Non-steroidal anti-inflammatory drugs with activity against either cyclooxygenase 1 or cyclooxygenase 2 inhibit colorectal cancer in a DMH rodent model by inducing apoptosis and inhibiting cell proliferation

W A Brown, S A Skinner, C Malcontenti-Wilson, D Vogiagis, P E O'Brien

Abstract

***Background*—Standard non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of colorectal cancer by 40–60% but the mechanism by which this occurs is uncertain. Selective cyclooxygenase2inhibitorsarepotentiallyidealchemopreventive agents as they are less toxic than standard NSAIDs. No study has compared the efficacy of these drugs at clinically relevant doses in a tumour model.**

***Aims*—To assess the efficacy of a range of NSAIDs with varying activity against the two cyclooxygenase isoforms in a rodent colorectal carcinogen model at anti-inflammatory doses and to explore the effect of NSAIDs on the rate of tumour apoptosis and proliferation.**

***Methods*—Colorectal tumours were induced in six week old Sprague-Dawley rats with five weekly doses of 1,2 dimethylhydrazine. Test agents were: indomethacin 2 mg/kg/day, meloxicam 0.6 mg/kg/day, celecoxib 6 mg/kg/day, and sulindac sulphone 40 mg/kg/day. Sulindac was tested at its chemoprotective dose of 20 mg/kg/day. After 23 weeks the number and volume of tumours per animal were recorded. Histology was performed. Tumour apoptosis was quantified on haematoxylin-eosin sections. Tumour proliferation was quantified using an immunohistochemical stain for bromodexoyuridine incorporation.**

***Results*—Test agents effectively reduced the number and volume of tumours developing in the treatment period. In all groups there was an increase in the rate of tumour apoptosis and a reduced rate of proliferation.**

***Conclusions*—These data suggest that the chemopreventive effect of NSAIDs is independent of their cyclooxygenase inhibitory profile. One potential mechanism for their action may be through induction of apoptosis and inhibition of proliferation.**

(*Gut* 2001;**48**:660–666)

Keywords: non-steroidal anti-inflammatory drugs; chemoprevention; colorectal cancer; apoptosis; bromodexoyuridine

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Accepted for publication
22 June 2000

Non-steroidal anti-inflammatory drugs (NSAIDs) reduce the risk of colorectal cancer by 40–60% in animal carcinogen models,[1–3] human observational studies,[4] and familial adenomatous polyposis cohort studies.[5] NSAIDs exert their therapeutic anti-inflammatory and antipyretic actions by inhibition of the enzyme cyclooxygenase and subsequent production of prostaglandins.[6 7] Two isoforms of cyclooxygenase have recently been identified: cyclooxygenase 1 (COX-1) is largely responsible for the constitutive production of prostaglandins and cyclooxygenase-2 (COX-2) is induced by proinflammatory stimuli.[8 9]

Based on the ability of NSAIDs to inhibit colorectal cancer, it is tempting to infer a role for cyclooxygenase and prostaglandins in the genesis of this disease. Prostaglandins have well established roles in tumour promotion and angiogenesis,[10] and both prostaglandins (particularly PGE_2) and COX-2 mRNA are overexpressed in human and rodent colorectal tumours.[11–14]

Immunohistochemical studies of colorectal tumours from both animal and human studies suggest that COX-2 protein is found in neoplastic epithelial cells[11 15] as well as in colonic interstitial tissue,[14] particularly interstitial macrophages.[16 17] In addition, COX-2 protein is detected in vascular endothelial cells, fibroblasts, and cancer cells.[11] COX-1 protein may also be detected immunohistochemically in neoplastic epithelial cells, inflammatory mononuclear cells, vascular endothelial cells, and fibroblasts.[11]

COX-2, rather than COX-1, appears to be the isoform that NSAIDs are targeting in colorectal cancer, as only one study (in carcinogen induced rodent colon tumours) has shown COX-1 to be upregulated.[18] Selective COX-2 inhibitors could prove potentially ideal agents for the prevention of colorectal cancer, given that they selectively target the relevant isoform of cyclooxygenase and have less toxicity than standard NSAIDs.[19]

Studies in rodent carcinogen models have found that these agents reduce the number of

Abbreviations used in this paper: NSAIDs, non-steroidal anti-inflammatory drugs; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; DMH, 1,2 dimethylhydrazine; ACF, aberrant crypt foci; Brd-U, bromodeoxyuridine; PBS, phosphate buffered saline; FCS, fetal calf serum.

Table 1 Test agents used: activity against cyclooxygenase and doses used. Comparison of the relative COX-1 and COX-2 activity of each test agent, as well as the methodological information regarding doses and number of animals used

Test agent	Activity against cyclooxygenase[a] ID_{50} COX-2/ID_{50} COX-1	n	Dose (mg/kg/day)
Sulindac	30	10	20
Indomethacin	22–60	10	2
Meloxicam	0.07–0.3	10	0.6
Celecoxib	<0.0008	8[b]	6
Sulindac sulphone	—	10	40

[a]While absolute ID_{50} values vary according to the cell system used, the ratio stays relatively constant, as does ranking.[17 22 51–55]
[b]Two animals from this group died after randomisation, one while being anaesthetised for blood collection, and the other had a large gastroduodenal tumour eroding his liver and hepatic vessels.

aberrant crypt foci (ACF)[20–22] and colorectal tumours.[23 24] When doses of selective COX-2 inhibitors based on the therapeutic anti-inflammatory dose were used, only a modest 20–25% reduction in the number of ACF was seen.[20–22] When the dose of the test agent was increased 10-fold, a more impressive 40% reduction in the total number of ACF was noted.[20 22] Only these supranormal doses have been tested in tumour models,[23 24] and while these doses are effective in animals, doses of this magnitude are unlikely to be practical for potential clinical applications.

Although selective COX-2 inhibitors carry fewer gastrointestinal side effects than standard NSAIDs,[19] they do have side effects, particularly affecting the renal tract[25] and the female reproductive system.[26] Toxicity is more likely when the dose is increased. Chemopreventive agents ideally have no toxicity. Therefore, it would be preferable if normal therapeutic anti-inflammatory doses of selective COX-2 inhibitors could be used.

We have previously compared the effect on the development of carcinogen induced ACF in animals treated with equivalent anti-inflammatory doses of predominantly COX-1 inhibitors (indomethacin and sulindac), a selective COX-2 inhibitor (meloxicam), and a highly specific COX-2 inhibitor (celecoxib), and found equivalent inhibition in all treatment groups.[27] The fact that the chemoprotective power of NSAIDs is not reliant on the cyclooxygenase inhibitory profile suggests that the effect of NSAIDs in colorectal cancer is not solely modulation of COX-2 and the cyclooxygenase-prostaglandin pathway.

This theory is supported by the efficacy of non-cyclooxygenase active agents such as sulindac sulphone.[2 28] Further supportive evidence comes from studies which demonstrate that while sulindac effectively inhibits tumorigenesis, tumour prostaglandin levels are unchanged.[29 30] One suggested mechanism for the action of these drugs is induction of apoptosis and regulation of cell proliferation.[31]

We have designed a study comparing NSAIDs with varying activity against the two cyclooxygenase isoforms at equivalent, clinically relevant, anti-inflammatory doses in a rodent carcinogen induced colorectal tumour model. We have compared their effects on tumour number and load, and quantified the effect on tumour apoptosis and proliferation.

Methods

TEST AGENTS

The agents chosen, dose used, and relative activity against different cyclooxygenase isoforms are shown in table 1. All drugs were pure substances.

COX-1 inhibitors used were indomethacin (Sigma Chemical Co, St Louis, Missouri, USA) and sulindac (Merck Sharp and Dohme, Sydney, Australia). Meloxicam (Boehringer Ingelheim, Sydney, Australia) is a selective COX-2 inhibitor. Celecoxib (Searle, St Louis, Missouri, USA) is a highly specific COX-2 inhibitor. Sulindac sulphone (Merck Sharp and Dohme) has no known anti-cyclooxygenase activity.

The doses of sulindac, sulindac sulphone, and indomethacin were based on previous work done by our group.[1–3] The normal adult human dose for indomethacin is 1–3 mg/kg/day and for sulindac 6 mg/kg/day (company information). The effective chemopreventive dose for indomethacin was therefore in the normal therapeutic range and calculations for COX-2 inhibitors were based around equivalency for this drug.

Sulindac may be considered a standard chemopreventive agent which has been well studied in both human and animal experiments. The dose we found to be effective was similar to that of other groups.[23] Therefore, the drug was included at its effective dose, even though it was outside the normal clinical range, to allow for comparison at a so-called "effective chemopreventive" dose.

The dose of meloxicam was calculated to be an equivalent anti-inflammatory dose to indomethacin on the basis of granuloma pouch studies (personal correspondence, Boehringer Ingelheim).

The dose of celecoxib was based on the published anti-inflammatory dose (Searle Monsanto, Chicago, Illinois, USA). The mean (SEM) serum level achieved with this dose of celecoxib at the end of the treatment period was 0.67 (0.07) μg/ml (assay kindly performed by Searle Monsanto). The reported minimum serum level required for maximal anti-inflammatory effect (therapeutic level) in rats is 0.3 μg/ml.[22]

TUMOUR MODEL

Induction of colonic tumours

Primary colonic tumours were induced with 1,2 dimethylhydrazine (DMH), as described previously.[29] DMH (Fluka Chemical Company, Castle Hill, NSW, Australia) is dissolved in isotonic saline and the pH adjusted to 7.0 with concentrated NaOH. Each animal received five weekly oral doses of DMH at 30 mg/kg per dose.

Treatment groups

Rats were randomised to a control group (n=12) or to treatment with test agents (table 1). Drugs were administered in food; control animals received food containing vehicle alone. The amount eaten was monitored, and the mixture adjusted to ensure delivery of the required daily dose. Treatment commenced on the day after the first dose of DMH and

continued for 23 weeks. A group of animals (n=10) not treated with DMH and who received the same food as control animals was also included. No tumours developed in these animals, and they will not be discussed further.

Tumour identification and measurement

One hour prior to colon removal, animals were injected intraperitoneally with the thymidine analogue bromodeoxyuridine (Brd-U) (Sigma-Aldrich, St Louis, Missouri, USA) at a dose of 200 mg/kg. They were anaesthetised with intraperitoneal pentobarbitone sodium 60 mg/kg (Boehringer Ingleheim, Sydney, Australia) and the colon removed via a midline laparotomy. The colons were opened along the mesenteric border, washed with isotonic saline, and pinned flat. The position, number, and volume of each tumour was recorded. The colons were fixed in 10% neutral buffered formalin (Sigma Scientific Co., St Louis, Missouri, USA) for 16–20 hours. Tumour tissue was processed, and embedded in paraffin. Sections of tissue (3 μm) were stained with haematoxylin and eosin for histological confirmation. Histological analysis was performed by two observers. Both observers were blinded to the treatment group.

Assessment of the rate of apoptosis within tumours

The rate of apoptosis in haematoxylin and eosin stained tumour tissue was measured using a modification of the technique described by Samaha and colleagues.[32] A sample of tumours from each group was studied with the observer blinded to the treatment group. Both the number of apoptotic cells and total number of tumour cells were counted in five random high power light microscopy fields (×1000 magnification under oil emersion) (Olympus CK2). The percentage of cells with apoptotic features was then calculated. The criteria used to recognise apoptotic cells were: shrunken size, loss of contact with surrounding tissue (at times forming the classically described halo), and nuclear condensation (fig 1).[31 33] This was the only phase of apoptosis that we were able to consistently recognise. Apoptotic bodies were not counted, as in this model it was difficult to distinguish them confidently from intraepithelial lymphocytes or debris resulting from formalin fixation.

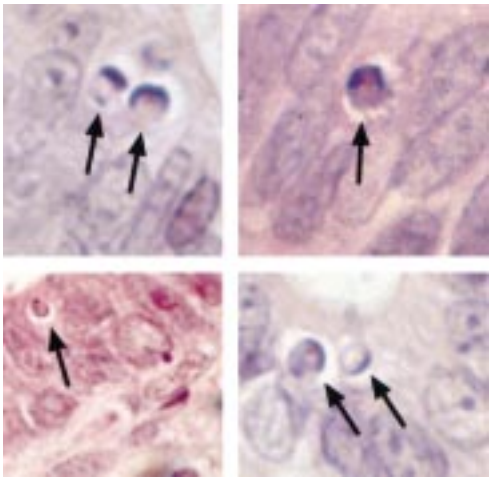

Figure 1 Photomicrograph of rodent colorectal tumour (×1000 magnification; oil emersion). Apoptotic tumour cells were recognised by their shrunken size, loss of contact with surrounding tissue (at times forming the classically described halo), and nuclear condensation. Arrows indicate examples of apoptotic tumour cells.

Assessment of the rate of proliferation within the tumour tissue

The rate of proliferation within tumour tissue was determined using an immunohistochemical stain for Brd-U. A sample of tumours from each group was studied with the observer blinded to the treatment group. Sections of paraffin embedded tissue (3 μm) were deparaffinised and taken to water through serial alcohols and washed in phosphate buffered saline (PBS) (pH 7.2). Sections were then placed in Antigen Retrieval Solution, pH 6.0 (Dako, Botany, NSW, Australia), and heated by microwave at 800 W for a total of 10 minutes. Endogenous peroxidases were blocked with 3% H_2O_2 in methanol for 15 minutes. This and all further incubations were carried out at room temperature. Non-specific binding was blocked with 20% fetal calf serum (FCS) in PBS for 30 minutes. Sections were incubated with primary antibody mouse anti-Brd-U (Dako, Botany, NSW, Australia) diluted 1:20 in 1% FCS in PBS. The secondary antibody used was peroxidase conjugated goat anti-mouse IgG (Dako, Botany, NSW, Australia) diluted 1:200 in 1% FCS in PBS. Incubation for each antibody step was for 30 minutes. Reaction products were amplified using DAB immunopure metal enhanced concentrate (Laboratory Supply, Milperra, NSW, Australia) diluted 1:10 in DAB phosphate buffer solution (Laboratory Supply, Milperra, NSW, Australia) for 10 minutes. After washing in tap water, nuclei were counterstained with haematoxylin for 30 seconds. The number of Brd-U positive cells (stain brown) and the total number of cells per high power field (×1000 magnification with oil emersion) were counted under light microscopy (Olympus CK2) in five random fields, and the percentage of cells in the S phase was calculated.

STATISTICAL ANALYSIS

The significance of differences in tumour number and volume per animal between treatment groups was determined via ANOVA with post hoc analysis by the method of least significant difference (LSD). Square root transformation of the volume data was required after exclusion of one statistical outlier from each of the meloxicam, celecoxib, and sulindac groups. The significance of differences in the percentage of apoptotic cells and proliferating cells between control and treatment groups was determined by two tailed Student's *t* tests of log transformed data. The statistical software package SPSS v 8.0 for windows (SPSS inc., Chicago, Illinois, USA) was used to analyse all data. A p value ≤ 0.05 was considered to be significant in all studies. All results are reported as mean (SEM).

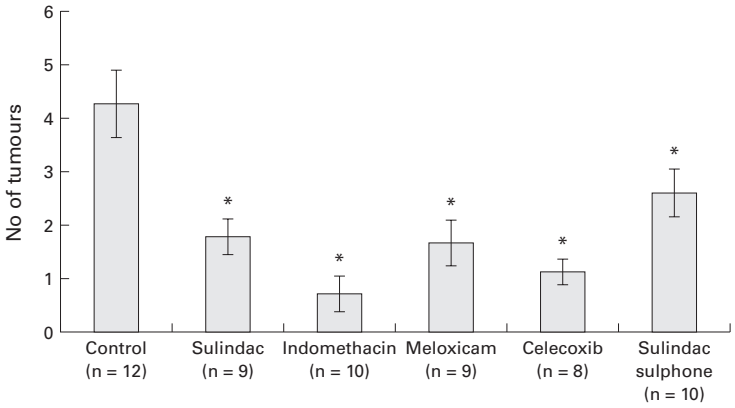

*Figure 2 Mean (SEM) number of tumours developing per animal over 23 weeks was significantly reduced compared with control animals in all treatment groups. *p<0.05.*

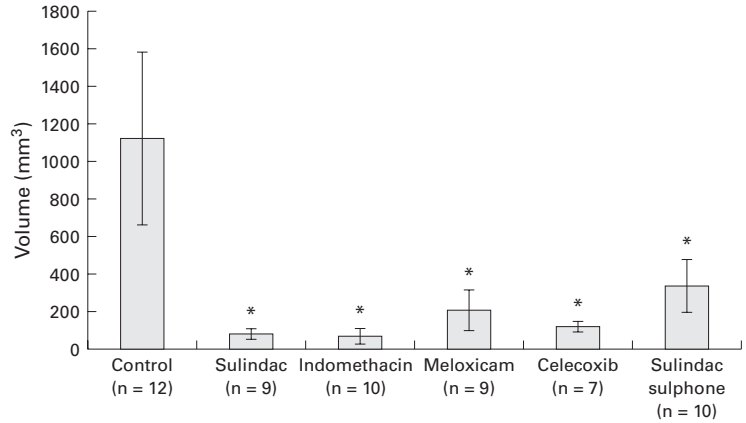

*Figure 3 Mean (SEM) volume of tumour (tumour load) developing per animal over 23 weeks was significantly reduced compared with control animals in all treatment groups. *p<0.05.*

Results

TUMOUR NUMBER

The mean number of tumours developing in control animals over the 23 week treatment period was 4.25 (0.64). This number was significantly reduced with all test agents, regardless of their COX inhibitory profile (fig 2). Of animals treated with predominantly COX-1 inhibitors, indomethacin treated animals developed a mean of 0.7 (0.33) tumours, an 83.5% reduction (p<0.001), and sulindac treated animals developed a mean of 1.9 (0.31) tumours, a 55.3% reduction (p<0.001). Of animals treated with predominantly COX-2 inhibitors, meloxicam treated animals developed a mean of 2.0 (0.49) tumours, a 52.9% reduction (p<0.001), and celecoxib treated animals developed a mean of 1.13 (0.23) tumours, a 73.5% reduction (p<0.001). Animals treated with sulindac sulphone developed a mean of 2.6 (0.45) tumours, a 38.8% reduction (p=0.009).

Multiple comparison testing showed that sulindac sulphone was significantly less effective than celecoxib (p=0.03) and indomethacin (p=0.004).

When the number of tumours was considered by histological type, we found that 37.3% of tumours in control animals were adenomas and 62.7% adenocarcinomas. This was significantly altered in animals treated with indomethacin and celecoxib, as no adenomas were found in these groups. In the other treatment groups the ratio of adenomas to adenocarcinomas was not significantly altered. In the sulindac group, 21.1% were adenomas and 78.9% adenocarcinomas. In the meloxicam group, 40.0% were adenomas and 60.0% adenocarcinomas. In the sulindac sulphone group, 42.3% were adenomas and 57.7% adenocarcinomas.

TUMOUR VOLUME

The mean volume of tumour (tumour load) developing per animal in the control group was 1119.5 (459.7) mm^3. This was significantly reduced in all treatment groups regardless of their cyclooxygenase inhibitory profile (fig 3). In the group treated with predominantly COX-1 inhibitors, indomethacin treated animals developed a mean of 59.0 (40.0) mm^3 tumour per animal, a 95% reduction (p<0.001), and sulindac treated animals developed a mean of 77.1 (32.1) mm^3, a 93.1% reduction (p=0.001). In the group treated with predominantly COX-2 inhibitors, meloxicam treated animals developed a mean of 201.8 (104.2) mm^3, an 82% reduction (p=0.002), and celecoxib treated animals developed a mean of 113.3 (29.9) mm^3, a 90% reduction (p=0.007). Animals treated with sulindac sulphone developed a mean of 324.6 (140.2) mm^3, a reduction of 71% (p=0.029).

Multiple comparisons between groups showed no one drug to be statistically more effective than another.

RATE OF APOPTOSIS PER TUMOUR

Mean percentage of apoptotic cells recognised by our criteria was 1.16 (0.10)% in tumours from control animals: 42 of the total 51 tumours in this group were examined.

The rate of apoptosis was significantly increased in tumours of animals treated with all test agents. Of the tumours developing in animals treated with predominantly COX-1 inhibitors, indomethacin treated animals (five of a total of seven tumours studied) had 3.14 (0.55)% apoptotic cells, a 2.7-fold increase (p<0.001), and those treated with sulindac (13 of a total of 19 studied) had 4.26 (0.47)% apoptotic cells, a 3.7-fold increase (p<0.001). In tumours developing in animals treated with predominantly COX-2 inhibitors, meloxicam treated animals (16 of a total of 20 tumours studied) had 3.03 (0.32)% apoptotic cells, a 2.6-fold increase (p<0.001), and those treated with celecoxib (eight of a total of nine tumours studied) had 2.57 (0.32)% apoptotic cells, a 2.2-fold increase (p<0.001). In tumours developing in animals treated with sulindac sulphone (23 of a total of 26 tumours studied), there were 2.29 (0.19)% apoptotic cells, a 2.0-fold increase (p<0.001).

The relative rates of apoptosis between adenomas and adenocarcinomas are shown in fig 4.

RATE OF PROLIFERATION PER TUMOUR

In tumours developing in control animals (11 tumours studied) there was a mean of 32.2 (3.2)% of cells in the S phase, as calculated

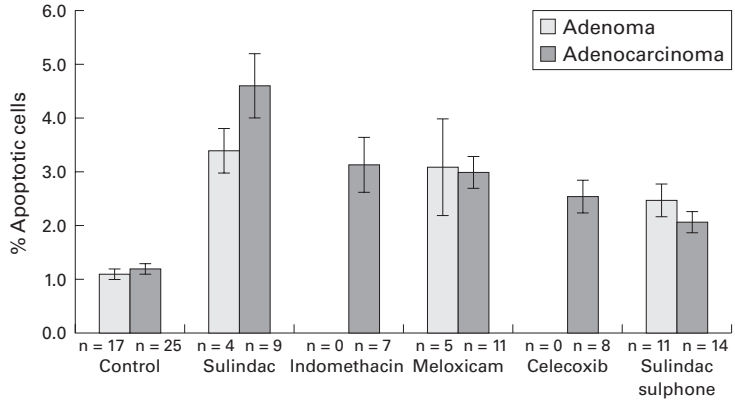

Figure 4 Mean (SEM) rate of apoptosis per tumour in all treatments was increased compared with the mean rate of apoptosis per tumour in control animals. Within treatment groups there was no significant difference in the mean rates of apoptosis according to their histology.

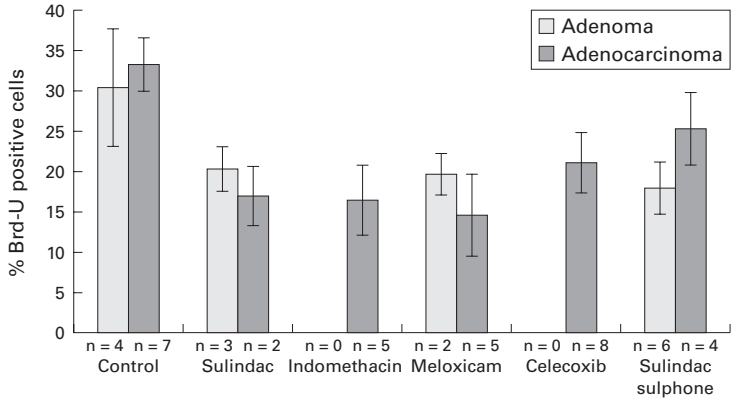

Figure 5 Mean (SEM) rate of cells in the S phase (proliferation index) per tumour in all treatments was decreased compared with the mean proliferation index per tumour in control animals. Within treatment groups, there was no significant difference in the mean proliferation index according to their histology.

from Brd-U labelling. The rate of proliferating cells was reduced in tumours of animals, regardless of the treatment group. Of the tumours of animals treated with predominantly COX-1 inhibitors, indomethacin treated animals (five tumours studied) had a mean of 16.5 (4.2)% of cells in the S phase, a 48.8% reduction (p=0.01), and sulindac treated animals (five tumours studied) 19.0 (2.1)%, a 40.8% reduction (p=0.024). Of the tumours of animals treated with predominantly COX-2 inhibitors, meloxicam treated animals (seven tumours studied) had a mean of 16.1 (1.9)% of cells in the S phase, a 49.9% reduction (p=0.002), and celecoxib treated animals (eight tumours studied) 21.2 (3.8)%, a 34.3% reduction (p=0.036). In tumours of animals treated with sulindac sulphone (10 tumours studied), there was a mean of 21.0 (2.8)% of cells in the S phase, a 34.6% reduction (p=0.024). The variance in the tumour numbers studied is due to both availability of tumour tissue and difficulty in staining tissue stored in 50% ethanol.

The relative rates of proliferation between adenomas and carcinomas in each treatment group are shown in fig 5.

Discussion

In this model of colon cancer, therapeutic anti-inflammatory doses of COX-2 inhibitors were as effective as a therapeutic dose of indomethacin, an effective chemopreventive dose of sulindac (three times the normal anti-inflammatory dose), and the non-cyclooxygenase active drug sulindac sulphone. Clinically, this has important implications as COX-2 selective drugs at standard anti-inflammatory doses are better tolerated than standard NSAIDs,[19] making them potentially better chemopreventive agents.

This is the first study to examine celecoxib and meloxicam at therapeutic anti-inflammatory doses in a carcinogen induced rodent tumour model. In the previous study of celecoxib at supranormal doses, there was a more profound effect on mean tumour number with 98% reduction compared with 73% reduction in our study. However, the degree of inhibition of tumour volume was similar with 87% reduction when supranormal doses of celecoxib were used and 90% reduction in our study.[23]

In ACF studies, therapeutic anti-inflammatory doses of celecoxib either have no effect[22] or a modest effect (22% reduction) (Brown, paper submitted). With supranormal doses however a 41% reduction in the number of ACF was noted.[22] Therefore, it is possible that therapeutic anti-inflammatory doses of highly selective COX-2 inhibitors are exerting their inhibitory effect later in the stepwise pathway of colorectal carcinogenesis (adenomas and carcinomas) whereas supranormal doses are having an effect at an earlier stage.

No adenomas developed in animals treated with either indomethacin or celecoxib. In this model a subtype of colorectal tumour has been described which does not seem to have a preceding polyp phase.[34] It is possible that NSAIDs are preventing tumours arising from the classic polyp-carcinoma sequence but are only able to inhibit tumours from this alternate pathway. This would explain the absence of adenomas and reduced number and volume of adenocarcinomas. Further studies are underway to test this hypothesis further.

From the point of view of understanding the mechanisms underlying colorectal carcinogenesis, these data add further weight to the contention that NSAIDs are functioning in a novel fashion, and that regulation of apoptosis and cell proliferation is a key end point.

In normal tissue, cell replication must be matched by cell death to maintain tissue homeostasis. The process of tumorigenesis in the colon is characterised by a stepwise increase in resistance to apoptosis followed by an increase in proliferation.[35 36] These data indicate that the effectiveness of NSAIDs against colorectal cancer can be explained, at least in part, by restoration of this critical balance between the rates of apoptosis and proliferation.

It has previously been demonstrated in this model[29] and in the *min*/+ mouse[30] that sulindac inhibits colorectal tumour development without reducing tumour prostaglandin levels. This suggests therefore that induction of tumour cell apoptosis and reduction in proliferation is not dependent on inhibition of prostaglandin

production. However, it is possible that cyclooxygenase inhibition may have other sequelae.

Regulation of the cyclin D/cdk4&6 checkpoint seems to be a critical step in colorectal carcinogenesis as it controls the length of time a cell spends in G1.[37] Inhibition of cyclooxygenase causes build up of arachidonic acid which in turn is converted to syringomyelin and finally ceramide.[38] Ceramide has been shown to not only regulate the cyclin D/cdk4&6 checkpoint by dephosphorylation of Rb[39] but also to separately induce apoptosis by a bcl-2 dependent mechanism.[40]

Inhibition of cyclooxygenase may also lead to positive feedback to the upstream regulators of cyclooxygenase transcription. Transcription of other related genes such as c-myc, bcl-2, and bax[41] could also be affected.

However, several lines of evidence suggest that the ability of NSAIDs to inhibit colorectal tumorigenesis is not dependant on cyclooxygenase inhibition. Drugs such as sulindac sulphone, 5-ASA, and olsalazine[42] also inhibit colorectal tumorigenesis by inducing tumour cell apoptosis and reducing tumour cell proliferation, yet these drugs do not inhibit cyclooxygenase. Furthermore, cell culture studies have shown that induction of apoptosis is independent of COX-2 protein expression.[43 44] Confirming this finding in vivo, we have recently found that increased expression of COX-2 in DMH induced rodent colorectal tumours is not altered by treatment with NSAIDs (unpublished data). The sum of these data suggests that NSAIDs are inhibiting colorectal cancer by a novel non-cyclooxygenase dependant mechanism.

The cyclin D/cdk4&6 checkpoint is inhibited by wild-type APC,[45] hDLG,[46] transforming growth factor β,[47] COX-2,[48] and cdk inhibitors, including p21 WAF1 and $p16^{INK4}$ which are also under the control of p53.[45] In carcinogen induced rodent models of colorectal cancer, 15% of tumours have mutated APC protein[49] and 75% activating β-catenin mutations.[50] Therefore, NSAIDs could be regulating this key checkpoint, either directly or through an effect on biological inhibitors, reducing the number of proliferating cells, increasing the time in G1, and allowing apoptosis to occur.

This study is the first to directly compare the efficacy of standard NSAIDs, a non-cyclooxygenase active agent, and COX-2 inhibitors in a whole animal tumour model of colorectal cancer, at clinically relevant doses. It is also the first to demonstrate an increased rate of apoptosis and a reduced rate of proliferation in tumours treated with these agents. These data should be useful in planning clinical assessment of these agents for chemoprevention of colorectal cancer while also improving our understanding of underlying mechanisms.

The authors thank Merck Sharpe and Dohme (sulindac and sulindac sulphone), Boehringer Ingleheim (meloxicam), and Searle Monsanto (celecoxib) for donating pure substance for use in this study. We would also like to thank Dr Eric Meyer and Ms Terri Fraterrigo at Searle Monsanto for arranging transportation and assay of rat sera for celecoxib. Statistical advice was kindly provided by Dr Michael Bailey, Department of Epidemiology, Monash University. This project was supported by an Anti-Cancer Council of Australia project grant. Dr Wendy Brown was supported by a Royal Australasian College of Surgeon Foundation Scholarship (1997) and NH&MRC Scholarship (1998–1999)

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