Antitumoral Drugs Sulindac Sulfide and Sulfone Inhibit Cell Growth by Inducing Apoptosis

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ABSTRACT

The nonsteroidal anti-inflammatory drug sulindac is known to inhibit chemical carcinogenesis in rodent models and cause regression of adenomas in patients with adenomatous polyposis coli. Sulindac is a prodrug that is metabolized to a pharmacologically active sulfide derivative that potently inhibits prostaglandin synthesis. Recent studies, however, have shown that a sulfide derivative of sulindac, which essentially lacks prostaglandin synthesis inhibitory activity, also inhibits chemical carcinogenesis, suggesting that reduction of prostaglandin levels is not necessary for the antineoplastic activity of this class of drugs. Both sulindac sulfide and the sulfone inhibit the growth of cultured tumor cells, although the cellular mechanism(s) responsible for the antineoplastic activity of sulindac derivatives is unknown. In this study, we investigated the effects of sulindac sulfide and sulfone on the proliferation, differentiation, and apoptosis of HT-29 human colon carcinoma cells. Sulindac sulfide and sulfone significantly reduced cell number in both preconfluent and confluent cultures of HT-29 cells with the sulfide showing approximately 4-fold greater potency. In addition to HT-29 cells, both drugs inhibited the growth of a variety of tumor cell lines derived from other tissues, as well as normal epithelial cells and fibroblasts. Neither sulindac sulfide nor sulfone inhibited cell proliferation under conditions where the drugs were growth inhibitory. Only under specific conditions involving mitogenic stimulation did sulindac sulfide and sulfone cause cell cycle arrest. Neither sulindac sulfide nor the sulfone induced differentiation of HT-29 cells, but both drugs strongly induced apoptosis. The apoptotic response to sulindac sulfide and sulfone was both time- and dose-dependent and involved a mechanism independent of their inhibitory effect on cell cycle progression. These data suggest that apoptosis is responsible for the cell growth inhibitory activity of sulindac sulfide and sulfone and represents a potential mechanism for the antineoplastic activity of these drugs.

INTRODUCTION

p.o. administration of the NSAID5 sulindac inhibits tumor formation in rodent models of chemically induced colon carcinogenesis (1, 2) and causes regression of polyps in patients with adenomatous polyposis coli (3–5). Sulindac is a prodrug that requires metabolism to a sulfide derivative (cis-5-fluoro-2-methyl-1-[p-methylthiobenzyliden]-3-indeny lacetic acid) that is known to be exclusively responsible for its anti-inflammatory activity (6, 7). The anti-inflammatory activity (8, 9) and gastrointestinal toxicity (10) of sulindac and other NSAIDs are attributed to the inhibition of cyclooxygenase and the blocking of prostaglandin synthesis.

Until recently, reports in the literature attributed the antineoplastic activity of sulindac and other NSAIDs to their ability to inhibit prostaglandin synthesis. This rationale was based on studies showing that prostaglandins are mitogenic (11) and that high concentrations of prostaglandins exist in certain tumors (12) and tumor cell lines (13). However, recent studies have unexpectedly demonstrated that the sulfone derivative of sulindac (cis-5-fluoro-2-methyl-1-[p-methylsulfonylbenzyliden]-3-indeny lacetic acid), which is known to be devoid of prostaglandin inhibitory activity (6, 7), prevented tumor formation in rodent models of chemical carcinogenesis (14). For example, Hixson et al.3 showed that sulindac sulfone inhibited tumor formation in the azoxymethane-induced colon cancer rodent model without significantly reducing PGE2 levels in the stomach and colon. In addition, Thompson et al. (14) demonstrated that sulindac sulfone has potency comparable to sulindac for inhibiting tumor formation in a mammary gland model using the carcinogen, 1-methyl-1-nitrosourea.

The above findings question the paradigm that inhibition of prostaglandin synthesis mediates the antineoplastic activity of sulindac, as well as other NSAIDs, and may explain several previous reports showing data that are inconsistent with an involvement of the prostaglandin pathway in carcinogenesis. For example, Pepin et al. (15) showed that sulindac inhibits chemically-induced lung tumors in mice and reported that only the sulfone metabolite could be detected in lung epithelium, despite high levels of sulfide in plasma. In addition, Carter et al. (16) evaluated two NSAIDs, indomethacin and carprofen, for their ability to reduce PGE2 levels and inhibit mammary tumors following chemical induction of carcinogenesis. Although both NSAIDs effectively reduced PGE2 levels in mammary epithelium, only indomethacin suppressed tumor formation. Lastly, DeMello et al. (17) reported that indomethacin inhibited the growth of cultured cells at concentrations significantly in excess to those which are required to inhibit prostaglandin synthesis. In these experiments, the addition of exogenous prostaglandins did not reverse the growth inhibitory effects of NSAIDs.

The cellular basis for the antitumor activity of NSAIDs is also controversial. Although a number of reports have shown that NSAIDs, as well as sulindac sulfone, inhibit the growth of cultured tumor cells (17–24), it is not clear if this effect involved a decrease in the rate of cell proliferation (cell cycle progression) or an increase in the rate of cell death by either an apoptotic or necrotic mechanism. Several reports describing effects on cultured cells suggested an antiproliferative effect, involving a block in the G1 phase of the cell cycle, is responsible for the growth inhibitory activity of NSAIDs (18–22). However, rodent studies have not been able to confirm an antiproliferative effect of NSAIDs. For example, Ahnen et al. (25) showed no inhibitory effect of either sulindac or sulindac sulfone on the BrdUrd-labeling index in colonic tumors or normal colonic mucosa from azoxymethane-treated rats. In the same model, neither drug inhibited the proliferation of colonocytes within aberrant crypt foci, which represent early precancerous lesions (26). Together, these reports emphasize the need for additional investigation of the cellular mechanism responsible for the antineoplastic activity of sulindac derivatives. In this paper, we determine if the growth inhibitory
MATERIALS AND METHODS

Drug Synthesis. The sulfide and sulfone metabolites of sulindac were synthesized as follows: cis-5-fluoro-2-methyl-1-(p-methylthiobenzylidene)-3-indenylacetic acid (sulfide) was prepared according to the procedure described by Shuman et al. (27) and was found to be 99.7% pure. cis-5-Fluoro-2-methyl-1-(p-methylsulfonylbenzylidene)-3-indenylacetic acid (sulfone) was prepared from sulindac sulfone (Sigma Chemical Co., St. Louis, MO). The reaction involved oxidation with H2O2 resulting in a 99.7% yield.

Drug Preparation. Stock solutions of sulindac sulfide and sulindac sulfone were made at 1000 X concentrations in DMSO and diluted with media for cell culture testing. The final concentration of DMSO for all treatments was maintained at 0.1%. All drug solutions were prepared fresh on the day of testing.

Cell Culture and Treatments. The human colon carcinoma cell line, HT-29, was used for the majority of experiments described. HT-29 cells were obtained from ATCC (Rockville, MD) and grown in RPMI media (Celox, Hopkins, MN) supplemented with 5% FCS (Gemini Bio-products, Inc., Calabasas, CA), and 2 mm glucose, 100 units/ml penicillin, 100 units/ml streptomycin, and 0.25 mg/ml amphotericin (GIBCO-BRL, Grand Island, NY). Cultures were maintained in a humidified atmosphere of 95% air and 5% CO2 at 37°C. The cultures were passaged at preconfluent densities with the use of trypsin and 0.25 μg/ml amphotericin (GIBCO-BRL, Grand Island, NY). Cultures were passaged at preconfluent densities listed above and allowed to grow to confluency (approximately 10 000 cells/cm²) before dosing.

Cell Proliferation. Drug effects on cell proliferation were evaluated after measuring cell cycle distribution by flow cytometry. Cell cycle distribution was determined based on DNA content, which was measured after labeling with propidium iodide as described previously (29). Cell suspensions from either preconfluent or confluent cultures were prepared by trypsinization and washed twice with PBS by centrifugation at 300 X g. For each plate, 6 wells were designated as no-treatment controls, 6 wells as vehicle (0.1% DMSO) controls, and the remaining wells for drug dilutions with 6 wells/drug concentration. The cultures were treated for 6 days and then fixed by the addition of cold trichloroacetic acid to a final concentration of 10%. The SRB assay was performed as described previously (28).

Cell Differentiation. Drug effects on HT-29 cell differentiation were determined by measuring ALP activity as described previously (30, 31). Floating and attached cells were collected by trypsinization and washed 3 times with PBS by centrifugation at 300 X g. The cells were lysed in a 1-ml solution containing 0.25% sodium deoxycholate for 30 min at room temperature. The lysate was then centrifuged at high speed (13 000 X g) for 15 min at 4°C. An aliquot (0.5 ml) of the supernatant was then removed and stored in liquid nitrogen until assayed for ALP activity as described previously (32). ALP activity was measured at 25°C with the use of the Alkaline Phosphatase kit (procedure no. 245; Sigma). Protein concentrations of extracts were determined with the use of a protein assay kit (Bio-Rad, San Francisco, CA).

RESULTS

Growth Inhibitory Activity. The growth inhibitory activity of sulindac sulfide and sulfone was initially determined on preconfluent HT-29 cell cultures after 7 days of treatment. Dose-response experiments as shown in Fig. 1a demonstrated by the SRB-binding assay that sulindac sulfide reduced cell number with an IC50 value of approximately 45 μM. A dose 4–5-fold higher of sulindac sulfone (IC50 = 200 μM) was required to reduce cell number to an equal level. Similarly, sulindac sulfide and sulfone also reduced the uptake of neutral red dye and total BrdUrd incorporation, indicating that both drugs decrease viable tumor cell number (data not shown). Time course experiments demonstrated that the growth inhibitory effect of the drugs was most apparent after 4–7 days of treatment (data not shown). In addition to preconfluent cultures, sulindac sulfide and sulfone also reduced cell number in confluent HT-29 cell cultures as shown in Fig. 1b, although approximately 3–4-fold higher concentrations of both drugs were required relative to preconfluent cultures. For example, the IC50 value for sulindac sulfide increased to approximately 120 μM, whereas a concentration of 480 μM sulindac sulfone was required to reduce cell number by 50%.

Sulindac sulfide and sulfone also inhibited the growth of a variety of other cell systems. As shown in Table 1, tumor cell types derived from various tissues were inhibited by sulindac sulfide and sulfone. In addition, the growth of normal cells, such as early passage bronchial epithelial cells, was also inhibited by sulindac sulfide and sulfone.

Effects on Cell Proliferation. Previous studies have suggested that NSAIDs inhibit cell growth by reducing the rate of cell proliferation (18–22). However, in both preconfluent and confluent HT-29 cell cultures, neither sulindac sulfide nor sulfone inhibited BrdUrd uptake when standardized on a per cell basis (data not shown). Because these measurements were made after relatively late treatment times (4–7 days), it was feasible that an early delay in cell cycle progression could account for the net reduction in cell number. To test this possibility, the percentage of proliferating cells in preconfluent cultures was measured after early drug treatment periods by flow cytometry.
cytometric analysis of DNA content with the use of culture conditions identical to those for growth inhibition experiments. As summarized in Table 2, neither sulindac sulfide nor sulfone decreased the percentage of cells in the S phase within 8–48 h of treatment in preconfluent cultures. Similar results were obtained in confluent cultures (data not shown).

Because a previous report demonstrated that sulindac sulfide was capable of inhibiting cell proliferation after mitogenic stimulation (23), we determined if sulindac sulfide and sulfone inhibited HT-29 cell proliferation under conditions involving mitogenic stimulation. To test this possibility, confluent HT-29 cell cultures were used that contained a high percentage (90%) of resting cells in G0-G1. Following media replenishment, a sizable fraction were synchronously stimulated to undergo proliferation. As shown in Fig. 2a, the percentage of cells in S phase increased from approximately 10 to 55% of the population within 24 h following replenishment with fresh media. The entry of cells into S phase was completely suppressed in cultures treated with sulindac sulfide and suppressed by approximately 70%

![Fig. 1. Dose-dependent inhibition of HT-29 cell growth in preconfluent (a) and confluent (b) cultures by sulindac sulfide and sulfone. Preconfluent or confluent HT-29 cells were established in 96-well microtiter plates. For preconfluent cultures, the indicated concentration of sulindac sulfide (○) or sulindac sulfone (●) was added to the plates 24 h after seeding without replacing the media. For confluent cultures, the indicated concentration of sulindac sulfide or sulfone was added 4 days after plating at a high density. Cells were treated for 7 days and assayed for SRB binding as described in “Materials and Methods.” Each determination represents average absorbance of six replicates. The effect of each treatment was calculated relative to vehicle (0.1% DMSO) control.](image)

with sulfone treatment. In untreated cultures the percentage of cells in G0-G1 decreased as the percentage of cells in S phase increased, whereas the percentage of cells in G2-M did not significantly change in cultures treated with the sulfide or sulfone (Fig. 2b). These results indicate that the inhibitory effect on cell cycle progression results from an arrest in G0 of the cell cycle. It is of interest that neither drug reduced the percentage of cells in S phase if it was added 48 h after media replenishment; at this time the percentage of cells in S phase returned to levels observed before stimulation (see below; Table 3).

**Effects on Cell Differentiation.** Altered differentiation could account for the growth inhibitory activity of sulindac sulfide and sulfone. This possibility was tested by measuring ALP activity, a marker of colonocyte differentiation (30, 31). As shown in Fig. 3, treatment with sodium butyrate, a known inducer of HT-29 cell differentiation, caused the cells to enlarge, which is characteristic of differentiated cells (data not shown). Cell size was actually decreased with sulfone treatment. In untreated cultures the percentage of cells in G0-G1 decreased as the percentage of cells in S phase increased, whereas the percentage of cells in G2-M did not significantly change in cultures treated with the sulfide or sulfone (Fig. 2b). These results indicate that the inhibitory effect on cell cycle progression results from an arrest in G0 of the cell cycle. It is of interest that neither drug reduced the percentage of cells in S phase if it was added 48 h after media replenishment; at this time the percentage of cells in S phase returned to levels observed before stimulation (see below; Table 3).

**Induction of Apoptosis.** Increased cell death by either an apoptotic or necrotic mechanism could account for the growth inhibitory activity of sulindac sulfide and sulfone. This possibility was tested by measuring ALP activity, a marker of colonocyte differentiation (30, 31). As shown in Fig. 3, treatment with sodium butyrate, a known inducer of HT-29 cell differentiation (30, 31), significantly increased the level of ALP activity, but neither sulindac sulfide nor sulfone increased ALP activity relative to controls. Light microscopic examination of treated cells revealed that sodium butyrate caused the cells to enlarge, which is characteristic of differentiated cells (data not shown). Cell size was actually decreased in cultures treated with either sulindac sulfide or sulfone. These data rule out a possible effect of sulindac sulfide and sulfone on differentiation.

**Table 1 Growth inhibition of various cell types by sulindac sulfide and sulfone**

<table>
<thead>
<tr>
<th>Cell type/tissue specificity</th>
<th>IC50 (µM)</th>
<th>Sulindac sulfide</th>
<th>Sulindac sulfone</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCT116, colon</td>
<td>45</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>MCF7/S, breast</td>
<td>30</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>UACC375, melanoma</td>
<td>50</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>A-427, lung</td>
<td>90</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Bronchial epithelial cells (normal)</td>
<td>30</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>NRK, kidney (normal)</td>
<td>50</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>NRK, kidney (transformed)</td>
<td>60</td>
<td>240</td>
<td></td>
</tr>
</tbody>
</table>

*a* Cell lines were established using the culture and treatment conditions as described in "Materials and Methods." IC50 values were determined by the SRB assay.

*b* NRK, normal rat kidney fibroblast; NRK, Kirsten sarcoma virus-transformed NRK.

**Table 2 Effect of sulindac sulfide and sulfone on DNA cell cycle distribution in preconfluent HT-29 cells**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
<th>G0-G1</th>
<th>G2 + M</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8</td>
<td>51.4</td>
<td>13.1</td>
<td>17.5</td>
</tr>
<tr>
<td>Sulfide (60 µM)</td>
<td>24</td>
<td>56.3</td>
<td>22.0</td>
<td>21.6</td>
</tr>
<tr>
<td>Sulfone (120 µM)</td>
<td>24</td>
<td>66.3</td>
<td>21.4</td>
<td>12.4</td>
</tr>
<tr>
<td>Sulfone (60 µM)</td>
<td>48</td>
<td>68.6</td>
<td>15.0</td>
<td>16.4</td>
</tr>
<tr>
<td>Sulfone (120 µM)</td>
<td>48</td>
<td>62.6</td>
<td>22.7</td>
<td>14.7</td>
</tr>
<tr>
<td>Sulfone (120 µM)</td>
<td>68.1</td>
<td>13.3</td>
<td>18.4</td>
<td></td>
</tr>
</tbody>
</table>

*a* Preconfluent HT-29 cells were established in 75-cm² flasks at a density of 50,000 cells/cm². After 24 h the cultures were treated with vehicle (0.1% DMSO), sulindac sulfide (60 µM), or sulindac sulfone (120 µM) for the indicated time and harvested by trypsinization.

*b* Cells were processed for DNA cell cycle analysis by propidium iodide labeling as described in "Materials and Methods." Data are expressed in percentage of cells in each cell cycle phase. Similar effects were observed in 3 separate experiments.

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**DISCUSSION**

Sulindac treatment is known to cause regression of colonic adenomas in patients with adenomatous polyposis coli (3–5), but the cellular and biochemical mechanism(s) responsible for this dramatic effect has not been established. Sulindac is a prodrug (a sulfoxide derivative) that is metabolized to a sulfide and sulfone derivative. Because of its ability to inhibit prostaglandin synthesis, the sulfide derivative is believed to be exclusively responsible for the anti-inflammatory activity of sulindac. We (25) and others (14) have reported that p.o. administration of the sulfone derivative has cancer chemopreventive

**Table 3. Effects of sulindac sulfide and sulfone on cell cycle distribution and apoptosis in paired cultures.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% cell cycle distribution</th>
<th>% apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>G0 + G2 /%</td>
<td>S /%</td>
</tr>
<tr>
<td>Vehicle</td>
<td>87.1 /%</td>
<td>8.9 /%</td>
</tr>
<tr>
<td>Sulindac sulfide (120 /M)</td>
<td>89.2 /%</td>
<td>6.3 /%</td>
</tr>
<tr>
<td>Sulindac sulfone (240 /M)</td>
<td>87.7 /%</td>
<td>6.9 /%</td>
</tr>
</tbody>
</table>

*Two groups of confluent HT-29 cell cultures were established in 25-cm² flasks. Both groups were treated with vehicle (0.1% DMSO), sulindac sulfide (120 /M), or sulindac sulfone (240 /M) 48 h after replenishment of fresh media. Cell cycle distribution was determined after 24 h of treatment in one group and apoptosis was determined after 4 days of treatment in the other group. Similar effects were observed in 3 separate experiments.*

**Fig. 3. Effects of sulindac sulfide and sulfone on HT-29 cell differentiation.** Confluent HT-29 cell cultures were established in 25-cm² flasks and treated for 7 days with vehicle (0.1% DMSO), 5 mm sodium butyrate, 60 /M sulindac sulfide, or 120 /M sulindac sulfone. Cell extracts were prepared from attached and floating cells and assayed for ALP activity as described in "Materials and Methods." Activity is expressed in units/min/mg of protein. Representative results from 1 of 3 separate experiments are shown.
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Efficacy in rodent models of chemical carcinogenesis, suggesting that an inhibitory effect on prostaglandin synthesis is not necessary for the antineoplastic activity of this class of drugs. Moreover, both sulindac sulfide and sulfone have been shown to inhibit the growth of cultured colon cancer cell lines (24). In this report, we extend these observations to other cell systems and investigate the cellular basis for the growth inhibitory activity of sulindac sulfide and sulfone. We hypothesized three non-exclusive mechanisms involving: (a) inhibition of cell proliferation; (b) altered differentiation; and/or (c) increase in the rate of cell death by either an apoptotic or necrotic mechanism. Data described here suggest that, although sulindac sulfide and sulfone cause an antiproliferative effect under certain circumstances, apoptosis is the likely cellular mechanism responsible for the antineoplastic properties of sulindac derivatives.

Available data suggest that inhibition of proliferation is not necessary for sulindac sulfide and sulfone to inhibit cell growth. For example, neither drug reduced BrdUrd uptake on a per cell basis in either preconfluent or confluent cultures after 4-7 days of treatment. In addition, neither drug perturbed cell cycle distribution after early treatment times. In contrast to these findings, previous reports by DuBois et al. (23) showed that sulindac sulfide does inhibit DNA synthesis after mitogenic stimulation with the peptide growth factor, transforming growth factor α. Because the experiment described by DuBois et al. (23) involved confluent cultures (quiescent cells) undergoing mitogenic stimulation, it was possible that sulindac derivatives may have a specific inhibitory effect on quiescent cells entering the cell cycle. Confluent HT-29 cell cultures, which contained a high percentage of cells in G0-G1 of the cell cycle and can be stimulated to undergo mitogenesis after media replenishment, were used to test this possibility. The results showed that both sulindac sulfide and sulfone specifically inhibited the mitogenic effect from media replenishment. Thus, both sulindac sulfide and sulfone have the potential to inhibit cell cycle progression under specific conditions. However, this effect cannot
fully explain the growth inhibitory activity of sulindac derivatives because the drugs inhibited growth under conditions where an antiproliferative effect did not occur.

Altered differentiation was considered as an alternative mechanism by which sulindac sulfide and sulfone inhibit tumor cell growth because certain differentiation agents (i.e., retinoids and vitamin D) have growth inhibitory properties. Despite this we found no evidence to support the possibility that differentiation is altered by sulindac derivatives. Because cell differentiation is often accompanied by an inhibition of DNA synthesis, the lack of effect of sulindac derivatives on differentiation is consistent with data showing that this class of drugs does not inhibit cell proliferation.

Increased cell death by either an apoptotic or necrotic mechanism is a third potential mechanism responsible for the growth inhibitory activity of sulindac derivatives. As background, necrosis and apoptosis are two types of cell death that display different morphological and functional characteristics (34). Necrosis involves the disruption of plasma membrane integrity, whereas apoptosis is considered to be a physiological process where plasma membrane integrity is maintained. To test the possibility that sulindac sulfide and sulfone induce apoptosis and/or necrosis, we used the acridine orange/ethidium bromide assay, which allowed for simultaneous measurement of both types of cell death based on chromatin structure, as well as membrane permeability. Sulindac sulfide and sulfone treatments were found to induce apoptosis in a dose- and time-dependent manner. The drugs induced apoptosis within the same dose and time range and occurred under cell culture conditions where both drugs inhibited cell growth. We, therefore, conclude that the growth inhibitory activity of sulindac metabolites on cultured tumor cells is caused by increasing the rate of apoptotic cell death and speculate that this cellular mechanism is responsible for the antineoplastic properties of sulindac and other NSAIDs.

The biochemical mechanism responsible for the ability of sulindac derivatives to induce apoptosis is not clear. It is likely that the mechanism does not require inhibition of prostaglandin synthesis because the sulfone derivative of sulindac, which lacks inhibitory activity on COX-1 or COX-2 (14), effectively induced apoptosis. Moreover, prostaglandins have been reported previously to induce apoptosis in ovarian epithelium (35). If inhibition of prostaglandin synthesis mediated the apoptotic activity of sulindac derivatives, one would predict that prostaglandins would inhibit, rather than induce, apoptosis.

Cancer chemotherapeutic drugs, such as cisplatin and etoposide, induce apoptotic cell death after arresting the cell cycle in G₂ phase (36). The tumor suppressor gene, p53, which regulates cell cycle progression, is necessary for chemotherapeutic drugs to induce apoptosis (37). Therefore, the ability of sulindac derivatives to arrest the cell cycle could explain the apoptotic activity of this class of drugs. We, however, observed apoptosis under conditions where neither sulindac sulfide nor sulfone altered cell cycle progression, suggesting that induction of apoptosis involves a p53-independent mechanism. It is provocative that certain chemopreventive drugs are able to mimic the apoptotic activity of chemotherapeutic drugs without arresting the cell cycle.

In conclusion, regulators of apoptosis represent a potential intracellular target for sulindac sulfide and sulfone, and possibly other NSAIDs that display cancer chemopreventive activity. Studies are in progress to determine if known regulatory mechanisms of apoptosis are altered by this class of drugs. Alternatively, new biochemical pathways that lead to apoptotic cell death may be discovered by additional exploration into the biochemical mechanism of action of sulindac derivatives and may provide insight to novel technologies for cancer chemoprevention.

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