SHORT COMMUNICATION

Prevention of 1,2-dimethylhydrazine-induced colon tumorigenesis by HMG-CoA reductase inhibitors, pravastatin and simvastatin, in ICR mice

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3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, pravastatin (Pr) and simvastatin (Si), suppressed 1,2-dimethylhydrazine (DMH)-induced colon cancer development in female ICR mice. All mice received an i.p. injection of 10 mg DMH/kg body wt once weekly for 15 weeks. Pr was administered at 0.01, 0.005 and 0.001% levels in drinking water, and Si at 0.01 and 0.002% levels in the diet. All animals had access to Pr or Si throughout the experiments which were terminated at weeks 25 or 30. Histologically most of the tumors were well-differentiated adenocarcinomas. The incidence of colon tumors examined at weeks 25 or 30 was reduced by 67% in the 0.01% Pr group, by 30% in the 0.005% Pr and 0.01% Si groups, and by 24% in the 0.001% Pr and 0.002% Si groups, compared with their respective controls. However, the differences did not reach statistical significance. The number of tumors per mouse was significantly reduced in all groups administered Pr and Si except the 0.001% Pr group as compared to their respective controls. The results from those three independent experiments seem to suggest that HMG-CoA reductase inhibitors may prevent colon tumorigenesis in laboratory animal model.

The relationship of serum cholesterol and colon cancer risk has been the subject of great controversy (1). Several epidemiologic studies have found an association between low serum cholesterol and colon cancer risk, while other studies failed to show such a relationship (2-4). However, they do not provide sufficient data on a causal association. It has been noted that cholesterol synthesis is required for growth of immortal and transformed cells, and that the competitive inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA*) reductase, a rate-limiting enzyme involved in cholesterol synthesis, inhibit the growth of human skin fibroblasts, glioma cells, and malignant lymphoma cells, and mouse L cells in culture (5-7). The addition of mevalonate, an intermediate product in the cholesterol biosynthetic pathway, but not cholesterol, reversed this inhibition, indicating that the inhibitors of HMG-CoA reductase and mevalonate may play a role in DNA replication and cell proliferation (8,9). Also, the enzyme inhibitors affected the growth of murine neuroblastoma and human pancreatic carcinoma transplanted in mice, and ascites hepatoma in rats (10-12). Another study in humans and rodents indicated that the enzyme inhibitors decrease biliary and fecal bile acids (13-15), which have been shown to play a role as tumor promoter and/or co-carcinogen in the colon (16,17). Thus, it is interesting to note that HMG-CoA reductase inhibitors could potentially participate in the inhibition of colon carcinogenesis.

The present study was carried out to determine whether HMG-CoA reductase inhibitors inhibit 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in mice. The results demonstrate that the HMG-CoA inhibitors such as pravastatin (Pr) (Sankyo Co., Tokyo) and simvastatin (Si) (Banyu Pharmaceutical Co., Tokyo) inhibit DMH-induced colon tumorigenesis in three independent experiments. To our knowledge, this is the first report that the suppression of colon carcinogenesis can be mediated by HMG-CoA inhibitors.

Female ICR/SiC mice (Shizuoka Laboratory Animal Center, Hamamatsu, Japan), 7 weeks of age at the start of experiments, were housed (five mice in a cage with sterilized woodchip bedding) in a specific-pathogen-free animal room under constant conditions with a 12 h light-dark cycle, a temperature of 22°C and a relative humidity of 50%. All animals had free access to drinking water and standard laboratory diet CE-2 (CLEA Japan Inc., Tokyo). Body weight was measured once weekly. All mice received an i.p. injection of the colon carcinogen DMH (Nakarai Tesque, Kyoto) in 0.1 ml of 0.9% NaCl solution once weekly for 15 weeks at a dose level of 10 mg/kg body wt/week. In experiment I, Pr was administered in drinking water at concentrations of 0% (control group) or 0.01% (Pr(h) group) for 25 weeks, whereas in experiment II, Pr was given in drinking water at concentrations of 0% (control group), 0.005% (Pr(m) group) or 0.001% (Pr(l) group) for 30 weeks. In experiment III, the mice were fed CE-2 diets containing Si at dose levels of 0% (control group), 0.01% (Si(m) group) or 0.002% (Si(l) group) for 25 weeks. The drinking water was changed every other day, and the diets once weekly. Because Pr is water soluble and Si is not, they were given in drinking water and in diet respectively. The dosage chosen was the maximum tolerated without any toxic effects in the chronic toxicity test in the high-dose group, and was reduced in the medium- and low-dose groups. In experiment II, the feces were collected from each group for 3 days at week 20 for analyses of bile acid and neutral sterols by the method described previously (18). Experiments I and III were terminated at week 25, and experiment II at week 30. When the animals were killed, blood was collected by heart puncture for serum cholesterol determination by the enzymatic method. At autopsy, the large bowel was cut open along its length and carefully inspected grossly. All tumors and grossly abnormal tissues were histologically examined after fixation in buffered formalin, and H & E staining. The data were analyzed statistically by chi-squared test and Student's t-test.

The body weight gain, water consumption and the amount
of food intake were similar in the Pr- treated, Si-treated and control groups of mice in all the experiments. The amounts of Pr ingested in experiments I and II were computed to be 25 mg/kg body wt/day in the Pr(h) group, 14 mg/kg body wt/ day in the Pr(m) group and 3.2 mg/kg body wt/day in the Pr(l) group, and in experiment III the intake of Si was 17 mg/kg body wt/day in the Si(m) group and 3.3 mg/kg body wt/day in the Si(l) group. The results summarized in Table I demonstrate that administration of Pr and Si reduced the colon tumor incidence by 67% in the Pr(h) group, by 30% in the Pr(m) and Si(m) groups, and by 24% in the Pr(l) and Si(l) groups; however, the differences did not reach statistical significance.

The mean number of tumors per mouse was significantly smaller in all treated groups, but not in the Pr(l) group, than in their respective control groups. The mean number of tumors per tumor-bearing mouse was also reduced slightly, but not significantly, in all treated groups except the Pr(l) group compared to their respective control groups. All tumors were located in the distal half of the colon (0—8 cm from the anus), and were polypoid in shape and ranged in size from 0.1 to 0.6 cm in diameter. Histologically, all the tumors were well-differentiated adenocarcinomas except seven anal squamous cell carcinomas, and extended into the mucosa or submucosa. Adenomatous tumors of the lung in one mouse and malignant lymphoma in one mouse were detected. There were no other tumors. No obvious toxicity due to Pr and Si was observed macroscopically and microscopically.

The results from these three experiments carried out at different periods of time show a similar trend in colon tumor development, suggesting that HMG-CoA reductase inhibitors are potential chemopreventive agents against colon tumorigenesis. These results provide an opportunity for efficacy studies in colon carcinogenesis.

Table I. Inhibitory effects of prevastatin (Pr) and simvastatin (Si) on DMH-induced colon tumorigenesis in ICR mice

<table>
<thead>
<tr>
<th>Experimental groups and treatments</th>
<th>No. of mice</th>
<th>No. of mice with tumors</th>
<th>No. of tumors per mouse</th>
<th>No. of tumors per tumor-bearing mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control: 0% Pr in drinking water</td>
<td>15</td>
<td>6 (40%)</td>
<td>0.9±0.4</td>
<td>2.3±0.6</td>
</tr>
<tr>
<td>Pr(h): 0.01% Pr in drinking water</td>
<td>15</td>
<td>2 (13%)</td>
<td>0.1±0.1</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control: 0% Pr in drinking water</td>
<td>34</td>
<td>21 (62%)</td>
<td>1.9±0.5</td>
<td>3.1±0.6</td>
</tr>
<tr>
<td>Pr(m): 0.005% Pr in drinking water</td>
<td>30</td>
<td>13 (43%)</td>
<td>0.7±0.2</td>
<td>1.6±0.3</td>
</tr>
<tr>
<td>Pr(l): 0.001% Pr in drinking water</td>
<td>30</td>
<td>14 (47%)</td>
<td>1.7±0.4</td>
<td>3.9±0.5</td>
</tr>
<tr>
<td><strong>Experiment III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control: 0% Si in diet</td>
<td>30</td>
<td>16 (53%)</td>
<td>2.1±0.5</td>
<td>3.9±0.7</td>
</tr>
<tr>
<td>Si(m): 0.01% Si in diet</td>
<td>30</td>
<td>11 (37%)</td>
<td>0.8±0.3</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>Si(l): 0.002% Si in diet</td>
<td>30</td>
<td>12 (40%)</td>
<td>0.7±0.2</td>
<td>2.1±0.7</td>
</tr>
</tbody>
</table>

*All mice received an i.p. dose of 10 mg DMH/kg body wt/week for weeks 1—15.
The mice had each treatment during weeks 1—25, 30 and 25 in experiments I, II and III respectively, and then all mice were killed for tumor detection.

**Mean±SEM.**

*Significantly different from respective controls: P < 0.05.*

Table II. Fecal bile acid concentration of ICR mice treated with DMH and prevastatin (Pr) in experiment II

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lithocholic acid</th>
<th>Deoxycholic acid</th>
<th>Hyodeoxycholic acid</th>
<th>Cholic acid</th>
<th>β-Muricholic acid</th>
<th>Others</th>
<th>Total bile acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.3±0.1</td>
<td>0.2±0.03</td>
<td>0.3±0.03</td>
<td>0.7±0.04</td>
<td>0.2±0.1</td>
<td>2.0±0.1</td>
<td>4.9±0.2</td>
</tr>
<tr>
<td>Pr(m)</td>
<td>0.3±0.03</td>
<td>0.2±0.04</td>
<td>0.5±0.05</td>
<td>0.3±0.04</td>
<td>0.2±0.02</td>
<td>1.2±0.1</td>
<td>2.9±0.3</td>
</tr>
<tr>
<td>Pr(l)</td>
<td>1.0±0.04</td>
<td>0.2±0.03</td>
<td>0.3±0.04</td>
<td>0.7±0.05</td>
<td>0.7±0.1</td>
<td>1.9±0.1</td>
<td>4.9±0.3</td>
</tr>
</tbody>
</table>

*See Table I or text. Six samples each were collected at week 20 and were analyzed.

**Mean±SEM, mmol/g dry feces.**

*Significantly different from other groups: P < 0.05.*

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7α-hydroxylase and fecal bile acids (13, 14). In experiment II, the concentrations of fecal bile acids, particularly a potent tumor promoter lithocholic acid, were significantly reduced in the Pr(m) group compared to the control group (Table II). However, the animals of the Pr(l) group did not show such a difference. The data are parallel with the results concerning tumor yield. Thus, it appears highly likely that Pr-modulated alteration of bile acid metabolism may be involved in the suppression of colon tumor development.

It has been found in a number of human and rat colon tumors and in preneoplastic lesions that an activation of K-ras oncogene seems to be an early event in the carcinogenesis process (21–25). Farnesyl isoprenylation of growth-regulating p21 proteins (oncogenic ras proteins) is a crucial step in transformation of the affected cells (26–28). It is believed that the anti-mitogenic effects of HMG-CoA reductase inhibitors result in inhibition of isoprenoid synthesis, an intermediate product of the cholesterol synthesis pathway. In the current study, however, cholesterol synthesis was not suppressed, and K-ras mutations (codons 12, 13 and 61) were not detected in 13 tumors and the pieces of normal-appearing mucosa from control mice in experiments II and III, as tested by polymerase chain reaction and single-strand conformation polymorphism analysis (29). There are no reports on the K-ras oncogene activation in carcinogen-induced colon tumors in mice, and K-ras mutations in rat colon tumors induced with oral heterocyclic amines were rare in one report (30). Thus, it is quite clear that the suppression of tumorigenesis in the current investigations was involved in another mechanism, different from the inhibition of p21 protein activation.

In conclusion, administration of Pr and Si inhibited colon tumorigenesis but to a limited degree in mice. However, the agents were given during the entire period of the experiments, including initiation and promotion stages of carcinogenesis. It is possible that the inhibitory effect on tumorigenesis is due to metabolic activation of the procarcinogen DMH. It was proposed in recent reports that lovastatin, which is an analog of Pr and Si and has a hypocholesterolemic activity equivalent to that of Pr and Si, disrupts a major growth factor signaling pathway associated with platelet-derived growth factor stimulation in vitro (31, 32). Thus, the mechanisms responsible for the colon tumor inhibition by Pr and Si treatments in the present study remain to be investigated further as well as optimal dosage of these agents, because the lowest dosage ingested in the current experiments was still 5–10 times higher than those used in humans as hypocholesterolemic drugs.

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References


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