Inhibition of rat colon tumors by sulindac and sulindac sulfone is independent of K-ras (codon 12) mutation

TANYA A. DE JONG,1 STEWART A. SKINNER,1 CATHY MALCONTENTI-WILSON,1 DAPHNE VOGIAGIS,1 MICHAEL BAILEY,2 IAN R. VAN DRIEL,3 AND PAUL E. O'BRIEN1
1Department of Surgery, 2Department of Epidemiology and Preventive Medicine, and 3Department of Pathology and Immunology, Monash University Medical School, Prahran, Melbourne 3181, Australia

De Jong, Tanya A., Stewart A. Skinner, Cathy Malcontenti-Wilson, Daphne Vogiagis, Michael Bailey, Ian R. van Driel, and Paul E. O'Brien. Inhibition of rat colon tumors by sulindac and sulindac sulfone is independent of K-ras (codon 12) mutation. Am. J. Physiol. Gastrointest. Liver Physiol. 278: G266–G272, 2000.—Nonsteroidal anti-inflammatory drug (NSAID) use reduces the risk of colorectal cancer by 40–50%. Previous studies suggest that effective inhibition of colorectal cancer by NSAIDs may be dependent on the presence or absence of a K-ras mutation. This study was aimed at determining the relationship between inhibition of colorectal cancer by sulindac and sulindac sulfone and the presence of activating K-ras mutations in the 1,2-dimethylhydrazine dihydrochloride rat model. Sulindac (20 mg·kg⁻¹·day⁻¹), sulindac sulfone (40 mg·kg⁻¹·day⁻¹), or vehicle was administered orally to male Sprague-Dawley rats for a 4-wk period beginning 20 wk after tumor induction. Tumor number and volume were measured before treatment by laparotomy and colonoscopy and again after treatment. Sulindac and sulindac sulfone treatment significantly reduced the number and volume of colorectal tumors compared with control rats. For K-ras (codon 12) mutation detection, frozen tumor tissue was collected at the endpoint. We found K-ras codon 12 mutations in 11 of 21 (52%) control tumors. The proportion of tumors with K-ras mutations in the sulindac-treated group (5 of 8 (62%); odds ratio = 1.51 (95% confidence interval = 0.29, 8.33)) and the proportion of sulindac sulfone-treated tumors (9 of 14 (64%); odds ratio = 1.63 (95% confidence interval = 0.41, 6.66)) were not significantly different from controls. Tumor inhibition did not correlate with K-ras (codon 12) mutation status, which suggests that the mechanism of inhibition of rat colorectal cancer by sulindac and sulindac sulfone is independent of K-ras mutation.

Colorectal cancer; 1,2-dimethylhydrazine dihydrochloride, non-steroidal anti-inflammatory drugs

COLORECTAL CANCER is one of the most common malignancies and is a major cause of cancer deaths in the western world (18). Chemoprevention of colorectal cancer by nonsteroidal anti-inflammatory drugs (NSAIDs) is arguably the most promising area for reducing the clinical problem. The NSAID sulindac has been shown in randomized, controlled trials to induce inhibition of polyps in patients with familial adenomatous polyposis (FAP) (10, 19, 26). A number of large case control and cohort studies have shown a reduction of 40–50% in colorectal cancer in subjects taking NSAIDs regularly, particularly aspirin (21, 27, 42). In addition, experimental studies in the rat demonstrate inhibition of benign and malignant tumors by a range of NSAIDs. We (6–8, 38) and others (2, 24, 25, 29) have shown that sulindac and sulindac sulfone (a sulindac metabolite that has no anti-inflammatory activity and no known cyclooxygenase activity) effectively inhibit tumor number and volume and number of aberrant crypt foci (ACF) in the 1,2-dimethylhydrazine (DMH) rat colorectal cancer model. DMH and its metabolite azoxymethane (AOM) cause colorectal cancer in rodents with a distribution and histology similar to that observed in humans (5). On a morphological level, this DMH-induced colorectal carcinogenesis appears to follow the classical ACF-adenoma-adenocarcinoma sequence (9, 32, 44).

K-ras is one of the oncogenes most frequently mutated in human cancers. The K-ras gene is found on human chromosome 12p and encodes an ~21-kDa G protein that is involved in growth factor signal transduction (12). Activated K-ras initiates a signal transduction cascade associated with increased or uncontrolled cell proliferation. Additionally, a recent study (45) showed that the presence of an activated K-ras is associated with a reduction in the frequency of apoptotic cells in human colorectal cancer. Thus activated K-ras may exert a dual effect on both cell proliferation and apoptosis in colorectal carcinogenesis.

Chemically induced ACF and carcinomas in rodents have been shown to frequently contain K-ras mutations (15, 20, 40, 43). Constitutive activation of K-ras by point mutation occurs with a frequency of ~40–60% in DMH-induced rat colorectal cancer. The majority of K-ras point mutations occur as G-to-A transitions, and ~70% of these occur in codon 12 (15, 43). Generation of the K-ras oncogene appears to be an early event in the progression of colorectal carcinogenesis (44). K-ras mutation is an early event in human sporadic polypoid adenoma formation with a reported K-ras codon 12, 13, and 61 point mutation frequency of 40–70% in carcinomas and 15–75% in adenomas (23). Codon 12 K-ras mutations have also been found in human sporadic microadenomas (33).
To date, only one in vivo study has examined the relationship between sulindac and K-ras activation in colorectal cancer. It was shown in an AOM model of colorectal rat cancer that sulindac selectively suppressed amplification of cells with a K-ras codon 12 mutation (37). However, cell proliferation was not measured. An in vitro study showed that an activated K-ras oncogene increases resistance to sulindac-induced apoptosis in rat enterocytes (1). This suggests that sulindac-induced apoptosis may be resisted in tumors with an activated K-ras. However, the exact mechanism of the chemopreventive action of sulindac has not yet been elucidated. It is not known whether sulindac mediates its chemopreventive effect via induction of apoptosis, via suppression of cell proliferation, or via an alternative mechanism, e.g., inhibition of ras signaling (14). The aim of our study was to determine whether, in the DMH rat model, sulindac and sulindac sulfone inhibition was selective for colorectal tumors with a K-ras (codon 12) mutation.

METHODS

DMH-induced colorectal tumor model

Animals. Six-week-old male Sprague-Dawley rats were obtained from Monash University Central Animal Services (Clayton, Australia). They were housed in a temperature-controlled environment with a 12-h light-dark cycle. Rats had access to standard rat chow (GR2+/+, Clark/King/Barastock) and tap water ad libitum throughout the course of study. All studies were conducted in accordance with National Health and Medical Research Council (Australia) guidelines and approved by the Monash University Standing Committee on Ethics in Animal Experimentation.

Induction of tumors, laparotomy, and measurement. Primary colorectal tumors were induced in rats (n = 30) with DMH using a protocol previously established in our laboratory (8). DMH (Fluka Chemical, Castle Hill, Australia) was dissolved in isotonic saline, and pH was adjusted to 7 with NaOH. Each animal received 5 × 30 mg/kg doses of DMH at weekly intervals by orogastric gavage. At 20 wk after the initial dose of DMH, rats were anesthetized with intraperitoneal injection of pentobarbital sodium (60 mg/kg; Boehringer Ingleheim, Sydney, Australia). The colon was removed via a midline laparotomy, opened along the mesenteric border, rinsed with isotonic saline, and pinned out flat. Tumors were counted macroscopically, volume measurements (height × width × length) were taken, and position (distance from cecum and rectum) was recorded. The change in total tumor volume over the treatment period was calculated as the summation of all tumor volumes within a group after treatment minus the summation before treatment. A sample from each tumor was snap-frozen in liquid nitrogen and stored at −80°C for subsequent DNA extraction. The remaining colon and tumors were fixed in 10% neutral buffered formalin (Sigma, St. Louis, MO) for 16–24 h and then stored in 50% (vol/vol) EtOH. Tumor tissues were processed and embedded in paraffin. Four-micrometer sections were then stained with hematoxylin and eosin (H & E).

Two independent observers, blinded to treatment group, assessed standard H & E sections using an Olympus light microscope under ×40 magnification. Only those tissues confirmed as adenoma or carcinoma on histology were included in the analysis and results of the study.

DNA extraction and detection of K-ras codon 12 G-to-A mutations. RNase-free DNA was extracted from rat colon (tumor and normal) tissue using the Qiagen Qiamp tissue kit according to the manufacturer’s instructions. A capillary spectrophotometer was used to measure optical density at 260 nm to determine the concentration of DNA in the samples. Extracted DNA was amplified by PCR using a technique similar to that described by Kumar and Dunn (17) to detect specific K-ras mutations. This uses a restriction fragment length polymorphism (RFLP) strategy. PCR is performed using a mismatched primer pair that generates a 75-bp product (for both wild type and mutant) with a HpH I restriction site generated in the PCR product only if a G-to-A substitution is present in the second base of codon 12 in the K-ras gene. Thus after digestion with HpH I, wild-type K-ras products are still 75 bp in size, whereas mutant K-ras products are cleaved into 46 bp and 29 bp, respectively. In brief, the following PCR protocol was used. DNA (200 ng) was amplified in a 100-µl reaction volume, using 0.5 µM of each primer [K-ras 1,5'-ACTTTGTGGTAGTTGGAG-3'; mismatch in bold; and K-ras 2,5'-TCCACAAATGTAGTTGGAGTG-3']. 1.5 mM Mg 2+, and 200 µM dNTPs. AmpliTaq Gold Taq polymerase (Perkin Elmer) was used, and cycling parameters were as follows: 94°C for 8 min (to activate AmpliTaq Gold) and then 50 cycles of 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C with a further 7 min at 72°C in the final cycle only. PCR products were visualized on 3% agarose gels stained with ethidium bromide. For each PCR, a control with no DNA and a positive mutant control were included. Any DNA samples that were negative for K-ras were subjected to PCR a second time for confirmation.

Ten-microliter aliquots of K-ras PCR product were then digested with HpH I (New England Biolabs, Beverly, MA) in a total volume of twenty microliters (NEB4 buffer) using one microliter (restriction enzyme supplied at 5 U/µl) of HpH I per
digestion. Digests were at 37°C for 4–5 h. A known mutant and a plasmid [pBluescript (K S+) ] were used as digestion controls, and a mock digest (no enzyme) was included. Hph I-digested and uncut PCR products were loaded in adjacent lanes, electrophoresed on native 16% PAGE gels, and analyzed by scanning using a Fluorimager 595, after staining with SYBR Green (Molecular Probes, Eugene, OR) (intercalator). Markers (10-bp ladder and 50-bp ladder) were included with each gel run. Mock digestions of K-ras PCR products and wild-type samples digested with Hph I gave only the 75-bp K-ras PCR product. In addition to the 75-bp K-ras PCR product, mutant K-ras 46-bp and 29-bp fragments were clearly visualized in those samples with the K-ras mutation.

Statistical Analysis

Statistical analysis was performed using the SAS (SAS Institute, Cary, NC) system. The outcome variables of tumor volume and tumor number were not normally distributed; thus statistical significance was determined using nonparametric techniques (Kruskal-Wallis and Wilcoxon 2-sample test). A P < 0.05 was considered to be significant, and, to compensate for multiple comparisons where necessary, a P < 0.02 was considered to be significant.

The number of tumors before treatment refers to all tumors present at laparotomy, including those that disappeared during the treatment period and for which there is no histological confirmation of tumor status. “Tumors” that were identified at laparotomy but were pronounced normal after histological analysis at the endpoint (after treatment) are excluded from all analysis. The number of tumors after treatment refers to the total number at the endpoint of the treatment period and for all analyses of tumor K-ras mutation status.

For statistical analysis of the difference in the proportion of K-ras mutations between treatment groups, comparisons were done using a test for equal proportions. To take into account the small sample size for the adenoma population, a two-tailed Fischer’s exact test was also used.

RESULTS

Colorectal tumors were induced in rats by oral administration of DMH. Sulindac (20 mg·kg\(^{-1}\)·day\(^{-1}\)), sulindac sulfone (40 mg·kg\(^{-1}\)·day\(^{-1}\)), or vehicle was orally administered to rats for a 4-wk period (20–24 wk after tumor induction). Tumor volume and number were measured at the beginning and at the end of the treatment period.

Treatment with sulindac and sulindac sulfone caused a reduction in the number of tumors in each of these groups, compared with an increase in tumor number in control rats (Table 1). This reduction in tumor number was highly significant for sulindac treatment compared with controls (P = 0.00037) but not for sulindac sulfone (P = 0.0607). For subsequent analysis of results, only tumors that were confirmed by histological analysis were included.

The effect of sulindac and sulindac sulfone treatment on tumor volume is illustrated in Table 2. Sulindac treatment led to a reduction in the total volume of tumor present (721-mm\(^3\) decrease) by the end of the treatment period. This was highly significantly different from the increase in volume (9,127-mm\(^3\) increase) seen in the control group during this period (P = 0.0002, Wilcoxon 2-sample t-test). The increase in tumor volume after 4-wk treatment with sulindac sulfone (365-mm\(^3\) increase) was also significantly less than the increase in the control group (P = 0.0182, Wilcoxon 2-sample t-test). These results indicate that both agents are effective inhibitors of tumor number and volume and that sulindac is more effective than sulindac sulfone.

K-ras Mutation Status of Colorectal Rat Tumors

DNA extracted from 43 rat colorectal tumor samples (33 carcinomas and 10 adenomas) was analyzed by RFLP-PCR and native PAGE gel analysis for the presence of K-ras codon 12 G-to-A point mutations. Figure 1 shows a representative analysis. K-ras wild-type samples and tumor samples with the K-ras mutation are depicted. Mutant K-ras 46-bp and 29-bp fragments were clearly visualized in those samples with the K-ras mutations (as well as the 75-bp K-ras PCR product). The paired, nondigested K-ras PCR products showed only the 75-bp product. Both wild-type K-ras samples (digested with Hph I) and their corresponding nondigested K-ras PCR products showed only the 75-bp K-ras PCR product.

Of 120 samples in total of normal mucosa, lymphoid tissue, mucosa adjacent to tumor, and tumor samples analyzed for K-ras codon 12 mutations, no normal tissues contained K-ras codon 12 mutations and only 2 of the tissues taken adjacent to the tumors contained a mutant K-ras. Mutant K-ras was found in 58% of carcinomas (19/33) and in 60% of adenomas (6/10) overall. Eleven of twenty-one tumors (52%) in vehicle-treated rats had a K-ras mutation. The proportion of tumors with a K-ras mutation was reduced to 36% (7/20) in rats treated with sulindac and to 33% (6/18) in sulindac sulfone-treated rats.

Table 1. Number of colon tumors per group

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Control (Vehicle)</th>
<th>Sulindac</th>
<th>Sulindac Sulfone</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>(n = 8)</td>
<td>(n = 10)</td>
<td>(n = 10)</td>
</tr>
<tr>
<td>Before treatment</td>
<td>20</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>After treatment</td>
<td>24</td>
<td>8</td>
<td>14†</td>
</tr>
</tbody>
</table>

Rats received sulindac, sulindac sulfone, or vehicle for 4-wk period as described in METHODS, and no. of tumors from all rats was determined. Histological confirmation of before-treatment values was not possible before treatment. *P = 0.0037, †P = 0.0607 vs. vehicle group.

Table 2. Effect of sulindac and sulindac sulfone on total tumor volume

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sulindac</th>
<th>Sulindac Sulfone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>3,384</td>
<td>1,592</td>
<td>1,653</td>
</tr>
<tr>
<td>After treatment</td>
<td>12,511</td>
<td>871</td>
<td>2,018</td>
</tr>
<tr>
<td>Change in volume</td>
<td>+9,127</td>
<td>−721*</td>
<td>+365†</td>
</tr>
</tbody>
</table>

Total tumor volume (in mm\(^3\)) is summation of all tumor volume within treatment group. *P = 0.0002, Wilcoxon 2-sample t-test; †P = 0.18, Wilcoxon 2-sample t-test.
tumors with K-ras mutations in the sulindac-treated group (5 of 8; odds ratio (OR) = 1.51; 95% confidence interval (CI) = 0.29, 8.33) was slightly increased compared with controls as was the proportion of sulindac sulfone-treated tumors (9 of 14; OR = 1.63; 95% CI = 0.41, 6.66).

Moreover, the change in tumor volume over the 4-wk treatment period was independent of K-ras mutation status (Table 3). In the sulindac-treated group, seven of eight tumors present after treatment had decreased in size over the treatment period and five of eight had K-ras mutations. Only one tumor increased in size during the sulindac treatment, and this tumor was K-ras wild type. All three new tumors that appeared during the sulindac treatment had K-ras mutations. Only samples that have K-ras codon 12 G-to-A mutation are cut by \( \text{HpH} 1 \) and show 46-bp and 29-bp mutant fragments (in addition to 75-bp K-ras PCR product). Fragment size is indicated. All uncut K-ras PCR products and K-ras wild-type samples show 75-bp product.

**Table 3.** K-ras status of tumors after treatment

<table>
<thead>
<tr>
<th>Tumors, new or increased in size</th>
<th>Control</th>
<th>Sulindac</th>
<th>Sulindac Sulfone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>10</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Mutant</td>
<td>11</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Tumors decreased in size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mutant</td>
<td>0</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>All tumors</td>
<td>10 (48%)</td>
<td>3 (38%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>Wild type</td>
<td>11 (52%)</td>
<td>5 (62%)</td>
<td>9 (64%)</td>
</tr>
</tbody>
</table>

Experimental design described in Table 1. Increase/decrease in size over 4-wk treatment period is indicated. New tumors are those not present at laparotomy that appeared during treatment period. Restriction fragment length polymorphism-PCR strategy was used to determine K-ras (codon 12) mutation status in rat colon tumor DNA as described in Methods. Mutant, G-to-A point mutation at K-ras codon 12.

**DISCUSSION**

Sulindac and its sulfone derivative inhibit tumor number and volume in DMH-induced colorectal rat tumors (8, 24, 25, 29, 38). These findings were confirmed in the present study. Our results indicated that both sulindac and sulindac sulfone (over a 4-wk treatment period) caused a significant decrease in rat colorectal tumor volume and that sulindac showed a greater inhibitory effect than sulindac sulfone.

DMH and its metabolite AOM cause colorectal cancer in rodents with a distribution and histology similar to those observed in human colorectal cancer (5) and are thus widely used as a model for human colorectal carcinogenesis. Chemically induced colorectal tumors in rodents have been shown to contain K-ras mutations (15, 20, 43) with an incidence similar to that observed in humans. In this study we have found activating K-ras (codon 12) point mutations in ~60% of tumors, which correlates well with the reported values ranging between 40 and 65% (15, 43). It has been demonstrated that all of the K-ras point mutations observed in the DMH model are G-to-A transitions, and >95% occur in codons 12 and 13, with ~70% occurring in codon 12 (43). Codon 12 mutations are considered the mutations most relevant to progression of colorectal cancer, because activating codon 12 point mutations, but not codon 13 mutations, have been found in all of the stages of colorectal tumor progression including ACFs, microadenomas, and carcinomas. The techniques (17, 43) used in our study detect these K-ras G-to-A point mutations in codon 12.

Activated K-ras has been shown to stimulate cell proliferation in cancers, and a recent study (45) showed that an activated K-ras was associated with a reduction in the frequency of apoptotic cells in human colorectal cancer. The balance between cell proliferation and cell death is critical in normal tissues. Increased cell proliferation and decreased frequency of apoptosis shift this balance in favor of development of cancer.

A recent in vitro study (14) has demonstrated that sulindac sulfide, a metabolite of sulindac, specifically inhibits signaling by the ras gene product p21ras by binding to the p21ras protein. It also blocks activation of p21ras GTPase by activation of p120GAP. The authors of that study suggest that sulindac sulfide will bind and inhibit the signaling of both wild-type and mutant p21ras (14). Given that oncogenic p21ras has unregulated GTPase activity, sulindac sulfide may have a greater effect on wild-type p21ras (where its binding also regulates GTPase activity) than on mutant p21ras protein. Thus sulindac (converted to sulindac sulfide in vivo) may have an inhibitory effect on both wild-type and mutant p21ras protein in colon tumors in the rat.

There are conflicting suggestions as to whether tumors with a K-ras mutation are more, or less, susceptible to sulindac treatment. A recent study (45) in human colorectal cancer samples showed that an activated K-ras is associated with a reduction in the
frequency of apoptotic cells. An in vitro study showed that a K-ras oncogene increases resistance to sulindac-induced apoptosis in rat enterocytes (1). This suggests that an activated K-ras oncogene could confer resistance to sulindac-mediated apoptosis, and thus sulindac would be a more effective inhibitor of colorectal tumors that do not have an activated K-ras, if sulindac mediates its chemopreventive effect via apoptosis. To date, only one in vivo study has examined a relationship between sulindac and K-ras activation in colorectal cancer (37). In an AOM-induced model of colorectal rat cancer, sulindac caused a reduction in the number of K-ras codon 12 mutations found in colon tumor and stool samples. The authors of that study suggested that sulindac may be specifically suppressing cells with a K-ras codon 12 mutation and that sulindac mediates its inhibitory effect in rat colorectal cancer via suppression of cell proliferation. However, they did not measure cell proliferation in their study (37).

Our results do not support either a specific suppression of cells with a K-ras codon 12 mutation by sulindac or selective inhibition of tumors without an activated K-ras. In the sulindac-treated group, seven of eight tumors present after treatment had decreased in size over the treatment period, and five of eight had K-ras mutations. Only one tumor increased in size during the sulindac treatment, and this tumor was K-ras wild type. Our study differs from the in vivo study described above (37) in that the duration of sulindac treatment was for 4 wk rather than 52 wk, and in our study sulindac was not administered before initiation of carcinogenesis. Data from our laboratory indicate that there is no difference in the inhibitory effect of sulindac whether administration is for 4 wk at the end or continuously for the 23-wk duration of the experiment (unpublished data).

No studies to date have examined the relationship between K-ras activation and sulindac sulfone inhibition in colorectal cancer, in vivo or in vitro. One study (41) examined the relationship between H-ras activation and sulindac sulfone inhibition of rat mammary carcinogenesis. This study showed effective reduction of incidence and number of cancers by sulindac sulfone. Interestingly, it demonstrated inhibition by sulindac sulfone of mammary carcinomas with wild-type or mutant codon 12 H-ras, with a greater inhibitory effect against carcinomas with a mutant H-ras genotype. Related in vitro studies suggested that induction of apoptosis may be the chemopreventive mechanism.

Specific carcinogens have been shown to induce unique mutations, and ras oncogenes present in chemically induced animal models have a narrow mutation spectrum. Several studies indicated that H-ras mutations are not detected in DMH-induced colorectal carcinogenesis (15, 20). It could be postulated that sulindac sulfone may exert its inhibitory effect with regard to K-ras codon 12 mutations in DMH-induced colorectal carcinogenesis in a manner similar to that for H-ras codon 12 mutations in mammary carcinogenesis. Our results do not support this, because carcinomas with both wild-type and mutated K-ras (codon 12) were inhibited by treatment with sulindac sulfone (Table 3). There was no greater inhibitory effect observed for colorectal tumors bearing an activated K-ras in our study. Overall, there is no apparent correlation between sulindac sulfone effect and K-ras mutation status in our model.

Given the relatively small number of tumors in the study, it is possible that there may be a difference in K-ras mutation frequency between the treated (sulindac group and sulfone group) and control (vehicle) groups that was unable to be detected. The magnitude of the difference in proportion of K-ras mutations between the groups is ~10% (52% in controls, 62% in sulindac-treated tumors, and 64% in sulindac sulfone-treated tumors). The number of tumors per group in our study ranged from 8 to 21. Because of the limited number of tumors available, this study did not have the power to detect a statistically significant change of this magnitude. However, if either sulindac or sulindac sulfone were selectively inhibiting a particular K-ras genotype, we would expect to see a skewing of these data such that the majority of tumors in a treatment group were of a single K-ras genotype. In our study, neither sulindac nor sulindac sulfone demonstrated selective inhibition of a particular K-ras genotype.

The exact mechanisms of the chemopreventive action of sulindac and sulindac sulfone have not yet been elucidated. Additionally, it has not been determined whether sulindac and its sulfone metabolite, with no known cyclooxygenase (COX) inhibitory activity, mediate their inhibitory effects via different or similar pathways. Previously, the majority of NSAIDs effective in inhibition of colorectal cancer were thought to work via inhibition of COX, and more specifically via inhibition of COX-2 (3, 16, 39). The ability of sulindac sulfone to inhibit colorectal cancer in experimental systems was unexpected given that it has no effect on either COX-1 or COX-2. Recently, we measured prostaglandin levels in tumor tissue after treatment with sulindac (8). We found that, although tumor growth is significantly inhibited by sulindac treatment, prostaglandin levels are unaffected. Currently, a growing body of evidence suggests that both sulindac and sulindac sulfone are mediating their inhibitory effects independently of COX activity (4, 13, 14, 30). There is evidence to suggest that restoration of apoptosis (22, 30, 31, 35) or inhibition of cell proliferation (11, 28, 36) are possible common mechanisms of action of sulindac and sulindac sulfone in mediating tumor inhibition.

In conclusion, the results of our study do not support selective inhibition by either sulindac or sulindac sulfone of colorectal rat tumors on the basis of K-ras mutation status. This suggests that the mechanism of effect of sulindac and of sulindac sulfone is independent of the K-ras status of the tumor.

The authors thank Eric Glare for technical expertise with native PAGE, and Merck, Sharp and Dohme, Sydney, Australia, for the gift of sulindac and sulindac sulfone (pure substance).

This work was supported by a grant from the National Health and Medical Research Council (Australia).
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