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Suppression of Intestinal Polyp Development by Nimesulide, a Selective Cyclooxygenase-2 Inhibitor, in Min Mice

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Nonsteroidal anti-inflammatory drugs (NSAIDs) suppress colon carcinogenesis in man and experimental animals. However, conventional NSAIDs inhibit both cyclooxygenase (COX) isoforms, COX-1 and COX-2, and cause gastrointestinal side-effects. Nimesulide, a selective inhibitor of COX-2, is much less ulcerogenic. We, therefore, examined its influence on the development of intestinal polyps in Min mice. Female Min mice at 4 weeks old were given 400 ppm nimesulide in their diet for 11 weeks. This treatment resulted in a significant reduction of the numbers of both small and large intestinal polyps, the total being 52% of that in untreated control Min mice. The size of the polyps in the nimesulide-treated group was also significantly decreased. The results suggest that nimesulide is a good candidate as a chemopreventive agent for human colon cancer with low toxicity.

Key words: Nimesulide — COX-2 inhibitor — Min mouse — Intestinal polyp

It has been reported that death rates from colon cancer are approximately 40% lower in persons taking aspirin 16 or more times per month compared to persons not on this medication.[1)] Consistent with the epidemiological data, aspirin and related nonsteroidal anti-inflammatory drugs (NSAIDs) including indomethacin, sulindac and piroxicam, have been found to inhibit chemically induced colon cancer in rodents.[2,3)] Germline mutations of the *APC* gene result in familial adenomatous polyposis (FAP), an autosomal dominant inherited predisposition to colorectal cancer. FAP patients develop many colorectal polyps, some of which progress to cancer. Although FAP patients with germline mutations of *APC* gene only account for a small percentage of human colorectal cancer cases, somatic mutations of the *APC* gene are frequently observed in the early stages of sporadic colon cancer.[4,5)] Administration of sulindac and indomethacin to FAP patients causes regression of colorectal adenomas in terms of the number and size.[6,7)] Like FAP patients, Min mice containing a nonsense mutation of the *Apc* gene at codon 850 develop intestinal adenomatous polyps,[8,9)] and this can similarly be suppressed by sulindac and piroxicam.[10–12)]

Two isoforms of cyclooxygenase (COX), COX-1 and COX-2, have been characterized in mammalian and avian species.[13)] COX-1 is constitutively expressed in most tissues to regulate prostaglandin production and to maintain stable physiological conditions including gastric cytoprotection and blood flow. In contrast to COX-1, COX-2 is inducible and has been indicated to produce prostanoids involved in inflammation and mitogenesis.[13)] In fact, overexpression of COX-2 is a feature of human colorectal cancers,[14,15)] azoxymethane-induced colorectal tumors in rats[16)] and intestinal polyps in Min mice[17)] and $Apc^{\Delta 716}$ knockout mice.[18)] COX-2-overexpressing cells are also known to be resistant to induction of apoptosis.[19)] NSAIDs such as indomethacin, sulindac and piroxicam inhibit both COX-1 and COX-2 with comparable potencies or strongly inhibit COX-1 rather than COX-2.[20)] Inhibition of COX-1 by these NSAIDs is a causal factor in their gastrointestinal side-effects, such as the development of gastritis, gastric ulcers and gastrointestinal bleeding. This is a major disadvantage for long-term application of NSAIDs as chemopreventive agents for colorectal cancers.

Nimesulide (4-nitro-2-phenoxymethanesulfonanilide, shown in Fig. 1) belongs to the sulfonamide class of NSAIDs and has been clinically used as an anti-inflammatory drug in some European countries since 1985.[21)] Its anti-inflammatory activity is almost the same as that of indomethacin, but its ulcerogenic potential is much weaker.[22)] Nimesulide was first demonstrated to be a selective inhibitor of COX-2 in 1995 by Taniguchi *et al.*,[23)] and indeed, no serious adverse effects have so far been reported after clinical administration of nimesulide. We recently found that this NSAID suppresses the development of aberrant crypt foci in the colons of rats treated with azoxymethane.[24)] As part of our continuing search

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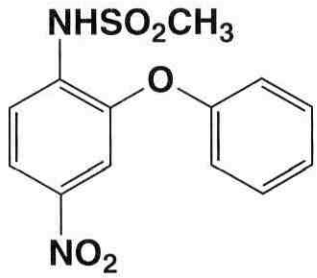

Fig. 1. Structure of nimesulide.

for a safe chemopreventive agent for colon cancer, we further examined the effects of this COX-2-selective inhibitor on the development of intestinal polyps in Min mice.

Nimesulide was provided by Helsinn Healthcare SA (Pazzallo-Lugano, Switzerland), and mixed with AIN-76A powder diet (Dyets, Inc., Bethlehem, PA) at a dose of 400 ppm. Twenty-one female C57BL/6J Min/+ (Min) mice at 4 weeks old, obtained from the Jackson Laboratory (Bar Harbor, ME), were allocated to a nimesulide treatment group (10 animals) and a control group given only the basal diet (11 animals). These two groups of mice were provided diet and water *ad libitum* throughout the experiment for 11 weeks.

At 15 weeks of age, all animals were killed under ether anesthesia. Their intestinal tracts were removed, filled with 10% formalin and a few minutes later opened, separated into 4 parts (proximal, middle and distal small intestine and large intestine), washed with PBS^-, held between paper towels and fixed with 10% formalin for 4 h at room temperature. The numbers and diameters of intestinal tract polyps were determined under $\times 5$ magnification with a stereoscopic microscope, detectable polyps being those more than 0.2 mm diameter. For histological examination, formalin-fixed intestines were embedded in paraffin and 5 μm sections were prepared and stained with hematoxylin and eosin (HE). Fig. 2 shows a polyp in the small intestine of the Min mouse, observed with a stereoscopic microscope (Fig. 2, A and B) and observed after HE staining (Fig. 2C).

Administration of nimesulide at a dose of 400 ppm in the diet did not affect feeding, body weight or behavior of the Min mice. The average body weight for the control group was 20.5 ± 1.1 g, and that for the nimesulide-treated group was 20.3 ± 1.7 g at 15 weeks of age. The daily intake of nimesulide was 49 mg/kg body weight on average during the experiment. Table I summarizes data for the number and distribution of intestinal polyps in untreated control and nimesulide-treated groups of Min mice. Untreated control Min mice had 49.5 ± 4.4 polyps

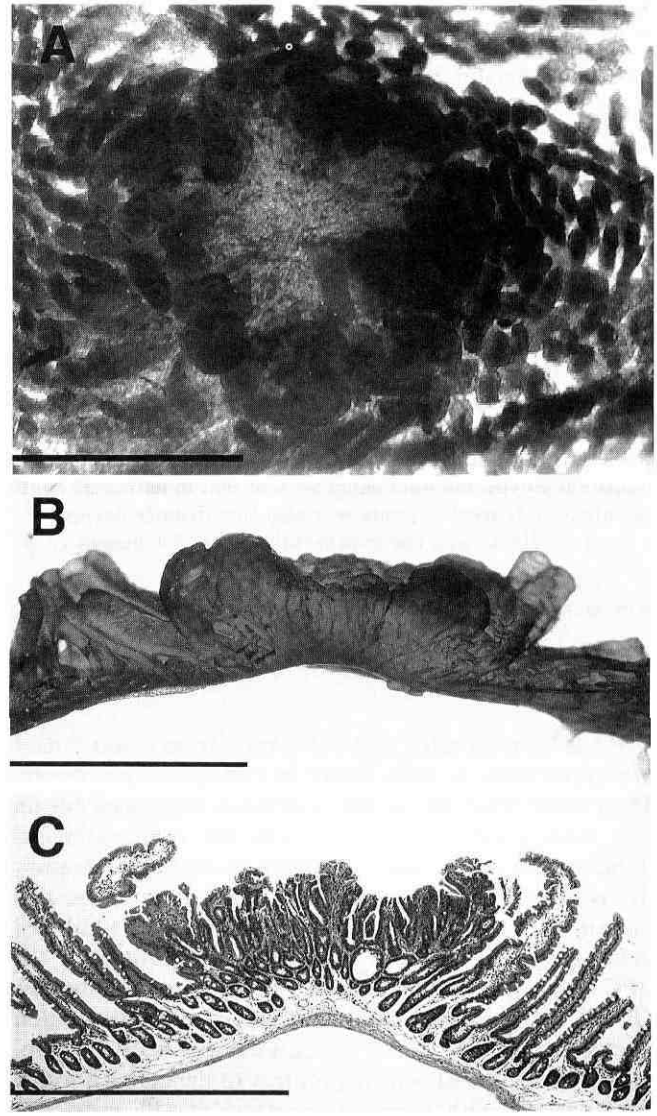

Fig. 2. Photographs of a small intestinal polyp in a Min mouse of the control group. Dissection micrographs taken under a stereoscopic microscope through a white heat lamp: A, viewed from the intestinal lumen; B, viewed from the side of the villi. Histological section: C, stained with HE. Length of bars for A–C are 1 mm.

per mouse at the age of 15 weeks. Most polyps were located in the proximal to distal small intestine and only a few polyps appeared in the colorectum. Administration of nimesulide reduced the total number of polyps to 52% of the control value. Polyp numbers observed in the middle and distal small intestines and the large intestine were significantly decreased to 44, 41 and 33% of those of the control group, respectively. Only a slight, non-

Table I. Effects of Nimesulide on Intestinal Polyp Development in Min Mice

	Control group	Nimesulide group
Small intestine		
Proximal	10.5±1.4	9.2±1.4
Middle	15.5±2.0	6.8±1.4[a)]
Distal	22.7±2.9	9.3±1.5[a)]
Large intestine	0.6±0.2	0.2±0.1[a)]
Total	49.5±4.4	25.5±3.8[a)]

Ten female Min mice at 4 weeks of age were fed a diet containing 400 ppm nimesulide. As controls, 11 female Min mice were maintained on basal diet. At 15 weeks of age, all mice were killed and their intestinal tracts were removed and examined under ×5 magnification to count the number of polyps. Data are expressed as mean±SE values. Polyp numbers indicate polyps per mouse per region.
a) Significantly different ($P<0.05$) from the control group by Student's *t* or Welch's *t* test.

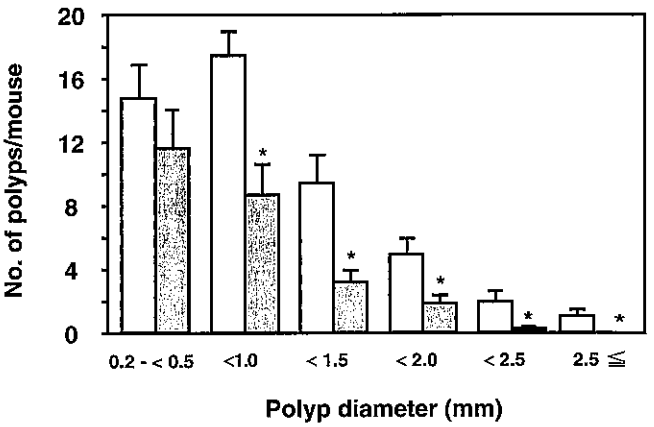

Fig. 3. Size distributions of intestinal polyps in Min mice. Polyps were classified in terms of their diameters in millimeters in both the control group (□) and the nimesulide-treated group (■). The number of polyps per mouse in each size class is expressed as mean±SE. * Significantly different ($P<0.05$) from the control group by Student's *t* or Welch's *t* test.

significant reduction was detected in the proximal part of the small intestine in the nimesulide-treated group. Fig. 3 shows data for the size distributions of the intestinal polyps developing in control and nimesulide-treated Min mice. Under a stereoscopic microscope, polyps with 0.2 to 3.8 mm diameter in the control group and polyps with 0.2 to 2.5 mm diameter in the nimesulide group was detected. The number of polyps in each size class was less in the nimesulide-treated group than the untreated control group. The above data suggests that nimesulide suppresses both the formation and growth of intestinal polyps in Min mice. Totals of 60 and 30 polyps in the small and large intestines of three mice each of the control and nimesulide-treated groups, respectively, were histologically examined. All these polyps were found to be adenomas (Fig. 2C). Moreover, no significant changes were observed in other organs, such as the liver, lung, spleen and kidney of nimesulide-treated mice.

The present study revealed that nimesulide, a COX-2 selective inhibitor, inhibits intestinal polyp development in Min mice. Besides nimesulide, several other chemicals including aryl methylsulfonamide and aryl methylsulfonyl derivatives have been reported to inhibit preferentially COX-2.[13, 18] Recently, an aryl methylsulfonyl derivative, MF tricyclic [3-(3,4-difluorophenyl)-4-(4-(methylsulfonyl)phenyl)-2-(5*H*)-furanone], was similarly shown to suppress intestinal polyp formation in *Apc*$^{\Delta 716}$ knockout mice.[18] Among various COX-2-selective inhibitors, only nimesulide is currently permitted for clinical use as an anti-inflammatory drug. Clinical studies have demonstrated that nimesulide shows much weaker side-effects in the gastro-intestinal tract than the conventional NSAID, indomethacin.[25] Thus, nimesulide may find application as a safer chemopreventive agent against FAP and sporadic human colon cancer than other NSAIDs. Experiments to test the inhibitory effects of nimesulide on azoxymethane-induced colon carcinogenesis in mice are under way in our laboratory.

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