Rat colorectal tumours treated with a range of non-steroidal anti-inflammatory drugs show altered cyclooxygenase-2 and cyclooxygenase-1 splice variant mRNA expression levels

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Non-steroidal anti-inflammatory drugs (NSAIDs) reduce tumour mass by increasing the rate of tumour cell apoptosis and decreasing cell proliferation. The classically recognized target for NSAID action are the two isoforms of the cyclooxygenase (COX) gene, which is responsible for prostaglandin production. In the rat, the COX-1 gene expresses an alternatively spliced mRNA COX-1 splice variant (SV) which may, at best, code for a truncated COX-1 protein. Previously, we reported that COX-1SV mRNA is differentially expressed in the ageing stomach. In this study, carcinogen treated rats were treated for 23 weeks with celecoxib, sulindac or sulindac sulfone, while untreated rats received vehicle alone. For each animal, the number and volume of tumour per animal was recorded and histology was performed. Using competitive polymerase chain reaction, we determined whether COX gene expression was altered in colorectal tumours and in regions of adjacent and distant macroscopically normal intestine, from vehicle or NSAID treated rats. In addition, we immunolocalized COX-1 and COX-2 in the same tumour and normal colonic tissue. Tumours from animals treated with vehicle or celecoxib expressed significantly elevated mRNA in comparison with the adjacent normal mucosa. In contrast, tumours from sulindac and sulindac sulfone treated rats expressed significantly less COX-2 mRNA than tumours from vehicle treated rats. The expression of COX-1 mRNA remained unchanged in all tissues examined. However, COX-1SV mRNA levels were elevated in colorectal tumours and reduced after NSAID treatment to the levels observed in normal colonic mucosa. Our results indicate that the anti-neoplastic actions of NSAIDs may be attributed to COX dependent and/or COX independent mechanisms of action. We also demonstrate the presence and differential expression of COX-1SV mRNA in colon tumours. COX-1SV mRNA represents 2% of the total COX-1 mRNA expressed and its role in colon cancer remains to be established.

Introduction

Epidemiological, clinical and animal studies have shown that aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) reduce the incidence of colorectal cancer by 40–60% (1–4). The pharmacological target of NSAIDs are the cyclooxygenase (COX) enzymes, which inhibit prostaglandin synthesis from arachidonic acid. COX-1 is considered to be responsible for a variety of physiological functions and is expressed in most tissues including the stomach (5). In contrast, COX-2 is an inducible immediate early gene associated with inflammation (6), ovulation and cancer (7). However, this distinction is not entirely accurate, since we and others have demonstrated that both COX-1 and COX-2 mRNA are constitutively expressed in a variety of human tissues including the stomach (8–10). There is also evidence of COX-1 mRNA inducibility in vitro and in vivo (10,11).

NSAIDs are known to have pharmacological mechanisms in addition to, or other than, the inhibition of prostaglandin production, which include the induction of apoptosis and the regulation of cell proliferation (12). For instance, blockade of COX by NSAID has been shown to cause accumulation of its substrate, arachidonic acid, which in turn is converted to sphingomyelin and subsequently to the pro-apoptotic agent ceramide (13). Furthermore, COX-1 and COX-2 null mouse embryo fibroblasts remain sensitive to the anti-proliferative and anti-neoplastic effects of NSAIDs (14). In a recent study, NSAIDs were found to inhibit PPARδ, which is normally regulated by the adenomatous polyposis coli (APC) gene in tumorigenesis (15).

Increased expression of COX-2 mRNA and protein have been reported in human colorectal cancer (16) and in carcinogen induced rodent models of colon cancer (17). Both transgenic mice with an APC mutation and multiple intestinal neoplasia mice (Min), which carry a chemically induced APC mutation, are highly susceptible to intestinal adenocarcinoma formation and express increased levels of COX-2 (18,19). Furthermore, disruption of the COX-2 gene in such mice significantly reduces the number of tumours formed (19). These results provide evidence for a role of COX-2 in early adenoma formation. Using an in vitro model of colon cancer, Tsuji et al. (20) demonstrated that COX-1 regulates angiogenesis in endothelial cells, while the activation of COX-2 leads to an increased production of angiogenic factors. This indicates that angiogenesis which is induced by colon cancer cells, is regulated by both COX isoforms (20,21).

Generally, COX-1 mRNA expression does not alter in colorectal cancer. However, in one study using semi-quantitative RT–PCR, COX-1 and COX-2 mRNA levels were elevated in rat colonic tumours (22). The rat COX-1 gene expresses an alternatively spliced mRNA COX-1 splice variant (SV) which was cloned from an immortalized rat tracheal epithelial cell line (23). COX-1SV does not code for a full-length protein and it is not known whether a truncated protein is synthesized. This alternatively spliced mRNA is the same length as that encoding COX-1 and only distinguishable in the first 150 base pairs (bp), where a sequence, thought to originate from the second intron, is substituted for the first two exons. In rat

Abbreviations: APC, adenomatous polyposis coli gene; COX, cyclooxygenase gene; COX-1SV, alternatively spliced mRNA COX-1 splice variant (SV); DMH, dimethylhydrazine; Min, multiple intestinal neoplasia mice; NSAIDs, non-steroidal anti-inflammatory drugs; PAP, peroxidase-anti-peroxidase; PBS, phosphate buffered saline.
tracheal epithelial cells more than 90% of total COX-1 mRNA was identified as COX-1SV (23). We have demonstrated that COX-1SV mRNA is expressed in vivo and is differentially expressed in the ageing rat stomach (10). Previous studies examining COX-1 mRNA expression in the rat have utilized northern analysis, RNase protection or RT–PCR techniques with primers and/or probes designed downstream of the splice junction (17,24–28). However, none of these techniques allow distinction between COX-1 and COX-1SV mRNA. It is well established that alternative splicing is biologically significant (29). Furthermore, if COX-1SV mRNA is present and unaccounted for, it may lead to an overestimation of intact COX-1 mRNA which encodes for functional protein.

Regardless of their efficacy as anticancer agents, the precise mechanism(s) for the protective effect of NSAIDs remains unknown. Long-term treatment with NSAIDs, beginning shortly after carcinogen administration, limits tumour growth and number. However, regardless of the NSAID administered in our tumour model, colorectal tumours still develop. We hypothesized that the mRNA expression levels of the two COX isoforms, including COX-1SV mRNA, would be altered in such tumours. By studying the tumours remaining after long-term NSAID administration, a better understanding of how NSAIDs inhibit tumour progression will prevail.

Materials and methods

Induction of colonic tumours

Primary colonic tumours were induced with dimethylhydrazine (DMH) as previously described (3). Each animal received five weekly oral doses of DMH, at 30 mg/kg per dose. All experiments were approved from the Monash University Animal Experimentation and Ethics Committee.

Treatment groups

Rats were randomized to control group or to groups treated with test agents (control, n = 12; celecoxib, n = 8; sulindac, n = 10; sulindac sulfone, n = 10). Drugs were suspended into a vehicle (1% methylcellulose) and administered in the food (see ref. 38). Control animals received food containing vehicle alone. Drugs were suspended into a vehicle (1% methylcellulose) and administered in the food (see ref. 38). Control animals received food containing vehicle alone. 

Treatment with test agents commenced on the day after the first dose of DMH and continued for 23 weeks. The effect of these drugs on tumour number, volume, apoptosis and cell proliferation have been reported and are published in detail elsewhere (38).

Tissue collection

Animals were not fasted prior to colon removal. They were anaesthetized with i.p. 60 mg/kg pentobarbitone sodium (Sigma–Aldrich, St Louis, MO, USA) 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. Colorectal tumours, tissue adjacent to the tumours and macroscopically normal colorectal tissue (away from the tumour) from vehicle or NSAID treated rats were divided into two. One was snap frozen in liquid nitrogen (stored at −80°C) and the other was fixed in formalin for histological processing.

RNA extraction

Total RNA was isolated from samples with the RNeasy Mini kit (Qiagen, Melbourne, Australia). Finally, the RNA was eluted with 40 µl Rnase-free water. The concentration of each 4 µl RNA sample was determined by capillary spectrophotometry (Helix, San Diego, CA, USA) using a Cary 1 spectrophotometer (Varian, Melbourne, Australia).

Primers

Primers were designed as previously described (10). Briefly, to specifically detect COX-1 mRNA which is translated to functional COX-1 protein and COX-1SV using competitive PCR, primers must be designed to span the splice junction. We designed one set of primers downstream of the splice junction to detect total COX-1 mRNA (functional COX-1 and COX-1SV). A second set of primers were designed specifically to span the COX-1SV splice junction as demonstrated by Kitzler et al. (23). Functional COX-1 mRNA was determined by subtracting COX-1SV mRNA from total COX-1 mRNA. The COX-1, COX-1SV and COX-2 primers amplified products of 645, 445 and 535 bp, respectively.

Construction of competitors

Competitors were constructed as previously described (10). Competitive templates for COX-1, COX-1SV and COX-2 were designed by PCR-mediated mutagenesis. Each competitive template is 20–30% shorter, yet otherwise identical to the target sequence, to allow distinction and quantitation of the amplified sequences. COX-1 competitor (448 bp) was diluted in 10 mM Tris–HCl pH 7.5 to the required competitor copies.

Reverse transcription

Up to 1 µg RNA was reverse transcribed in a volume of 20 µl containing 1× GeneAmp PCR buffer, 5 mM MgCl2, 1 mMol dNTP, 1 U RNase inhibitor, 2.5 U MuLV reverse transcriptase and 2.5 U random hexamers (all from Perkin-Elmer, Foster City, CA). Reactions were incubated at 15°C for 1 min, the temperature was increased to 42°C for 9 min followed by 42°C for 1 h, 85°C for 5 min and 4°C for 1 min in a thermal cycler (MJ Research, Watertown, MA, USA).

Competitive PCR

Each aliquot of cDNA used in the three competitive PCR assays originated from the same reverse transcription reaction. Competitive PCR was performed using 2 µl (for COX-1), 4 µl (for COX-1SV) and 2 µl (for COX-2) cDNA in a total volume of 50 µl per reaction. The reaction consisted of 2 U AmpliTaq Gold, 1× GeneAmp PCR buffer, 2.5 mM MgCl2 and 200 µmol/l dNTP (all from Perkin-Elmer), 0.2 µmol/l upper and lower primers and 1000 (COX-1), 100 (COX-1SV) or 10 000 (COX-2) copies of competitor. A thermostirling program of 94°C for 8 min, then 40 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, finally 72°C for 7 min and 4°C for 1 min. PCR products were visualized on ethidium bromide stained, 2% agarose gels then scanned using a Fluorimager 575 (Molecular Dynamics, Sunnyvale, CA). PCR product band volumes were quantitated with ImageQuantNT Software (Molecular Dynamics). Native–competitor product ratios were calculated and the levels of gene expression were reported as copies of mRNA per microgram of total RNA.

Validation of competitive PCR

To ensure that competitor and target sequences compete equally in the PCR, increasing concentrations of each competitor were titrated against a constant aliquot of target cDNA known to be positive for the gene of interest. The three competitive PCR assays have been validated previously (10).

Immunohistochemistry

Deparaffinized sections 3 µm thick were washed in phosphate buffered saline (PBS, pH 7.2). Antigen retrieval was achieved using microwave heating at 800 W for 10 min in antigen retrieval solution, pH 6 (Dako, NSW). Endogenous peroxidases were blocked with 3% (v/v) H2O2 in methanol for 15 min and after washing they were incubated with 20% (v/v) normal rabbit serum (NRS) in PBS for 30 min. The sections were incubated in PBS and 1% NRS with primary antibodies at 1:1000 for COX-1 or COX-2 (polyclonal antiserum raised in a goat against the mouse COX-1 protein or the rat COX-2 protein; Santa Cruz Biotechnology, CA, USA) for 1 h. On each slide two sections were present, one was exposed to primary antiserum and one was exposed to PBS, 1% (v/v) NRS. After washing in PBS, 3×5 min, sections were incubated with rabbit anti-goat immunoglobulin G (Dako) for 30 min at room temperature, washed with PBS and incubated for a further 30 min with goat peroxidase–anti-peroxidase (PAP) complex (1:60 in PBS and 1% NRS). After washing, sections were incubated with diaminobenzidine tetrahydrochloride (Sigma) for 10 min, rinsed in distilled water for 5 min, then counterstained with Harris haematoxylin (Sigma) for 30 s, then rehydrated and mounted.

Assessment of COX-1 and COX-2 staining patterns

All slides were examined using light microscopy (Olympus CK2) with the observer blinded to the treatment group. The type of cells with COX-1 or COX-2 staining within normal caecal and rectal tissue, tumour and peri-tumour (histologically normal tissue adjacent to the tumour) was noted.

Statistical analysis

Values are expressed as mean ± SEM. The Mann–Whitney U non-parametric test was used to compare differences between groups. Correlation studies were performed using Spearman’s correlation coefficient.

Results

Competitive PCR

For each of the competitive PCR assays, a known number of competitor molecules were co-amplified with the target sequence and mRNA quantitation was achieved by comparing
the ratio of competitor versus the target sequence, as previously described (10,30).

Representative samples of tumour, peri-tumour (histologically normal tissue adjacent to the tumour) and normal colorectal tissue from rats treated with vehicle or NSAIDs were subjected to competitive PCR with the addition of a known copy number of COX-1 (1000 copies; Figure 1A), COX-1SV (100 copies; Figure 1B) or COX-2 (10 000 copies; Figure 1C) competitor.

Expression of COX-1 mRNA did not significantly change between tumour, peri-tumour and normal colonic tissues specimens. Nor did NSAID treatment significantly alter the expression of COX-1 mRNA (Figure 2A). COX-1SV mRNA levels (Figure 2B) were significantly elevated in vehicle treated tumours (mean = 2.4×10³ copies/µg RNA; P < 0.05) compared with paired normal colorectal specimens (mean = 7×10² copies/µg RNA) while vehicle treated peri-tumours expressed an intermediate level (mean = 1.2×10³ copies/µg RNA) of COX-1SV. Tumours treated with NSAIDs appeared to express less COX-1SV than untreated tumours. Significant differences were observed between vehicle treated tumours and peri-tumours treated with celecoxib (mean = 1.1×10³ copies/µg RNA; P < 0.05). Tumours treated with sulindac also expressed reduced levels of COX-1SV mRNA which approached significance (mean = 11.5×10² copies/µg RNA). While tumours treated with sulindac sulfone expressed significantly less COX-1SV (mean = 7.66×10² copies/µg RNA; P < 0.05) in comparison to vehicle treated tumours. Like COX-1SV, COX-2 mRNA (Figure 2C) was elevated in vehicle treated tumours (mean = 6.3×10³ copies/µg RNA; P < 0.05) when compared with vehicle treated normal mucosa (mean = 2.3×10³ copies/µg RNA). COX-2 expression in celecoxib treated tumours (mean = 7.5×10³ copies/µg RNA) was similar to that observed in vehicle treated tumours, while the levels of COX-2 mRNA were significantly reduced in sulindac (mean = 2.4×10⁵ copies/µg RNA; P < 0.05) and sulindac sulfone (mean = 1.7×10⁵ copies/µg RNA; P < 0.05) treated tumours, when compared with vehicle treated tumours.

Immunohistochemistry

In normal colonic tissue COX-1 immunostaining was predominately localized in the peri-nuclear region of the interstitial tissue (Figure 3A). Peri-nuclear staining could also be seen within the colonocytes. While colonocytes at the lumen of the colon showed cytoplasmic COX-1 staining. Staining was also seen in platelets and vascular endothelial cells (data not shown). Animals receiving carcinogen, but no NSAID treatment, had greater COX-1 immunostaining in the interstitial tissue than in the cells of epithelial origin (colonocytes and tumour cells, Figure 3B). A similar COX-1 immunostaining pattern was observed in tumour tissue from NSAID treated rats (data not shown). The level of COX-1 immunostaining in tumour cells, colonocytes and interstitial tissue did not change dramatically between control and NSAID treated animals.

In normal colonic tissue, COX-2 immunostaining was demonstrated in the cytoplasm of colonocytes and in the interstitial tissue (Figure 3C). Within tumour cells from animals receiving carcinogen but no NSAID treatment, COX-2 immunostaining was observed in the cytoplasm and the peri-nuclear regions, with variable staining in the nucleus. Within interstitial cells of both tumour and normal adjacent tissue, staining was predominately seen in the cytoplasm and peri-nuclear regions (Figure 3D). The colonocytes of macroscopically normal tissue

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**Fig. 1.** Expression of COX in the rat colon by competitive PCR. Representative samples of tumour, peri-tumour (histologically normal tissue adjacent to the tumour) and normal colorectal tissue from rats treated with vehicle or NSAIDs.

**Fig. 2.** Quantitation of COX expression in tumours. Peri-tumours and normal mucosa of rats treated with vehicle or NSAIDs, expressed as mRNA copies/µg RNA. Values are means ± SE for 8–12 rats per group. (A) COX-1 was expressed in all tissues examined and no significant differences were observed between groups. (B) COX-1SV mRNA was significantly elevated in vehicle treated tumours in comparison with the normal colonic mucosa. Tumours treated with NSAIDs also expressed reduced COX-1SV mRNA levels. (C) COX-2 mRNA was significantly elevated in vehicle treated tumours in comparison with the normal colonic mucosa. Tumours treated with sulindac and sulindac sulfone, but not celecoxib, expressed reduced COX-2 mRNA levels.
adjacent to tumours had cytoplasmic staining and nuclear staining was also observed deep in the crypt. Additional staining was observed in the muscularis propria, vascular smooth muscle and endothelial cells as well as adipocytes (data not shown). The COX-2 immunostaining intensity in tumour cells was higher than that observed in normal epithelial cells. A similar COX-2 immunostaining pattern was observed in tumour tissue from NSAID treated rats (data not shown).

Correlation studies
A positive correlation was observed between COX-1SV and COX-2 mRNA expression (Spearman’s correlation coefficient, 0.64; \( P = 0.001 \)).

An inverse correlation was observed between COX-2 mRNA expression and the previously investigated parameter, rate of tumour apoptosis (Spearman’s correlation coefficient, \(-0.485\); \( P < 0.05 \)).

No correlation was observed between COX expression, tumour size or tumour type (adenoma and adenocarcinoma).

Discussion
We have reported the in vivo mRNA expression of COX-1, COX-1SV and COX-2 in rat colorectal tumours after long-term NSAID administration. The expression of COX-2 mRNA was significantly elevated in tumours from animals treated with vehicle or celecoxib when compared with the adjacent normal mucosa. While tumours from sulindac and sulindac sulfone treated rats expressed significantly less COX-2 than tumours from vehicle and celecoxib treated rats. Expression of COX-1SV was elevated in tumours from vehicle treated rats compared with all other groups investigated. COX-1 mRNA expression did not alter in the tissue examined. This is the first study distinguishing COX-1 and COX-1SV in rat colorectal tumours and although COX-1SV levels represent 2% of the total COX-1 expressed, previous studies would have reported additive results. Additionally, we immunolocalized COX-1 and COX-2 in the same tumour and normal colonic tissue.

Similar to COX-2 mRNA, COX-1SV expression levels are elevated in colorectal tumours. However, it is important to emphasize that the mean levels of COX-2 mRNA observed in vehicle treated tumours were 260-fold higher than the mean levels observed for COX-1SV. Importantly tumours from animals treated with two traditional NSAIDs and a selective COX-2 inhibitor expressed significantly lower levels of COX-1SV mRNA than tumours receiving vehicle alone, indicating that COX-1SV may be regulated by NSAID treatment. Interestingly, we observed a positive correlation between COX-2 and COX-1SV mRNA. A biological role for COX-1SV has not been established. COX-1SV mRNA can at best code for a truncated protein, since it is missing the open reading frame normally located in exon 1 (23). Previously we reported that COX-1SV mRNA was differentially expressed in the ageing
rat stomach (10). To date, the role of COX-1SV remains unknown and will only be established if COX-1SV mRNA codes for an alternative COX-1 protein. Additionally its existence in the human remains unknown.

In the rat colorectal tumour model, administration of NSAIDs reduces the number and volume of tumours developing (12,31–35) and significantly increases the rate of apoptosis, while reducing the rate of cell proliferation (36–38). In this study we have utilized the same, well characterized tumours to determine COX expression. Sulindac and sulindac sulfone significantly reduced the expression of COX-2 mRNA. We also observed an inverse association between COX-2 mRNA and the previously investigated parameter, apoptosis. Other studies have also indicated that increased expression of COX-2 appears to be linked to the inhibition of apoptosis. For example, cell lines overexpressing COX-2 show alterations in cellular adhesion and resistance to apoptosis (13,39). Such phenotypic changes could be reversed by the addition of the COX inhibitor, sulindac sulfide (39).

It is likely that overexpression of COX-2 contributes to the development and progression of colonic neoplasms (13,20). Therefore the level of COX-2 expressed within a tumour may be a factor which determines tumour growth. Unlike sulindac and sulindac sulfone, the COX-2 specific inhibitor celecoxib did not reduce COX-2 mRNA levels in our tumour specimens, although tumour volume was previously shown to be reduced (38). Therefore celecoxib may have a different mechanism of action.

NSAIDs and prostaglandins have been shown to transcriptionally modulate COX expression (40). In carciongin induced rat mammary tumours, ibuprofen is an effective chemopreventive agent which reduces mRNA and protein levels of both COX-1 and COX-2 (41). At NSAID concentrations that induce apoptosis in transformed chicken embryo fibroblasts, induction of COX-1 and COX-2 mRNA levels occur. These authors also demonstrated that NSAIDs have a dose-dependant effect on COX-1 mRNA levels (42). Thus in some cell types, chronic NSAID treatment may actually increase rather than decrease COX activity (42). Thus the differences in COX expression observed in our study and the previous studies may be due to the NSAID dose administered and the distinct tumour model utilized. It is important to note that the tumours harvested at the end of the current study developed in the constant presence of NSAIDs which were administered initially, 1 day after carcinogen treatment ceased. This suggests that the tumours which developed did so under selective pressure. Thus the timing of NSAID administration during the carcinogenic process may influence the COX expression profile.

We have immunolocalized COX-1 and COX-2 in normal colonic and tumour tissues. In the normal colon, COX-1 was localized predominantly to the cytoplasm and the peri-nuclear regions of the interstitial tissue. COX-2 immunostaining was observed within the cytoplasm and peri-nuclear regions, additional nuclear staining was detected within tumour cells. Consistent with these observations, both COX isoforms are located in the endoplasmic reticulum and contiguous outer membrane of the nuclear envelope (43). COX-2, however, is also located within the nuclear membrane, where it is ideally positioned to participate in mitogenesis under pathophysiologic conditions (44).

Whether COX-2 is produced within tumours or interstitial tissue is a subject of ongoing debate. COX-2 is immunolocalized to neoplastic epithelial cells in genetically engineered mice (18), while other studies have localized COX-2 only in the interstitium (19,45,46). The distribution of COX-2 in the current study is similar to the distribution described in a human colorectal carcinoma study, where COX-2 was localized to inflammatory mononuclear cells, vascular endothelial cells, fibroblasts and cancer cells (47). These data suggest that the DMH rat model of colorectal carcinogenesis reflects more accurately the distribution of the COX isoforms observed in humans.

Several studies have questioned whether the chemopreventive action of NSAIDs in colon cancer requires the inhibition of COX. Zhang et al. (14) have demonstrated that the apoptotic and anti-proliferative actions of NSAIDs also occur in embryonic fibroblasts from COX-1 and/or COX-2 deficient embryos following malignant transformation. Furthermore, colon cancer cells with no COX-1 or COX-2 activity are growth inhibited as effectively by NSAIDs as cells producing the COX isoforms (48). Therefore, the anti-neoplastic actions of NSAIDs appear to be independent of COX-1 or COX-2 inhibition. However, a large number of studies have demonstrated that the inhibition of cyclooxygenase is required for the chemopreventive effect of NSAIDs (49). Rigas and Shiff (49) present a model which assumes that NSAIDs inhibit at least two different steps of the neoplastic process. Inhibition of either step (COX dependent or COX independent) leads to the inhibition of colon cancer. Therefore, the pleiotropic nature of NSAIDs appears to affect several biochemical pathways and this may explain their remarkable effectiveness in colon cancer chemoprevention.

We have demonstrated the presence of an alternatively spliced COX-1 mRNA (COX-1SV) in colon cancer. Unlike COX-1 mRNA, COX-1SV is elevated in colorectal tumours and reduced after NSAID treatment to the levels observed in the normal colonic mucosa. However, the function of COX-1SV in tumorigenesis has not been established and only represents 2% of the total COX-1 mRNA expressed in rodent colorectal cancer. We also demonstrate that long-term NSAID administration reduces the levels of COX-2 mRNA in tumours from sulindac and sulindac sulfone, but not celecoxib, treated rats. These data are consistent with recent studies demonstrating that the anti-neoplastic effects of NSAIDs are attributed to both COX dependent and/or COX independent mechanisms of action. Studies are underway to further our understanding of the molecular mechanisms of NSAID action and the significance of COX-1SV in colorectal cancer.

References


