Chemopreventive Efficacy of Sulindac Sulfone against Colon Cancer Depends on Time of Administration during Carcinogenic Process

Bandaru S. Reddy, Toshihiko Kawamori, Ronald A. Lubet, Vernon E. Steele, Gary J. Kelloff, and Chinthalapally V. Rao

Division of Nutritional Carcinogenesis, American Health Foundation, Valhalla, New York 10595 [R. S. R., T. K., C. V. R.], and Chemoprevention Branch, National Cancer Institute, Bethesda, Maryland 20892 [R. A. L., V. E. S., G. J. K.]

ABSTRACT

Epidemiological and model studies with laboratory animals have provided evidence that nonsteroidal anti-inflammatory drugs reduce the risk of colon cancer. Sulindac, a nonsteroidal anti-inflammatory drug, has been shown to inhibit azoxymethane (AOM)-induced colon carcinogenesis in rats when administered continuously before, during, and after carcinogen treatment (initiation and postinitiation periods) or when given continuously beginning 14 weeks after carcinogen administration (promotion/progression stage). The present study was designed to investigate the chemopreventive efficacy of sulindac sulfone (exisulind), the sulfone metabolite of sulindac, when administered during the promotion/progression stage of colon carcinogenesis in comparison to the effect during the initiation and postinitiation periods. We have also studied the modulating effect of exisulind on colonic tumor apoptosis. At 5 weeks of age, groups of male F344 rats were fed diets containing 0%, 0.06%, and 0.12% exisulind. At 7 weeks of age, groups of animals were injected s.c. with AOM (15 mg/kg body weight, once weekly for 2 weeks). Animals intended for the promotion/progression study and receiving 0% exisulind were switched to an experimental diet containing 0.12% exisulind at 14 weeks after the second AOM treatment. All rats remained on their respective dietary regimens until the termination of the study, 50 weeks after the second AOM injection. Colon tumors were evaluated histopathologically for tumor type. Administration of 0.06% and 0.12% exisulind during the initiation and postinitiation periods significantly inhibited the incidence and multiplicity of invasive and/or noninvasive adenocarcinomas of the colon. The inhibition of colon tumorigenesis by exisulind was associated with a significant retardation of body weight gain shortly after sulindac administration and increased apoptosis in the colon tumors. In contrast, administration of the higher dose (0.12%) of exisulind during the promotion/progression stage had only minimal effects on colon tumorigenesis and apoptosis in the colon tumors, suggesting that early administration, but not late administration, may be required for chemopreventive efficacy of this drug.

INTRODUCTION

Cancer of the colon, a leading cause of cancer mortality in both men and women in Western countries, including the United States and Canada, is preventable when diagnosed at the early stage of development and followed by preventive dietary strategies including potential chemopreventive agents (1, 2). Accumulating evidence from epidemiological, human intervention, and/or laboratory animal model studies indicates that chronic ingestion of NSAIDs, such as aspirin, piroxicam, or sulindac, appears to reduce the risk of colorectal cancer (3), decrease the incidence of colon adenomas (4), reduce the number and size of colonic polyps in patients with familial adenomatous polyposis (5), and protect against chemically induced colon carcinoma (6–10) and spontaneous intestinal adenomas, at least in animal models (11–13). Many chemopreventive agents including NSAIDs are known to possess several anticancer mechanisms that may operate in a coordinated manner during the initiation and postinitiation stage that confers upon them a broader spectrum of chemopreventive activities during different stages of carcinogenesis. Studies in our laboratory have demonstrated that sulindac and piroxicam administered during the promotion/progression stage significantly inhibited the development of AOM-induced colonic adenocarcinomas (7, 14). Although the precise mechanism by which NSAIDs inhibit colon tumorigenesis is not fully known, the available data support the hypothesis that it involves the inhibition of PG synthesis through the modulation of COX activity and/or induction of apoptosis in colonic tumors (13, 15–17).

Sulindac is an anti-inflammatory drug which, after absorption, undergoes two major biotransformations in the liver and in colonic contents by microflora (7, 18–20). These transformations include reversible reduction to the sulfide metabolite, the most potent inhibitor of PG production, and irreversible oxidation to the sulfone metabolite, which has no anti-inflammatory activity and does not inhibit COX (Refs. 19–21; Fig. 1). After oral administration of sulindac to rats, sulindac sulfone (exisulind) and sulindac sulfide were identified in the cecum, stool specimens, and serum (7). Of the two major metabolites of sulindac, sulindac sulfide is known to be a potent inhibitor of both COX-1 and COX-2 enzymes, whereas the sulfone derivative does not directly inhibit either of these enzymes. Administration of sulindac sulfide at a dose of 0.5 mg/day clearly inhibited spontaneous intestinal tumors in the C57B1/6J Min mice, whereas exisulind at the same dose level had no protective effect on intestinal tumorigenesis in this genetic model (22). The inhibition of spontaneous intestinal tumors in this laboratory animal model by sulindac sulfide was associated with a decrease in mucosal PGE2 levels (22). Similarly, Wechter et al. (23) found that exisulind at a dose of 50 mg/kg had minimal effect against spontaneous intestinal tumorigenesis in the APC Min mouse model. On the other hand, a recent study by Piazza et al. (20) showed that dietary administration of 0.1% and 0.2% exisulind given continuously during the initiation and postinitiation stages (before, during, and after carcinogen treatment) significantly inhibited AOM-induced colon tumorigenesis in rats.

The present study was therefore designed to replicate prior studies in the AOM colon cancer model with exisulind and to specifically investigate whether this agent was effective when administered beginning 14 weeks after carcinogen treatment (during the promotion/progression stage). This study design allows us to elucidate the role of this agent in inhibiting the progression and growth of premalignant colon tumors. This is important with regard to the possible clinical use of any agent in the secondary prevention of colon cancer. The multistep nature of carcinogenesis provides many opportunities for chemoprevention interventions with agents targeted to specific mechanisms involved in cancer initiation, promotion, and progression. In addition, we assessed the effect of exisulind on apoptosis in colon tumors because several NSAIDs have been shown to inhibit colon carcinogenesis through the induction of apoptosis (13, 15, 22, 24).
CHEMOPREVENTIVE EFFICACY OF SULINDAC SULFONE

MATERIALS AND METHODS

Materials. AOM (CAS 25843-45-2) was purchased from Ash Stevens (Detroit, MI). Exisulind (Fig. 1) was provided by the National Cancer Institute (Rockville, MD). Weanling male F344 rats were obtained from the Charles River Breeding Laboratories (Kingston, NY). The ingredients of the semipurified diet were bought from Dyets, Inc. (Bethlehem, PA) and stored at 4°C before preparation of experimental diets. Exisulind was added to a modified AIN-76A diet at 0.06% and 0.12%. The modified AIN-76A diet consisted of 20% casein, 0.3% d.l.-methionine, 52% corn starch, 13% dextrose, 5% corn oil, 5% alphacel, 3.5% AIN mineral mixture, 1% AIN vitamin mixture, and 0.2% choline bitartrate (8). All control and experimental diets containing exisulind were prepared weekly in our laboratory and stored in a cold room.

Determination of the MTD of Exisulind. The purpose of this MTD study was to determine the tolerable dose of exisulind in F344 rats. MTD is defined as the highest dose that causes no more than 10% weight decrement compared to the appropriate control diet group and does not produce mortality or any clinical signs of toxicity that would be predicted to shorten the natural life span of the animal. At 5 weeks of age, groups of male F344 rats (six rats in each group) were fed the AIN-76A diet containing 0%, 0.05%, 0.1%, 0.15%, 0.2%, and 0.3% exisulind. Body weights were recorded once weekly for 8 weeks. All animals were killed after 8 weeks, and the organs were examined grossly for any abnormalities. The body weights of animals fed the control diet and diets containing 0.05% and 0.1% exisulind were comparable throughout the study (data not shown). The animals fed 0.15% and 0.2% exisulind showed a retardation of body weight gain ranging from 3–5% beginning the second week, but later on, the body weight loss was about 5–6%. On the other hand, the animals fed 0.3% exisulind exhibited significant toxicity, and the body weight loss reached 12% at the end of the study. The results of this short-term study suggest that the MTD of exisulind is around 0.2%.

Experimental Procedure. The experiment was designed to evaluate the efficacy of 0.06% and 0.12% exisulind administered continuously from 2 weeks before, during, and after carcinogen treatment to the end of the study (designated as the initiation and postinitiation stages; Fig. 2). In addition, 0.12% exisulind was given starting 14 weeks after carcinogen treatment (designated as the promotion/progression stage) until the end of the study. Our past experience with AOM-induced colon carcinogenesis suggests that the colonic neoplasms would have developed by week 14 after carcinogen administration (14). The dose selection was based on our MTD study and previous chemoprevention studies that have used 0.05–0.2% exisulind in animal model assays (20). The experimental methods were as described previously (7). Weanling male F344 rats were quarantined for 1 week and had free access to the AIN-76A diet. As shown in Fig. 2, beginning at 5 weeks of age, groups of animals in the initiation and postinitiation study had access to their respective control and experimental diets containing 0.06% and 0.12% exisulind, whereas the rats for the assays testing efficacy in the promotion/progression stage were fed the control diet. Beginning at 7 weeks of age, the rats intended for carcinogen treatment were injected s.c. with AOM at a dose rate of 15 mg/kg body weight once weekly for 2 weeks, and those intended for vehicle treatment received an equal volume of normal saline. Starting 14 weeks after the second AOM treatment, groups of animals designated for the intervention only during promotion and progression and maintained on control diet began to receive the experimental diet containing 0.12% exisulind (Fig. 2). These dietary regimens were continued until termination of the experiment 50 weeks after the second AOM treatment. Body weights were recorded every week for the first 10 weeks and then recorded every 4–6 weeks. As scheduled, all rats were sacrificed by CO2 euthanasia, and all organs were examined grossly. Colon tumors were evaluated histopathologically. For this evaluation, they were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed by routine procedures with H&E staining. The sections were examined for tumor...
types according to the classification of Pozharisski (25) that is routinely used in our laboratory (26). Upon termination of this study, more than 90% of the colon tumors had developed into adenocarcinomas. All adenocarcinomas were classified as invasive or noninvasive. The noninvasive adenocarcinomas of the colon were growing out of the mucosal layers into the intestinal lumen. Invasive adenocarcinomas penetrated the muscularis mucosa deeply into the intestinal wall and beyond.

The effect of exisulind on apoptosis in colon tumors was determined by established morphological methods (15). Apoptotic cells were evaluated in the paraffin-embedded sections stained with H&E using a light microscope. Apoptotic cells were identified by cell shrinkage, nuclear condensation, and formation of apoptotic bodies (15). The number of cells/microscopic field ranged from 25–40. Crypts were chosen randomly, and 300 cells in the colon were analyzed by the $\chi^2$ test, and tumor incidence was analyzed statistically by $t$ test, and tumor incidence was analyzed statistically by $t$ test, and tumor incidence was analyzed statistically by $t$ test, and tumor incidence was analyzed statistically by $t$ test, and tumor incidence was analyzed statistically by $t$ test, and tumor incidence was analyzed statistically by $t$ test.

RESULTS

General Observation. As summarized in Table 1, the administration of 0.12% exisulind in saline-treated animals significantly retarded body weight gain (13–16%) beginning 1 week after the first treatment with the drug and continued until termination of the study (P < 0.05 to P < 0.0001). In AOM-treated animals, body weights were lower for rats given 0.06% or 0.12% exisulind from weeks 1–24 and 1–36, respectively, after the first treatment with the drug and continued until termination of the study (P < 0.05; $^a$, P < 0.01; $^b$, P < 0.001; and $^c$, P < 0.0001).

Apoptosis. As compared to the control diet, the continuous administration of 0.06% and 0.12% exisulind during the initiation and postinitiation stages significantly reduced the incidence and multiplicity of invasive adenocarcinoma of the colon (P < 0.01). The incidence and multiplicity of noninvasive adenocarcinoma were not affected at this dose level (P > 0.05). In the rats given 0.12% exisulind during the initiation and postinitiation stages, there was a significant inhibition of incidences and multiplicities of both invasive and noninvasive adenocarcinomas of the colon (P < 0.05 to P < 0.001). This reduction in colon tumorigenesis may be confounded by the retardation of body weight gain in animals given sulindac sulfone (Table 1). However, it is noteworthy that administration of 0.12% exisulind during the promotion/progression stage had minimal effects on the incidences and multiplicities of invasive, noninvasive, and total adenocarcinomas of the colon (P > 0.05).

Tumor Data. Table 2 summarizes the AOM-induced colon tumor incidence (percentage of rats with tumors) and multiplicity (number of tumors/rat). There was no evidence of colon tumors in saline-treated animals fed the control and experimental diets. AOM induced adenomas in the colon in about 10% of the rats fed the control diet and adenocarcinomas in about 90% of the rats fed the control diet. Among the latter, 76% of animals showed invasive adenocarcinomas, and 41% of animals exhibited noninvasive adenocarcinomas. Administration of 0.06% exisulind during the initiation and postinitiation stages significantly inhibited the incidence and multiplicity of invasive adenocarcinoma of the colon (P < 0.01). The incidence and multiplicity of noninvasive adenocarcinoma were not affected at this dose level (P > 0.05). In the rats given 0.12% exisulind during the initiation and postinitiation stages, there was a significant inhibition of incidences and multiplicities of both invasive and noninvasive adenocarcinomas of the colon (P < 0.05 to P < 0.001). This reduction in colon tumorigenesis may be confounded by the retardation of body weight gain in animals given sulindac sulfone (Table 1). However, it is noteworthy that administration of 0.12% exisulind during the promotion/progression stage had minimal effects on the incidences and multiplicities of invasive, noninvasive, and total adenocarcinomas of the colon (P > 0.05).
DISCUSSION

The overall objective of these studies is to identify effective and safe chemopreventive agents that will facilitate the development of cancer-preventive strategies and their application in clinical setting. The specific objective of the present investigation, which is part of a large-scale preclinical efficacy study of NSAIDs as chemopreventive agents against cancer of the colon, was to confirm the chemopreventive efficacy of exisulind observed previously in the AOM colon cancer model and to determine the chemopreventive efficacy of this agent administered during the promotion/progression stage of colon carcinogenesis. In particular, this preclinical model may provide baseline information for possible evaluation of this agent for its suitability in late intervention/prevention protocols of colonic premalignant lesions among high-risk individuals. The results of this study demonstrated that the administration of 0.06% or 0.12% exisulind in the diet during the initiation and postinitiation periods significantly inhibited colon tumorigenesis. These results are in agreement with a previous study that had shown that dietary administration of 0.1% and 0.2% exisulind during the initiation and postinitiation stages significantly suppressed colon carcinogenesis (20).

The results of the present study also demonstrate that administering 0.12% exisulind 14 weeks after carcinogen treatment (the promotion/progression stage) had a minimal effect on colon tumor inhibition. A question arises as to why colon carcinogenesis is inhibited when exisulind is administered throughout the experiment (the initiation and postinitiation periods), but not when it is administered beginning 14 weeks after AOM treatment (the promotion/progression period). In this connection, it is noteworthy that the administration of NSAIDs such as piroxicam, sulindac, and curcumin, a naturally occurring anti-inflammatory agent, during the promotion and progression stages is equally as effective when they are administered continually during both the initiation and postinitiation phases (7, 14, 27). This result implies that the efficacy of these NSAIDs is primarily during the latter stages of carcinogenesis (the promotion/progression phase) in contrast to the efficacy of exisulind, which is relatively ineffective during these phases. This result emphasizes two aspects: (a) the effects of exisulind are quite distinct from those of most of the NSAIDs; and (b) these differential effects argue strongly that the efficacy of sulindac is not primarily mediated by its sulfone metabolite, exisulind.

The colon tumor inhibition by sulindac has been attributed to its ability to block PG synthesis via COX and to enhance apoptosis (7, 13, 15). Several studies have shown that the levels of PGs and COX activities, specifically, COX-2 activity, are elevated in colon tumors (28–30). Oshima et al. (31) demonstrated that induction of COX-2 is a very early event in the sequence of polyp formation in the colon. Therefore, suppression of PG production via modified COX activity by NSAIDs, including sulindac, provided a mechanistic approach for chemoprevention of colon cancer because PGs affect numerous aspects of tumorigenesis including cell proliferation and immune responsiveness. In contrast, exisulind lacks anti-inflammatory activity and does not inhibit PG synthesis (20, 22). Thus, the mechanism by which exisulind exerts its chemopreventive activity would appear to be distinct from that of the NSAIDs, a result indirectly confirmed by the different time of sensitivity of sulindac and exisulind exhibited here.

Several reports refer to apoptosis as one of the mechanisms of NSAID-induced tumor inhibition (13, 15, 22, 24, 32). The present study demonstrates that continual administration of exisulind throughout the initiation and postinitiation stages increases apoptosis in the colon tumors, whereas there is no effect on apoptosis when this sulindac metabolite is administered during the promotion/progression stage. This interesting but unexpected result warrants further study. Of interest in this regard is our previous observation that the inhibition of colon carcinogenesis by sulindac, the parent compound of sulindac sulfone, given during the promotion/progression stage was associated with the induction of apoptosis in colon tumors (15).

In conclusion, this study has explored for the first time whether the sulindac metabolite exisulind exhibits chemopreventive efficacy when administered to rats during the promotion/progression stage of colon carcinogenesis. This study confirms the chemopreventive effects of exisulind when administered throughout the carcinogenic process but shows the limited chemopreventive efficacy of exisulind during the promotion/progression phase, in contrast to the effects of its parent compound sulindac and many other NSAIDs. These results demonstrate clear differences between exisulind and sulindac.

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