic adenomas and histologically normal mucosa of the colon. *Cancer Res* 1993, **53**, 2374–2378.
93. Arvind P, Papavassilio ED, Tsiolias GJ, *et al*. PGE2 downregulates the expression of HLA DR antigen in human colon adenocarcinoma cell lines. *Biochemistry* 1995, **34**, 5604–5609.
94. Arvind P, Papavassilio ED, Goldin E, *et al*. Aspirin and aspirin like drugs induce the expression of HLA DR in HT29 colon adenocarcinoma cells. *Int J Oncol* 1996, **8**, 1207–1211.
95. Chan TA, Morin P, Vogelstein B, *et al*. Mechanisms underlying NSAID mediated apoptosis. *Proc Natl Acad Sci* 1998, **95**, 681–686.
96. Singh S, Aggarwal BB. Activation of transcription factor NFKB is suppressed by Curcumin (Diferulolymethane). *J Biliological Chemist* 1995, **270**(42), 24995–24999.
97. Siebenlist U, Franzo G, Brown K. Structure, regulation and function of NF-kB. *Ann Rev Cell Biol* 1994, **10**, 405–455.
98. Baeuerle PA, Henkel T. Function and activation of NF-kB in the immune system. *Ann Rev Immunol* 1994, **12**, 141–179.
99. Baeuerle PA, Henkel T. Function and activation of NF-kB in the immune system. *Ann Rev Immunol* 1994, **12**, 141–179.
100. Van Antwerp DJ, Martin SJ, Verma IM, Green DR. Inhibition of TNF-induced apoptosis by NF-kB. *Trends in Cell Biology* 1998, **8**, 107–111.
101. Mayo MW, Wang CY, Cogswell PC, *et al*. Requirement of NF-kappa B activation to suppress p53-independent apoptosis induced by oncogenic Ras. *Science* 1997, **278**, 1812–1815.
102. Chen F, Sun S, Kuhn D, *et al*. Involvement of NF-KB in silica-induced cyclooxygenase II gene expression in rat alveolar macrophages. *Am J Physiol* 1997, **272**, L779–L786.
103. Kipp E, Ghosh S. Inhibition of NF-kappa-B by sodium salicylate and aspirin. *Science* 1994, **265**, 956–959.
104. Schwenger P, Bellosta P, Vieter I, *et al*. Sodium salicylate induces apoptosis via p38 mitogen activated protein kinase but inhibits TNF induced c-Jun N-terminal kinase/stress activated protein kinase activation. *Proc Natl Acad Sci USA* 1997, **94**, 2869–2873.
105. Wallace JL, McCafferty DM, Carter L, *et al*. Tissue selective inhibition of prostaglandin synthesis in rat by tepoxalin:anti-inflammatory without gastropathy? *Gastroenterology* 1993, **105**, 1630–1636.
106. Kopp E, Ghosh S. Inhibition of NF-kB by sodium salicylate and aspirin. *Science* 1994, **265**, 956–959.
107. Kubba AK, Watson D, Ansari B, *et al.* Curcumin induces apoptosis,causes cell cycle arrest and down regulates NF-kB signal in colorectal cancer cells. *Gut* 1999, **44**(Suppl. 1), A91 (W364).
108. Ansari B, Kubba AK Campbell FC, *et al.* Sulindac sulfide TNF signaling NF-kB inhibition and colorectal cancer. *Gut* 1999, (Suppl. 1).
109. Matsuhashi N, Nakajima A, Suzuki A, *et al*. Long-term outcome of sulindac therapy for sporadic adenomatous colorectal polyps. *Gastroenterology* 1998, **114**(4), G2637.
110. Aoki T, Boland CR, Brenner DE. Aspirin modulation of malignant biomarkers in rectal mucosa of high risk subjects. *Gastroenterology* 1996, **110**, A484.