Suppression of Intestinal Polyps in Msh2-deficient and Non-Msh2-deficient Multiple Intestinal Neoplasia Mice by a Specific Cyclooxygenase-2 Inhibitor and by a Dual Cyclooxygenase-1/2 Inhibitor

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Centre for Cancer Genetics, Samuel Lunenfeld Research Institute, Departments of Surgery [G. L., C. A., K. H., S. G.] and Pathobiology and Laboratory Medicine [M. R.], Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada. Apgen Institute, Ontario Cancer Institute, Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada. 1/COX-2 inhibitors such as sulindac. Various epidemiological studies and small clinical trials have shown a chemoprotective effect for NSAIDs1 (1) in subjects at average risk for colorectal cancer (reviewed in Refs. 1, 2), as well as in individuals with the inherited colorectal cancer syndrome, familial adenomatous polyposis (3, 4).

The exact mechanism(s) by which NSAIDs reduce colorectal cancer risk and mediate regression of adenomatous colorectal polyps is not entirely clear. Several non-COX-dependent mechanisms have been proposed for the inhibition of colon tumors by NSAIDs, but most of these have been in vitro studies at suprapharmacological concentrations that are unlikely to be achieved in clinical studies. However, it is clear that the inhibition of COX activity and reduction of prostaglandins by NSAIDs can be achieved at clinically relevant concentrations in in vitro and in vivo models that target prostaglandins play a role in cell proliferation, tumor growth, and immunoresponsiveness (1, 5, 6). It is now well recognized that two COX isozymes exist, which are encoded by different genes. COX-1 is constitutively expressed in many mammalian tissues (7, 8), whereas COX-2 expression is inducible and stimulated in inflammatory cells such as monocytes and macrophages by cytokines, mitogens, growth factors, and tumor promoters (9–11). NSAIDs inhibit both COX isoforms, and this accounts for their therapeutic and side-effect profiles (12–14). Although both COX isozymes carry out the same catalytic reaction, producing the precursor prostaglandin PGH2, COX-2 is responsible for the production of the majority of the proinflammatory, hyperalgesic, and pyreatic prostaglandins, whereas COX-1 produced prostaglandins have mainly physiological roles (15–17). Of great clinical significance is that the deleterious side effects related to the administration of NSAIDs, including upper gastrointestinal ulceration and bleeding, are mediated by COX-1 inhibition (18, 19).

Both COX isozymes are present in colon tumors from rodents and humans (20–24). A number of lines of evidence suggest that COX-2 is involved in colorectal carcinogenesis. COX-2 mRNA and protein are elevated in chemically induced colon tumors (22) and in intestinal tumors from Min and ApcΔ716 mice (23, 25). COX-2 is also overexpressed in human colorectal polyps and cancer (20, 21, 24). Tsujii et al. (26) demonstrated that colonic epithelial cells with increased COX-2 expression develop altered adhesion properties and resist apoptosis. ApcΔ716 mice with COX-2 null alleles have significantly fewer intestinal polyps than control ApcΔ716 mice, and the specific COX-2 inhibitors MF-tricyclic and rofecoxib inhibit the number and size of intestinal tumors in these mice (25, 27). Both the epithelial and stromal compartments have been shown to overexpress COX-2 (21, 23–25, 28, 29), and recent data suggest that stromal COX-2 may influence tumor growth by an angiogenic effect (30). On the basis of some of these observations, in addition to the reduced side-effect profile of specific COX-2 inhibitors (31), there is now clinical studies at suprapharmacological concentrations that are unlikely to be achieved in clinical studies. However, it is clear that the inhibition of COX activity and reduction of prostaglandins by NSAIDs can be achieved at clinically relevant concentrations in in vitro and in vivo models that target prostaglandins play a role in cell proliferation, tumor growth, and immunoresponsiveness (1, 5, 6). It is now well recognized that two COX isozymes exist, which are encoded by different genes. COX-1 is constitutively expressed in many mammalian tissues (7, 8), whereas COX-2 expression is inducible and stimulated in inflammatory cells such as monocytes and macrophages by cytokines, mitogens, growth factors, and tumor promoters (9–11). NSAIDs inhibit both COX isoforms, and this accounts for their therapeutic and side-effect profiles (12–14). Although both COX isozymes carry out the same catalytic reaction, producing the precursor prostaglandin PGH2, COX-2 is responsible for the production of the majority of the proinflammatory, hyperalgesic, and pyreatic prostaglandins, whereas COX-1 produced prostaglandins have mainly physiological roles (15–17). Of great clinical significance is that the deleterious side effects related to the administration of NSAIDs, including upper gastrointestinal ulceration and bleeding, are mediated by COX-1 inhibition (18, 19).

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considerable interest in the use of these agents for chemoprevention and treatment of colorectal cancer.

The COX-2 specific inhibitor celecoxib has been shown to inhibit the initiation and progression of azoxymethane-induced colon tumors in rats (32, 33). Although this study provides important in vivo support for the potential use of COX-2 inhibitors as chemopreventive agents in colorectal cancer, chemical carcinogen-induced rodent tumor models may be less relevant models of human colorectal neoplasia compared with genetic models that develop spontaneous tumors. A variety of murine Apc knockout mouse models (such as the Min and ApcΔ716 mouse) develop spontaneous small-bowel polyps and small numbers of large-bowel polyps. A number of studies have shown the positive effects of both dual COX-1/COX-2 inhibitors (34–39), as well as celecoxib (40), in inhibiting primarily small-intestine polyps in Min mice. Our DNA mismatch-repair Min mouse model (Apc+/−;Msh2+/−) has genetic features of both familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer, and most importantly, rapidly develops numerous small- and large-bowel adenomas, as well as colonic aberrant crypt foci (41). The purpose of this study was to determine, for the first time, the effects of COX inhibitors on intestinal adenomas and colonic aberrant crypt foci in this accelerated polyposis, mismatch-repair-deficient Min mouse model, in addition to a standard Min mouse model.

MATERIALS AND METHODS

All experiments were performed in accordance with protocols approved by the Samuel Lunenfeld Research Institute Animal Care Committee.

Mice. Min mice (original breeding pair from The Jackson Laboratory, Bar Harbor, ME) were bred on the C57BL/6J strain (42). In our laboratory, these mice develop ~30–50 adenomas in the small intestine and 4–6 colorectal adenomas by 180–200 days of age, at which time they become moribund and die of anemia and intestinal obstruction. Min mouse adenomas demonstrate loss of the wild-type Apc allele (43). The mismatch-repair-deficient Msh2 mouse (Msh2+/−) was created by gene targeting (44). Heterozygous (Msh2+/−) mice are phenotypically normal, whereas homozygous (Msh2+/−) mice develop diffuse and fatal lymphoblastic lymphoma and, occasionally, invasive intestinal adenocarcinoma (44, 45). Male Min mice were crossed with female Msh2+/− mice to generate Apc+/−;Msh2+/− mice. Male mice of this genotype were then crossed with female Msh2+/− mice to make Msh2-deficient Min mice (Apc+/−;Msh2−/−). This latter genotype demonstrates an accelerated intestinal adenoma phenotype with many polyps appearing by 1 month of age and typically >300 small-bowel adenomas and up to 15–20 large-bowel polyps by 3–4 months of age, when they become moribund (41). These mice also develop dysplastic colonic aberrant crypt foci, a feature unique to this mouse model. In contrast to Min mouse adenomas, which demonstrate loss of heterozygosity of the wild-type Apc in all polyps, loss of heterozygosity is found in only 30% of adenomas from Apc+/−;Msh2−/− mice (41). Apc+/−;Msh2−/+ and Apc+;Msh2−/+ are phenotypically similar to standard Min mice (41, 42).

Ear punch tissue was used to extract DNA for genotyping. This served as a template for PCR-based assays that were used to determine Apc and Msh2 status as described previously (41).

Diets. MF-tricyclic was chosen as the specific COX-2 inhibitor and sulindac as a dual COX-1/COX-2 inhibitor for this study. MF-tricyclic has >500-fold selectivity ratios for COX-2 versus COX-1 inhibition in recombinant cell assays (25). Sulindac and MF-tricyclic were added to mouse chow (Purina Test Diets, Richmond, IN) to achieve doses of ~13 mg/kg of mouse weight/day. At this concentration, plasma concentrations of MF-tricyclic and sulindac sulfide (the active component of sulindac) were estimated to be ~0.5 μM. For MF-tricyclic, this concentration results in >50% inhibition of COX-2, but not inhibition of COX-1 in human whole blood assays. For sulindac sulfide, this concentration results in ~50% inhibition of COX-1 and <50% inhibition of COX-2 in human whole blood assays (46). The diets were prepared monthly and stored at 4°C. Laboratory Autoclavable Rodent Diet (cat. no. 5010) was used as control diet.

Study Design. Weanling Apc+/−;Msh2−/− mice (3 weeks of age) were placed on diets containing sulindac, MF-tricyclic, or no drug. Littermates were distributed among the study groups and weighed weekly. After 4 weeks on diet, mice were sacrificed by cervical dislocation, and intestines were removed immediately and flushed with Ringer’s solution to remove food and fecal debris. The intestines were opened longitudinally, laid flat on Whatman paper, and fixed for at least 3 h in 10% neutral-buffered formalin. Fixed intestines were stained with methylene blue and examined in a blinded fashion for polyps and aberrant crypt foci by both gross inspection and light microscopy as described previously (41). For purposes of counting, the small bowel was arbitrarily divided into three equal segments from proximal to distal (SB1, SB2, and SB3). Representative lesions were harvested for histopathological and immunoblot analysis and snap frozen. Blood (1–2 ml) was also obtained from mice at necropsy and used to extract serum for measurement of drug levels.

Weaning Min mice were also placed on the three diets as described above. These mice were either wild-type or heterozygous at the Msh2 locus because they were littermates of the Apc+/−;Msh2−/− mice. These mice were sacrificed after 22 weeks on diet, and tissues were processed as described above for Apc+/−;Msh2−/− mice.

Drug Levels. Drug concentrations were measured in terminal bleeding plasma samples taken from all mice following sacrifice. Blood was collected in heparinized tubes, and plasma was separated and frozen at −70°C prior to preparation for high-performance liquid chromatographic analysis. Plasma (100 μl) was mixed with an equal volume of acetonitrile and centrifuged at 10,000 × g for 15 min; a 25-μl aliquot of the supernatant was analyzed by reverse-phase high-performance liquid chromatographic separation on a HP1090 system (Hewlett-Packard, Palo Alto, CA) with an Eclipse XDB-C18 rapid resolution column (75 × 4.6 mm; 3.5-μm bead size; Hewlett Packard) for MF-tricyclic, a Symmetry C18 column (150 × 3.9 mm; 5-μm bead size; Waters, Milford, MA) for sulindac, or an Inertsil phenyl column (100 × 3 mm; 5-μm bead size; MetChem Technologies Inc., CA) for sulindac sulfide and sulfoxide, using a 65:35 (0.1% trifluoroacetic acid in water-0.1% trifluoroacetic acid in acetonitrile) solvent at a flow rate of 1 ml/min with monitoring at 220 nm for MF-tricyclic and 330 nm for sulindac and its metabolites. Drug concentrations were determined by comparison to standard curves constructed for each compound under identical conditions.

Western Analysis. Normal mouse bowel and autologous polyp tissue were excised, immediately frozen in liquid nitrogen, and stored at −70°C. Frozen tissues were thawed in ice-cold homogenization buffer (50 mM potassium phosphate (pH 7.1) containing 0.1 mM NaCl, 2 mM EDTA, 0.4 mM phenylmethylsulfonyl fluoride, 60 mg/ml soybean trypsin inhibitor, 2 mg/ml leupeptin, 2 mg/ml aprotinin, and 2 mg/ml pepstatin, (all from Sigma Chemical Co., St. Louis, MO)]. Tissues were disrupted twice, for 15 s each, on ice with a tissue tearer (IKA Laborteknik, Staufen, Germany). Samples were homogenized by sonication at 4°C using a Cole Parmer 4710 series ultrasonic homogenizer (Cole Parmer Instrument Co., Chicago, IL.). Debris was removed by centrifugation at 1,000 × g for 15 min at 4°C, and the resultant supernatants were subjected to centrifugation at 100,000 × g for 45 min at 4°C. Membrane fractions were resuspended in homogenization buffer and then sonicated to obtain a homogeneous membrane suspension. Protein concentrations were determined for each sample by a protein assay kit (Bio-Rad, Mississauga, Ontario, Canada).

Membrane fractions were mixed with 0.5 volume of SDS sample buffer [20 mM Tris-HCl (pH 6.8) containing 0.4% (v/v) SDS, 4% glyceral, 0.24 mM β-mercaptoethanol, and 0.5% bromphenol blue], boiled for 5 min, and analyzed by SDS-PAGE on 9 × 10 cm precast 4–20% Tris-glycine acrylamide gels (NOVEX, San Diego, CA; Ref. 7). Proteins were electrophoretically transferred to nitrocellulose membranes as described previously (7). Primary antisera to COX-1 and COX-2 were used at a final dilution of 1:7000. The secondary horseradish peroxidase-linked goat antirabbit IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA) was used at a dilution of 1:5000. Immunodetection was performed using enhanced chemiluminescence according to the manufacturer’s instructions (Amersham Pharmacia Biotech, Quebec, Canada).

Statistical Analysis. Mice weights, polyps, and aberrant crypt foci counts in various groups were compared by ANOVA. The F statistic was considered significant at P < 0.05. When a significant difference was found between groups, post hoc analyses, including the least significant difference and
Student-Newman-Keuls tests, were conducted to detect significant differences between means of individual groups. Similar significant differences were found with both post hoc analysis tests, and results are presented using the least significant difference test. The experiments had >80% statistical power, and a two-tailed α of 0.05, with at least 10 animals/group based on the following a priori settings: (a) an expected control mean small-bowel polyp number of 300 (SD ± 35), and effect modification of ± 50 polyps in the experimental groups of Apc+/−Msh2+/− mice; (b) an expected control mean small-bowel polyp number of 40 (SD ± 10), and effect modification of ± 10 polyps in the experimental groups of Min mice (same power calculations for large-bowel polyps and aberrant crypt foci in Apc+/−Msh2+/− mice). Post hoc power calculations were made using the means and SDs observed in the present study to derive estimates for effect size for one-way ANOVA, and powers were in excess of those calculated using a priori estimates. All power calculations and analyses were performed with SPSS software.

**RESULTS**

**Study Groups.** Mice with combinations of Apc and Msh2 mutant backgrounds that develop spontaneous intestinal polyps were used in the present study as summarized in Table 1. Food intake was similar among the three groups (data not shown). Of note, the Apc+/−Msh2+/− mice weighed less than Min mice (Apc+/−Msh2+/− or Apc+/−Msh2+/−) of comparable age.

**Drug Levels.** Serum drug levels at sacrifice (mean ± SD, µg/ml) were as follows: sulindac, 0.45 ± 0.53, sulindac sulfide 0.58 ± 0.34, sulindac sulfone, 2.48 ± 0.90, and MF-tricylic, 0.56 ± 0.18.

**Polyp Numbers in Apc+/−Msh2+/− Mice.** The numbers of small-bowel polyps, large-bowel polyps, and aberrant crypt foci in Apc+/−Msh2+/− mice fed control diet or diet containing sulindac or MF-tricylic are shown in Fig. 1. There was an overall statistically significant difference in the mean number of small-bowel polyps among the three groups (Fig. 1A; mean ± SD): control, 341 ± 43; sulindac, 278 ± 80; MF-tricylic, 178 ± 29 (P < 0.001 by ANOVA). More specifically, mice on sulindac and MF-tricylic had fewer small-bowel polyps compared with mice fed control diet (P = 0.042 and P < 0.001, respectively). The reduction in small-bowel polyps in the sulindac and MF-tricyclic groups was uniform throughout the three segments of the small bowel (Fig. 1B). Despite the significant effects of the COX inhibitors on small-bowel polyps in Apc+/−Msh2+/− mice, there was no difference in the number of large-bowel polyps or aberrant crypt foci among the three groups (Fig. 1C).

**Polyp Numbers in Min (Apc+/−Msh2+/− and Apc+/−Msh2+/+) mice.** Similar to the above results, there was also a statistically significant difference in the number of small-bowel polyps in Min mice placed on the three different diets: control, 39 ± 21; sulindac, 27 ± 17; MF-tricylic, 15 ± 13 (P = 0.007 by ANOVA). Both sulindac and MF-tricylic in diets led to a reduction in polyp numbers compared with mice on the control diet (P = 0.057 and P = 0.002 respectively). There was also a statistically significant difference in the number of large-bowel polyps among the groups (control, 4.8 ± 1.8; sulindac, 5.0 ± 3.2; MF-tricylic, 2.3 ± 2.3; P = 0.009) because of reduction in large-bowel polyps in the MF-tricyclic group (P = 0.004; Fig. 2). There was no difference in numbers of aberrant crypt foci among the groups, although Min mice develop very few aberrant crypt foci.

**Western Analysis.** COX-1 protein was identified in both normal bowel and in small- and large-bowel polyps from mice of both genotypes used in this study. A representative immunoblot is shown in Fig. 3. COX-2 expression was much less than COX-1 protein expression. COX-2 protein was evident in polyps, with none or only trace amounts in normal bowel from both Apc+/−Msh2+/− and Apc+/−Msh2+/− (Min) mice. Small-bowel polyps from Min mice and large-bowel polyps from Apc+/−Msh2−/− mice demonstrated higher concentrations of COX-2 protein than the other groups.

**DISCUSSION**

The primary objective of this study was to test the effects of a specific and dual COX-1/COX-2 inhibitor on spontaneous small-intestine polyps and colonic aberrant crypt foci and polyps in a DNA mismatch-repair-deficient Min mouse (Apc+/−Msh2−/−). This model has genetic and phenotypic features of both familial adenomatous polyposis (based on the germline Apc mutation), and hereditary nonpolyposis colorectal cancer (based on germline Msh2 loss). Moreover, the combination of both mismatch-repair deficiency and Apc-mediated tumorigenesis has relevance to sporadic colorectal cancer in humans. A secondary objective of the study was to test the same COX inhibitors in Min mouse littermates (Apc+/−Msh2+/− and Apc+/−Msh2+/+) of the mismatch-repair-deficient mice. Our results provide the first evidence for the beneficial effects of COX inhibitors, especially the specific COX-2 inhibitor, MF-tricyclic, on inhibiting the development of intestinal polyps in mismatch-repair-deficient Min mouse.

**Table 1. Number and sex of mice in the study groups**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Control</th>
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<th>MF-tricyclic</th>
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<td>Apc+/−Msh2+/−</td>
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<td>11 (7 M, 4 F)</td>
<td>9 (5 M, 4 F)</td>
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<tr>
<td>Apc+/−Msh2+/+ or Apc+/−Msh2−/− mice (Min)</td>
<td>17 (6 M, 11 F)</td>
<td>11 (5 M, 6 F)</td>
<td>10 (5 M, 5 F)</td>
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**Table 2. Effect of drugs on Apc+/−Msh2−/− and Min mice**

<table>
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<tr>
<td>Sulindac</td>
<td>11</td>
<td>12.5 (3.8)</td>
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<tr>
<td>MF-tricyclic</td>
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<td>10.8 (3.4)</td>
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<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of mice</th>
<th>Weight (g)</th>
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<td></td>
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<tr>
<td>MF-tricyclic</td>
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<td>12.8 (2.7)</td>
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COX INHIBITORS IN MISMATCH-REPAIR-DEFICIENT MICE

deficient Min mice. Apc−/−Msh2−/− mice had 50% fewer small-bowel polyps, compared with controls, when weaning mice were fed a diet containing MF-tricyclic. In agreement with previous studies with specific COX-2 inhibitors and dual COX-1/COX-2 inhibitors, we show here reduction in intestinal polyps in non-mismatch-repair-deficient Min mice (27, 33–40). In addition, the specific COX-2 inhibitor MF-tricyclic decreased the numbers of large-bowel adenomas in Min mice.

Both sulindac and MF-tricyclic inhibited the development of small-bowel polyps, but not large-bowel polyps or the numerous colonic aberrant crypt foci that arise in Apc−/−Msh2−/− mice. Interestingly, the COX-2-specific inhibitor, MF-tricyclic, inhibited the development of some large-bowel polyps in Min mice. The lack of effect of sulindac on large-bowel polyps in Min mice is consistent with previous studies of aspirin and piroxicam in these mice, in which the greatest chemopreventive effects were observed in the small intestine of Min mice (35, 37, 38). The reasons for this differential effect of various agents on intestinal neoplasia in Min mice has never been explained. It is difficult to reconcile the discrepancy in effects of COX inhibitors, especially MF-tricyclic, on small- and large-bowel polyps and aberrant crypt foci in Apc−/−Msh2−/− mice. A number of possibilities can be proposed, including genetic differences between small- and large-bowel lesions, variations in stromal cell makeup in the small- and large-bowel neoplasms, physiological differences in drug delivery within various regions of the intestine, and variability in COX-2 expression within the small- and large-bowel neoplasms in these mice. The latter explanation is unlikely because COX-2 expression in small- and large-bowel polyps did not correlate with sensitivity to COX inhibitors, i.e., large-bowel adenomas did not express COX-2 to any lesser degree than small-bowel polyps (Fig. 3). This observation highlights the present controversy regarding the precise mechanism(s) by which COX inhibitors mediate their antineoplastic effects (47–49). It is also worth pointing out that neither the standard Min mouse nor the mismatch-repair-deficient Min mouse are perfect surrogates for studies of human colorectal neoplasia because both animal models develop primarily small-bowel adenomas.

We observed ∼50% inhibition of small-bowel adenomas by the specific COX-2 inhibitor MF-tricyclic in both Apc−/−Msh2−/− and Apc−/−Msh2+/− mice. This is similar to the effects of low-dose celecoxib in Min mice recently reported by Jacoby et al. (40). In the latter study, the polyp inhibitory effects were greater in the distal small bowel. We did not observe a differential effect of sulindac or MF-tricyclic in any specific region of the small bowel of Apc−/−Msh2−/− mice.

There is considerable observational evidence that COX-2 inhibitors may be very useful chemopreventive agents for both high-risk individuals as well as the general population at average risk for colorectal cancer. COX-2 is up-regulated and overexpressed in a large fraction of human colorectal cancer cell lines (50), resected colorectal cancers (20, 21), and in a subset of premalignant colorectal adenomas (20). A large body of epidemiological data also support the effectiveness of dual COX-1/COX-2 inhibitors in reducing risk of colorectal cancer (1, 2). These retrospective studies have provided impetus for clinical trials of COX inhibitors in humans at various risk for colorectal polyps and colorectal cancer. A Phase III prospective randomized polyp prevention trial assessing the effectiveness of aspirin is under way. Since the original, somewhat serendipitous observation that the dual COX-1/COX-2 inhibitor sulindac mediates regression of rectal polyps in familial adenomatous polyposis, prospective trials have provided more direct evidence for the polyp inhibitory effects of

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Fig. 1. A, effects of dual COX-1/COX-2 inhibitor (sulindac) and COX-2-specific inhibitor (MF-tricyclic) on inhibition of small-bowel polyps in Apc+/−Msh2−/− mice. Mice were weaned at 3 weeks and then fed diets containing no drug (control; n = 12), sulindac (n = 11), or MF-tricyclic (n = 9). After 4 weeks on diets, mice were sacrificed and small-bowel polyps counted. Columns, mean; bars, SD. #: significantly fewer polyps compared with control; *, significantly fewer polyps compared with control and sulindac. B, effects of dual COX-1/COX-2 inhibitor (sulindac; ) and COX-2-specific inhibitor (MF-tricyclic; ) on inhibition of polyps in different regions of the small bowel in Apc+/−Msh2−/− mice. Same mice as in A. Note the uniform effect of drugs in the proximal (SB1), middle (SB2), and distal (SB3) third of the small bowel. : control. Bars, SD. C, effects of dual COX-1/COX-2 inhibitor (sulindac) and COX-2-specific inhibitor (MF-tricyclic) on inhibition of large-bowel polyps ( ) and colonic aberrant crypt foci (large bowel ACF, ) in Apc+/−Msh2−/− mice. Same mice as in A. No significant differences between any of the groups Bars, SD.

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Fig. 2. Effects of dual COX-1/COX-2 inhibitor (sulindac) and COX-2-specific inhibitor (MF-tricyclic) on inhibition of small-bowel polyps in Min (Apc+/−Msh2−/+ and Apc+/−Msh2+/+) mice. Mice were weaned at 3 weeks and then fed diets containing no drug (control; n = 17), sulindac (n = 11), or MF-tricyclic (n = 10). After 22 weeks on diets, mice were sacrificed and small- (−) and large-bowel (+) polyps counted. Horizontal bars indicate means, *, significantly fewer polyps compared with controls.
We thank Dr. Robert Gryfe for assistance with statistical analyses.

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REFERENCES


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