SHORT COMMUNICATION

Sulindac suppresses tumorigenesis in the Min mouse


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The Min mouse provides a genetically defined model for inherited and sporadic forms of human colorectal tumorigenesis. To test the suitability of this model for the evaluation and optimization of chemopreventive agents, we examined the effects of sulindac on tumorigenesis in Min mice as this compound can inhibit colorectal tumorigenesis in human familial adenomatous polyposis patients. Treatment of Min mice with sulindac in their drinking water (84 mg/l) or diet (167 and 334 p.p.m.) resulted in a significantly decreased average tumor load. The conservation of sulindac activity in the Min mouse provides an opportunity to explore the mechanism of sulindac suppression as well as to test other potential chemopreventive agents.

Alterations of the adenomatous polyposis coli (APC) gene play a critical role in the development of both inherited and sporadic forms of colon cancer. Germline mutations of the APC gene result in familial adenomatous polyposis (FAP), an autosomal dominant inherited predisposition to colorectal cancer (1–4). Patients with FAP develop hundreds of colorectal adenomas, some of which inevitably progress to cancer. Although FAP patients with germline mutations of APC account for less than 1% of colorectal cancer in the USA, somatic mutations of the APC gene occur in the vast majority of sporadic colorectal cancers (5–7). Such alterations can be found in the smallest lesions examined, suggesting that they are an early event, if not the initiating event, in colorectal tumorigenesis (8,9). Like human FAP patients, mice with germline mutations of the murine homolog of the APC gene develop intestinal neoplasias (10–12). The Min mouse was the first such mouse described (10) and contains a nonsense mutation of the Apc gene at codon 850 (11). Min mice on a standardized sensitive genetic background (C57BL/6) develop an average of 30–60 macroscopically visible intestinal tumors by the age of 90 days (13). Thus, these mice provide a good model for colorectal tumorigenesis at both the genotypic and phenotypic levels.

A growing number of studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of colorectal cancer. Several epidemiological studies have found that aspirin use is associated with a reduced risk of death due to colorectal cancer (14–19). Additionally, colorectal tumorigenesis can be suppressed in FAP patients by treatment with the NSAID sulindac (20–24). To practically demonstrate the utility of the Min mouse model in chemoprevention, we evaluated whether sulindac could suppress tumorigenesis in Min mice.

In an initial study, we administered sulindac in the drinking water. Ninety nine Min mice were randomly divided among three treatment groups, maintaining sexual balance in each group. Mice were maintained from weaning (28 days of age) until necropsy (94 days of age) on buffered drinking water (4 mM sodium phosphate, pH 7.4) containing 0, 21 or 84 mg/l sulindac (Sigma). Mice had ad libitum access to both buffered water and chow (Purina 5010). Water consumption studies conducted prior to initiation of the study indicated that the sulindac did not have an adverse effect on the drinking behavior and that the expected drug levels would correspond to doses of ~0, 5 and 20 mg/kg/day sulindac. After sacrifice, the entire intestines were removed, opened, spread out on filter paper with the lumen side up and fixed in 3.7% formaldehyde. Macroscopically visible tumors along the entire length of the small and large intestines were counted by an observer blind to the treatment. While sulindac at 21 mg/l produced no effect, sulindac at 84 mg/l produced a statistically significant decrease in tumor number (P < 0.001, Student's t-test; Table I). In a fourth treatment group, 36 animals were exposed to sulindac starting in utero; their pregnant mothers were maintained on sulindac 7 days prepartum and throughout nursing. In this case, whole litters were randomly assigned to treatment groups and necropsied at 104 days of age. Interestingly, this treatment produced a significantly improved response over the same dose given from weaning to 90 days (P < 0.05, one-tailed Student's t-test; Table I). This finding suggests that the timing of drug administration can affect the efficacy of sulindac treatment. This is consistent with studies that suggest that the first 2 weeks of life are especially critical for tumor initiation in Min mice (25).

To confirm these promising initial results, a second study was initiated to explore the efficacy of higher doses of sulindac. While no toxicity was observed after administration of sulindac at the highest doses used in the first study, the solubility of sulindac limited the maximum dosage. Therefore, sulindac was administered in the diet, rather than in the drinking water, for the second study. Furthermore, to eliminate potential differences due to variation in standard chow preparations, the mice were maintained on the purified diet AIN-93G (26,27). Although no contemporaneous studies have been done, this study and others (28; unpublished data) suggest that Min mice fed purified diets develop fewer tumors than Min mice maintained on standard chow. One hundred and two mice were randomly divided among three treatment groups maintaining sexual balance in each group. Mice were maintained from weaning (27 days of age) until necropsy (86 days of age) on a diet containing 0, 167 or 334 p.p.m. sulindac. Diet consumption

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Fig 1. Effects of sulindac in the diet on Min mice. Min mice were fed a diet containing sulindac as described in the text and outlined in Table II. The mean body weights of the mice in each treatment group were plotted at 30, 60 and 90 days. The error bars are the standard deviations of the means.

Table I. Effects of sulindac in the drinking water on intestinal tumorigenesis in Min mice

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>No. of mice</th>
<th>Intestinal tumors</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (buffered water)</td>
<td>34</td>
<td>48.6 ± 27.0</td>
<td></td>
</tr>
<tr>
<td>Low dose (21 mg/l)d</td>
<td>33</td>
<td>52.3 ± 28.5</td>
<td>*P = 0.6</td>
</tr>
<tr>
<td>High dose (84 mg/l)d</td>
<td>32</td>
<td>29.7 ± 17.9*</td>
<td>*P = 0.00008</td>
</tr>
<tr>
<td>High dose (84 mg/l)</td>
<td>36</td>
<td>22.3 ± 17.2*</td>
<td>*P = 0.000007</td>
</tr>
<tr>
<td>0–104 days*</td>
<td></td>
<td></td>
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</tbody>
</table>

*See text for details of treatment.  
| Mean number of tumors ± SD.  
| Two-tailed Student's t-test versus control.  
| Pregnant female mice were started on sulindac ~7 days prepartum. Mother and pups were maintained on sulindac through weaning and pups remained on sulindac until necropsy at 104 days.  
| Significant at the *P < 0.001* level versus control.

studies performed before and twice during the study indicated that the sulindac did not have an adverse effect on the feeding behavior and that the expected drug levels corresponded to doses of ~0, 40 and 80 mg/kg/day sulindac. Although there was a slight decrease (8%) in the mean weights of the high treatment group at 60 days, there was no significant difference in the weights at 90 days, suggesting minimal or no toxicity (Figure 1). However, both concentrations of sulindac produced a striking drop in tumor number (Table II), in agreement with the drinking water study. Histopathological examination of Swiss rolls (29) of the gastrointestinal tract from representative Min mice confirmed the macroscopic observations. Sulindac reduced both the number of adenomas and microscopic dysplastic foci (Table III).

Table II. Effects of sulindac in the diet on intestinal tumorigenesis in Min mice

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>No. of mice</th>
<th>Intestinal tumors</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (0 p.p.m.)</td>
<td>36</td>
<td>32.7 ± 17.5</td>
<td></td>
</tr>
<tr>
<td>Low dose (167 p.p.m.)</td>
<td>32</td>
<td>12.4 ± 5.5*</td>
<td>*P = 0.00000008</td>
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<tr>
<td>High dose (334 p.p.m.)</td>
<td>34</td>
<td>9.8 ± 3.4*</td>
<td>*P = 0.000000005</td>
</tr>
</tbody>
</table>

*See text for details of treatment.  
| Mean number of intestinal tumors ± SD.  
| Two-tailed Student's t-test versus control diet.  
| Significant at the *P < 0.001* level versus control diet.

The studies described above demonstrate that sulindac can inhibit the spontaneous intestinal tumorigenesis that occurs in the Min mouse. These results parallel studies in humans which demonstrate that sulindac can reduce both the number and size of adenomatous polyps in FAP patients (20–24). They are also consistent with studies in rodents which demonstrated the ability of sulindac and other NSAIDs to inhibit chemically induced tumorigenesis (30–36). Studies in tissue culture and patients suggest that sulindac and other NSAIDs promote apoptosis in epithelial cells, providing a potential explanation for their anti-neoplastic effects (37–40). Because the Min mouse model shares both genetic and phenotypic features with human FAP (11), it should provide a good system for studying NSAID and for the evaluation of other compounds for the treatment of FAP. This study and recent studies demonstrating the ability of Bowman–Birk inhibitor (28) and piroxicam
Sulindac suppresses tumorigenesis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of mice</th>
<th>Adenomas</th>
<th>Significance</th>
<th>Dysplastic focal</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>7.1 ± 5.4</td>
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<td>5.9 ± 4.3</td>
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<tr>
<td>Low dose</td>
<td>(0 p.p.m.)</td>
<td>1.8 ± 1.0*</td>
<td>P = 0.01</td>
<td>3.7 ± 2.3</td>
<td>P = 0.18</td>
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<tr>
<td>High dose</td>
<td>(167 p.p.m.)</td>
<td>1.0 ± 1.4*</td>
<td>P = 0.006</td>
<td>2.3 ± 2.5*</td>
<td>P = 0.04</td>
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<tr>
<td></td>
<td>(334 p.p.m.)</td>
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*For each mouse, a single eosin and hematoxylin stained section of a Swiss roll of the entire length of the intestines was examined microscopically. The number of observed tumors is less than those determined grossly because only a portion of the intestines was present in the examined section.


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