Y-700, a Novel Inhibitor of Xanthine Oxidase, Suppresses the Development of Colon Aberrant Crypt Foci and Cell Proliferation in 1,2-Dimethylhydrazine-Treated Mice

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Received July 12, 2004; Accepted October 18, 2004

Y-700, 1-[3-cyano-4-(2,2-dimethylpropoxy)phenyl]-H-pyrazole-4-carboxylic acid, is a newly synthesized inhibitor of xanthine oxidase. This study found that feeding of Y-700 suppressed the development of colonic aberrant crypt foci, precursor lesions of colon cancer, and cell proliferation in 1,2-dimethylhydrazine-treated mice, accompanied by reduced serum urate. These results suggest that Y-700 is a useful agent for the prevention of colon tumorigenesis and that xanthine oxidase plays an important role in the development of colon cancer.

Key words: colon aberrant crypt foci; xanthine oxidase; 1-[3-cyano-4-(2,2-dimethylpropoxy)phenyl]-H-pyrazole-4-carboxylic acid (Y-700); reactive oxygen species; cell proliferation

Excessive amounts of reactive oxygen species (ROS) increase oxidative stress, a major cause of tissue damage: ischemia-reperfusion injury, DNA mutation, and possibly cancer.1–4) There is evidence that treatment of tissue with allopurinol, the known inhibitor of xanthine oxidase (XO), is effective in attenuating ischemic tissue injury.5) Moreover, other inhibitors of XO activity from natural compounds such as 1’-acetoxychavicol acetate and some polyphenols have been reported to suppress the development of colon tumorigenesis,6–9) but the involvement of XO in these ROS-mediated lesions is still unclear because some of these compounds have also been reported to be direct scavengers of superoxide anion.6–9) It is necessary to examine whether an XO inhibitor possessing no oxygen-radical scavenging activity can suppress the lesions.

Recently, Y-700, 1-[3-cyano-4-(2,2-dimethylpropoxy)phenyl]-H-pyrazole-4-carboxylic acid, has been introduced as a novel XO inhibitor.10,11) Y-700 possesses no oxygen-radical scavenging activity (Fukunari et al., unpublished data). In this study, we investigated whether Y-700 suppresses the development of colon aberrant crypt foci, precursor lesions of colon cancer, and colon cell proliferation in 1,2-dimethylhydrazine-treated mice.

Male CD-1 (ICR): Crj mice (4 week old, Charles River Japan, Hino, Japan) were housed three to four to a metal cage in a room with controlled temperature (24 ± 1 °C) and a 12 h light–dark cycle (light period, 08:00–20:00 h). They had free access to diets and deionized water. The mice were maintained according to the “Guide for the Care and Use of Laboratory Animals” established by Hiroshima University. In experiment 1, after feeding commercial stock diet (MF, Oriental Yeast, Tokyo, Japan) for 1 week, the mice (initial average body weight: 26 g) were divided into five groups of 6–7 mice each. Y-700 was added to the diet (M-powder, Oriental Yeast) at a level of 5, 10, 15, or 20 mg/kg. The feeding experimental period was five weeks. In experiment 2, the mice (initial average body weight: 25 g) were divided into three groups of 12–14 mice each. Y-700 was added to the diet (M-powder, Oriental Yeast) at a level of 10 or 20 mg/kg. The feeding period was 10 weeks. In experiments 1 and 2, the animals were given 1,2-dimethylhydrazine (DMH, 10 mg/kg body weight, Nacalai Tesque, Kyoto, Japan, 10 g/l of 0.1 mol/l phosphate buffer, pH 6.8; 2 ml/kg body weight) by injection in a hind leg once a week for the initial 3 weeks of the experimental feeding. Blood was collected by decapitation under anesthesia with diethyl ether, and serum was obtained by centrifugation.

At termination of the studies, the colon was removed, slit open longitudinally from cecum to anus, placed on a paper towel, and fixed in 10% formaldehyde neutral buffered solution. The fixed colons were stained with methylene blue, and the number of ACF per colon, the number of AC per colon, and the number of AC per colon was counted.
focus were determined according to the method described previously. In addition to ACF-bearing areas, areas of flat mucosa with no visible ACF were embedded in paraffin. Immunohistochemical analysis of BrdU labeling was performed for the normal colonic mucosa. The BrdU staining method is described elsewhere. \(^{13}\) BrdU-positive cells in the colonic mucosal epithelium were counted under the light microscope at a magnification of \(\times 200\) in the rectum, distal colon, and proximal colon respectively. Serum urate level was determined using an enzymatic kit (Wako Pure Chemical Industries, Osaka, Japan). Several organs were immediately removed and weighed. Data were analyzed by Student’s \(t\)-test.

In experiment 1, consumption of Y-700 at 10–20 mg/kg diet caused significant suppression in the development of ACF (Fig. 1, \(P < 0.05\)). Essentially the same inhibitory effect of Y-700 on the development of AC was also observed (data not shown). Serum urate levels were significantly reduced in the groups fed Y-700 (5–20 mg/kg diet), implying the in vivo suppressive efficacy of the compound on urate biosynthesis. There was a significant correlation between the count of ACF and serum urate level (\(r = 0.87, P < 0.01\)). Final body weight was unaffected by dietary addition of Y-700. The weight of organs, including liver, kidney, epididymal adipose tissue, heart, lung, and spleen, was unaffected by dietary Y-700. In experiment 2, consumption of Y-700 on the 10 and 20 mg/kg diets caused significant suppression of the colon ACF and serum urate levels (Table 1). The colon labeling index of BrdU as an indicator of cell proliferation was also significantly suppressed by Y-700 on the 10 and 20 mg/kg diets. Dietary Y-700 had no influence on final body weight (data not shown).

The present study provides evidence that a highly specific XO inhibitor, Y-700, caused significant suppression of DMH-induced colonic ACF and mucosal cell proliferation in mice. These alterations were significantly associated with serum urate, implying an association of XO with ACF and cell proliferation. Recent clinical study conducted on healthy male volunteers indicates that Y-700 displays a potent and long-lasting hypouricemic action without notable adverse effects. \(^{14,15}\) Thus Y-700 might be a useful agent for prevention of colon carcinogenesis as well as hyperuricemia of gout.

ROS induce proliferation of various cell types, including fibroblasts and aortic endothelial cells. \(^{16}\) Antioxidants, including alpha-tocopherol, inhibit proliferation of many types of cells such as vascular smooth muscle, \(^{17}\) fibroblasts, \(^{18}\) and various cancer cell lines. \(^{19}\) In the present study, Y-700 effectively suppressed the development of DMH-induced ACF associated with cell proliferation of colonic mucosa. These findings suggest that ROS generated by XO might act as important mediators of mitogenic signaling in colon cancer.

Table 1. Effects of Dietary Y-700 on Colon Aberrant Crypt Foci (ACF), Cell Proliferation and Serum Urate in 1,2-Dimethylhydrazine-Treated Mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Y-700, 10 mg/kg</th>
<th>Y-700, 20 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon ACF (n/mouse)</td>
<td>61.6 ± 6.9</td>
<td>47.3 ± 6.3*</td>
<td>48.9 ± 3.7*</td>
</tr>
<tr>
<td>BrdU-labeling index (%)</td>
<td>7.8 ± 1.4</td>
<td>4.0 ± 0.8*</td>
<td>3.6 ± 1.1*</td>
</tr>
<tr>
<td>Serum urate (mg/100 ml)</td>
<td>1.91 ± 0.08</td>
<td>1.08 ± 0.06*</td>
<td>1.14 ± 0.07*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 12–14). *Significantly different from control group by Student’s \(t\)-test (\(P < 0.05\)).

Fig. 1. Effect of Dietary Level of Y-700 on Colon Aberrant Crypt Foci (ACF) in 1,2-Dimethylhydrazine-Treated Mice.

Each value is mean ± SE (n = 6–7). *Significantly different from control group (\(P < 0.05\)) by Student’s \(t\)-test.
700 is now in progress, and the structures of the major metabolites in urine and feces appeared to be the acyl-glucuronide of Y-700 in humans (Yamada et al., manuscript preparing). Accordingly, it appears unlikely that the suppressive effect of Y-700 on colon ACF and cell proliferation is mediated through radical scavenging activity of Y-700 itself or its metabolites.

References