Suppressive effects of nimesulide, a selective inhibitor of cyclooxygenase-2, on azoxymethane-induced colon carcinogenesis in mice

Masato Fukutake1, Seiichi Nakatsugi1,2, Takashi Isoi1,2, Mami Takahashi1, Toshihisa Ohta1, Souichi Mamiya1, Yasuaki Taniguchi3, Hidetaka Sato4, Kazunori Fukuda1, Takashi Sugimura1 and Keiji Wakabayashi1,5

1Cancer Prevention Division, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104, 2Osaka Research Laboratory, Sawai Pharmaceutical Co. Ltd, 8-14 Ikue 1-chome, Asahi-ku, Osaka 555, 3Tosu Research Laboratory, Hisamitsu Pharmaceutical Co. Inc., 408 Tashiro Daikan-machi, Tosu, Saga 841 and 4Japan Food Research Laboratories, Tama Laboratory, 6-11-10 Nagayama, Tama-shi, Tokyo 206, Japan

The effects of nimesulide, a selective inhibitor of cyclooxygenase-2 (COX–2) on azoxymethane (AOM)-induced colon carcinogenesis were investigated in mice. AOM at a dose of 10 mg/kg body wt was administered to male ICR mice once a week for 6 weeks. The animals were fed onAIN-76A powder diet containing nimesulide at doses of 200 or 400 p.p.m., starting the day before the first carcinogen treatment until the end of the experiment, at week 30. Administration of nimesulide reduced the incidence of colon carcinomas to 32 and 25% for the AOM + 200 and 400 p.p.m. nimesulide groups, respectively, compared with the AOM + basal diet group (50%). Multiplicities of colon carcinomas in the 200 and 400 p.p.m. nimesulide-treated groups were 0.70 ± 0.28 and 0.35 ± 0.11, respectively, being significantly smaller than the AOM alone value (1.79 ± 0.47). The sizes of the colon carcinomas in the nimesulide-treated groups were also decreased. No significant influence on liver and lung tumor development was apparent. Thus, nimesulide exerted a suppressive effect on AOM-induced colon carcinogenesis in mice.

Introduction

Colon neoplasia is currently one of the major causes of death from cancer in all developed countries and thus the search for effective chemopreventive agents has become very important (1). Epidemiological studies have shown that prolonged use of aspirin is associated with a reduced risk of colorectal cancer (2). Consistent with these data, several non-steroidal anti-inflammatory drugs (NSAIDs) suppressed the development of chemically induced colon carcinomas in rats and intestinal polyps in Min mice with a nonsense mutation of the Apc gene (3–8). In addition, clinical trials have demonstrated that one NSAID, sulindac, causes regression of adenomas in patients with familial adenomatous polyposis (9,10). Thus, clear benefit from cancer in all developed countries and thus the search for effective chemopreventive agents has become very important (1). Epidemiological studies have shown that prolonged use of aspirin is associated with a reduced risk of colorectal cancer (2). Consistent with these data, several non-steroidal anti-inflammatory drugs (NSAIDs) suppressed the development of chemically induced colon carcinomas in rats and intestinal polyps in Min mice with a nonsense mutation of the Apc gene (3–8). In addition, clinical trials have demonstrated that one NSAID, sulindac, causes regression of adenomas in patients with familial adenomatous polyposis (9,10). Thus, clear benefit has been found with NSAIDs as chemopreventive agents against colon carcinogenesis. However, they also have severe side-effects, causing gastritis, gastric ulcers and gastrointestinal bleeding, and this is recognized as a serious problem for clinical application.

The target of NSAIDs is cyclooxygenase (COX), the rate limiting enzyme in the conversion of arachidonic acid to prostanoids. Recently, COX was classified into two isozymes, COX-1 and COX-2 (11,12). COX-1 is considered as a housekeeping gene and prostanoids synthesized via the COX-1 pathway are responsible for various functions such as protection of the gastric mucosa, vasodilatation in the kidney and aggregation of platelets. In contrast, COX-2 is an immediate-early gene, which contributes to pro-inflammatory prostaglandin synthesis. Furthermore, levels of COX-2 mRNA and protein, but not those of COX-1, have been found to be elevated in chemically induced rat colon carcinoma tissues and in human colon carcinomas, when compared with normal mucosa (13–16). Thus, COX-2 has been suggested to be involved in inflammation and carcinogenesis. From these observations, the adverse effects of NSAIDs that inhibit both COX-1 and COX-2 (17) might be avoided by selectively targeting COX-2.

Nimesulide (4-nitro-2-phenoxyethanesulfonanilide), a selective inhibitor of COX-2 belonging to the sulfonamide class (18), is used clinically as an anti-inflammatory drug in several European countries. This compound is a potent anti-inflammatory agent with less ulcerogenic effects than other NSAIDs and severe side-effects have not been recognized (19–22). Recently, we have demonstrated that nimesulide suppresses the formation of aberrant crypt foci (ACF), putative precancerous lesions of the colon, induced by a colon carcinogen, azoxymethane (AOM) in rats (23). Moreover, this COX-2 inhibitor effectively reduced the development of intestinal polyps in Min mice (24). As part of our search for a safer chemopreventive agent for colon cancer, in the present study we examined the effects of nimesulide on AOM-induced colon carcinogenesis in mice.

Materials and methods

Chemicals

Nimesulide was provided by Helsinn Healthcare SA (Pazzalo-Lugano, Switzerland). AOM was purchased from Sigma (St Louis, MO).

Animals

Male ICR mice, purchased from Charles River Japan (Atsugi, Japan) at 6 weeks of age, were housed in plastic cages in an air-conditioned room with a 12 h light–dark cycle. Water and basal diet (AIN-76A; Dyets, Bethlehem, Switzerland). AOM was purchased from Sigma (St Louis, MO).

Experimental methods

A total of 145 male ICR mice, 6 weeks old, were divided into three AOM-treated groups (30 for AOM + basal diet, 40 for AOM + 200 p.p.m. nimesulide and 45 for AOM + 400 p.p.m. nimesulide) and two vehicle groups (30 for saline + basal diet, 20 for saline + 400 p.p.m. nimesulide). The mice received i.p. injections of AOM in sterile saline at a dose of 10 mg/kg body wt or saline only once a week for 6 weeks. The animals in the nimesulide-treated groups were fed the diets containing 200 and 400 p.p.m. nimesulide starting the day before the first carcinogen treatment until the end of the experiment at week 30. Diets containing nimesulide were prepared once a month by mixing with AIN-76A powder diet, and kept at 4°C until use. Fresh diet was provided to the mice once a week. During these processes, dietary

Abbreviations: ACF, aberrant crypt foci; AOM, azoxymethane; COX, cyclooxygenase; NSAIDs, non-steroidal anti-inflammatory drugs.

© Oxford University Press 1939
The significance of differences in the incidences of tumors was analyzed by the χ² test and other differences by Student’s t-test.

Results

Administration of nimesulide in the diet did not affect the feeding and behavior of mice. Based on dietary intake, the daily intake of nimesulide was estimated to be 20.9 and 38.8 mg/kg body wt on average for the 200 and 400 p.p.m. nimesulide-treated groups, respectively. The mean body weight gain was slightly decreased in the nimesulide-treated groups, respectively, being lower than the 50% basal diet group. No intestinal tumors were observed in the body weights among the groups given the basal diet. Two animals in the AOM + basal diet, three animals in the AOM + 200 p.p.m. nimesulide, and five animals in the AOM + 400 p.p.m. nimesulide group died, whereas one animal in the saline group, 54.0 ± 1.92 g in the AOM + 200 p.p.m. nimesulide group, 49.8 ± 1.05 g in the AOM + 200 p.p.m. nimesulide group, 48.5 ± 0.93 g in the AOM + 400 p.p.m. nimesulide group, 54.0 ± 1.92 g in the saline + basal diet group and 52.9 ± 1.57 g in the saline + 400 p.p.m. nimesulide group. Body weight gain was slightly decreased by the AOM treatment, but no significant differences were observed in the body weights among the groups given the carcinogen. Two animals in the AOM + basal diet group, three animals in the AOM + 200 p.p.m. nimesulide group, five animals in the AOM + 400 p.p.m. nimesulide group and one animal in the saline + 400 p.p.m. nimesulide group died, mostly before week 7. These animals were not included in the effective numbers. Data for the incidences (percentage of mice with cancers), multiplicities (number of cancers per mouse) and sizes of colorectal cancers are summarized in Table I. The incidences were reduced to 32 and 25% by the 200 p.p.m. and 400 p.p.m. nimesulide treatments, respectively, being lower than the 50% for the AOM + basal diet group. No intestinal tumors were found in the groups not receiving AOM. The numbers of colorectal carcinomas per mouse were significantly lower in the AOM + 200 p.p.m. nimesulide group (0.70 ± 0.28) and the AOM + 400 p.p.m. nimesulide group (0.35 ± 0.11) than in the AOM + basal diet group (1.79 ± 0.47). The average sizes of the colorectal carcinomas were also smaller in the AOM + 200 p.p.m. nimesulide group (9.00 ± 4.04 mm³) and in the AOM + 400 p.p.m. nimesulide group (1.99 ± 0.83 mm³) than in the AOM + basal diet group (20.6 ± 7.36 mm³). Moreover, carcinomas >5 mm diameter were not found in the nimesulide-treated groups. Colorectal carcinomas were located mainly in the distal colon and were not found in the proximal colon. The mean numbers of carcinomas in the middle colon, the distal colon and the rectum were higher in the AOM alone group than in the 200 and 400 p.p.m. nimesulide-treated groups (Figure 1).

Table II summarizes the results of histological examination of AOM-induced colorectal carcinomas. Most were well differentiated or moderately differentiated adenocarcinomas. Signet ring cell and mucinous type carcinomas were rare, being observed in the middle colon and the rectum, respectively. The proportions of histological types did not differ significantly among the groups. When colorectal carcinomas were classified

![Table I. Suppression of colorectal carcinoma development by nimesulide](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice with colorectal carcinomas (%)</th>
<th>No. of colorectal carcinomas/mouse</th>
<th>Carcinoma volume/mouse (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOM + basal diet</td>
<td>14/28 (50)</td>
<td>1.79 ± 0.47</td>
<td>20.6 ± 7.36</td>
</tr>
<tr>
<td>AOM + 200 p.p.m. nimesulide</td>
<td>12/37 (33)</td>
<td>0.70 ± 0.28</td>
<td>9.00 ± 4.04</td>
</tr>
<tr>
<td>AOM + 400 p.p.m. nimesulide</td>
<td>10/40 (25)</td>
<td>0.35 ± 0.11</td>
<td>1.99 ± 0.83</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent percentages.

Nimesulide was confirmed to be stable. Dietary intake for each group was measured every 2 weeks. Thirty weeks after the first carcinogen treatment, all surviving mice were killed. The major organs, including the intestines, were removed and fixed in 10% formalin neutral buffer solution. The number, size and location of all intestinal tumors detectable without a microscope were examined. Intestinal tumor sizes were determined as previously described (25). All tumors and organs demonstrating apparent abnormalities were embedded in paraffin and sections were stained with hematoxylin and eosin for histological examination.

Statistical analysis

The significance of differences in the incidences of tumors was analyzed by the χ² test and other differences by Student’s t-test.

![Table II. Histological types of AOM-induced colorectal carcinomas in mice](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>Histological type</th>
<th>Total no. of carcinomas</th>
<th>Well differentiated</th>
<th>Moderately differentiated</th>
<th>Signet ring cell</th>
<th>Mucinous</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOM + basal diet</td>
<td></td>
<td>50</td>
<td>42 (84)</td>
<td>7 (14)</td>
<td>0</td>
<td>1 (2)</td>
</tr>
<tr>
<td>AOM + 200 p.p.m. nimesulide</td>
<td></td>
<td>26</td>
<td>19 (73)</td>
<td>6 (23)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>AOM + 400 p.p.m. nimesulide</td>
<td></td>
<td>14</td>
<td>12 (86)</td>
<td>2 (14)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Histograms of colorectal carcinomas and their multiplicities (number of cancers per mouse) are shown in Figure 1. The incidences of colorectal carcinomas were reduced to 32 and 25% by the 200 p.p.m. and 400 p.p.m. nimesulide treatments, respectively, being lower than the 50% for the AOM + basal diet group. No intestinal tumors were found in the groups not receiving AOM. The numbers of colorectal carcinomas per mouse were significantly lower in the AOM + 200 p.p.m. nimesulide group (0.70 ± 0.28) and the AOM + 400 p.p.m. nimesulide group (0.35 ± 0.11) than in the AOM + basal diet group (1.79 ± 0.47). The average sizes of the colorectal carcinomas were also smaller in the AOM + 200 p.p.m. nimesulide group (9.00 ± 4.04 mm³) and in the AOM + 400 p.p.m. nimesulide group (1.99 ± 0.83 mm³) than in the AOM + basal diet group (20.6 ± 7.36 mm³). Moreover, carcinomas ≥5 mm diameter were not found in the nimesulide-treated groups. Colorectal carcinomas were located mainly in the distal colon and were not found in the proximal colon. The mean numbers of carcinomas in the middle colon, the distal colon and the rectum were higher in the AOM alone group than in the 200 and 400 p.p.m. nimesulide-treated groups (Figure 1).

Table II summarizes the results of histological examination of AOM-induced colorectal carcinomas. Most were well differentiated or moderately differentiated adenocarcinomas. Signet ring cell and mucinous type carcinomas were rare, being observed in the middle colon and the rectum, respectively. The proportions of histological types did not differ significantly among the groups. When colorectal carcinomas were classified.

![Fig. 1. Location of AOM-induced colorectal carcinomas in mice with or without nimesulide treatment. Data for AOM + basal diet ( ), AOM + 200 p.p.m. nimesulide ( ) and AOM + 400 p.p.m. nimesulide ( ) groups are presented as mean ± SE values. *Significantly different from the corresponding control at *P < 0.05.](image)
as non-invasive and invasive carcinomas, based on depth of invasion, the mean numbers of both non-invasive and invasive carcinomas per mouse were decreased by treatment with nimesulide.

In addition to colorectal cancers, tumors were also observed in the liver and lung in AOM-treated groups. The incidences of liver tumors were 27% in the AOM + basal diet group, 27% in the AOM + 200 p.p.m. nimesulide group and 30% in the AOM + 400 p.p.m. nimesulide group and no differences were observed among the three groups. The incidences of lung tumors were 29% in the AOM + basal diet group, 35% in the AOM + 200 p.p.m. nimesulide group and 43% in the AOM + 400 p.p.m. nimesulide group. Nimesulide-treated groups tended to show larger lung tumor incidences, but without statistical significance. The majority of these liver and lung tumors were adenomas, with a few adenocarcinomas. In addition, one adenoma was observed in the lung of mouse in the saline + 400 p.p.m. nimesulide group.

Discussion

The present study demonstrates that the COX-2 selective inhibitor nimesulide decreases AOM-induced colorectal carcinomas in ICR mice at concentrations of 200 and 400 p.p.m. in the diet. In contrast, no suppression of tumor development in the liver and lung was noted. We previously reported that nimesulide inhibits AOM-induced ACF formation in the colon of rats (23) and the development of intestinal polyps in Min mice (24). Thus, nimesulide appears to be an effective chemopreventor of intestinal carcinogenesis.

It is suggested that inhibition of tumors by NSAIDs involves the common property of COX suppression and the resultant reduction in levels of prostaglandins in tissues. Recently, evidence has accumulated suggesting that COX-2 may be a major target of NSAIDs for their chemopreventive effects. COX-2 is inducible by a variety of factors, which include cytokines, growth factors and tumor promoters (12). Several reports have demonstrated increased COX-2 expression in human and rodent colorectal carcinomas (13–16) and it has been demonstrated that overexpression of COX-2 may confer a survival advantage on cells by inhibition of apoptosis and a change in cellular adhesion to the extracellular matrix (26,27). COX-2 selective inhibitors have therefore become a focus of attention as effective colon cancer chemopreventive agents.

Nimesulide has been clinically used as an anti-inflammatory drug and it shows potent anti-inflammatory, but less ulcerogenic, activity than other NSAIDs in rats (22). Clinical studies have also provided evidence that nimesulide is better tolerated by the gastrointestinal tract than indomethacin (21). Consistent with these observations, no gastric abnormalities or other adverse effects of nimesulide were observed in the present long-term carcinogenesis study. Recently, the chemopreventive activity of other COX-2 inhibitors was reported: MF tricyclic reduced the number and size of intestinal polyps in ApcΔ716 knockout mice (28) and celecoxib suppressed AOM-induced colon carcinogenesis in rats (29). Several other chemicals, including NS-398, Dnp 697 and SC-58125, have also been shown to be selective COX-2 inhibitors (30–32). Among these, however, only nimesulide has already been employed clinically. Therefore, nimesulide could be a promising candidate in clinical trials to assess the chemopreventive potential against colon cancer in man.

Acknowledgements

This work was supported in part by a grant from the Program for Promotion of Fundamental Studies in Health Sciences of the Organization of Drug ADR Relief, R&G Promotion and Product Review of Japan and a Grant-in-Aid from the Ministry of Health and Welfare for the Second-Term Comprehensive 10-Year Strategy for Cancer Control, Japan.

References


Received on February 17, 1998; revised on June 23, 1998; accepted on July 17, 1998.