Antihypertensive and Antihyperlipidemic Effects of Funoran

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Studies were conducted to determine the mechanism of blood pressure- and cholesterol-lowering effects of funoran on rats fed with a saline solution and cholesterol diet. Clofibrate (COIB) was used as a reference hypolipidemic drug. Funoran caused significant reduction of systolic blood pressure (SBP). Funoran and CPIB significantly reduced serum total cholesterol (TC), free cholesterol (FC), triglyceride (TG), LDL-cholesterol (LDL), and atherogenic index (AI) levels in these rats. The increase of sodium, water excretion and sodium-potassium ratio in urine in the funoran group was more significant in the experimental rats than in the control group. Moreover, the ratio of Na and K in serum decreased with the funoran diet. The CPIB diet enhanced cholesterol level in the liver while the funoran diet suppressed the level, but in feces the former diet did not change the cholesterol level while the latter diet increased it. These results suggest that the enhanced ability to excrete sodium in urine by the funoran diet is an important factor for reducing blood pressure and that the antihyperlipidemic effect of funoran was not caused by the mobilization of peripheral cholesterol on the liver, but by the enhanced excretion of cholesterol into feces.

Key words: antihypertension, antihyperlipidemia, polysaccharide, funoran

Seaweed polysaccharides have various biological activities.1–30 Gloiopeltis tenax is a red alga belonging to the Endocladiaceae. It has been reported that a funoran fraction with medium sulfate content and non-gelling property obtained from this red alga exhibits a remarkable antitumor activity against Sarcoma 180-carrying mice,44 but no further investigations on the biological activities of funoran have been performed. We therefore studied the antihypertensive and antihyperlipidemic activities of a funoran fraction. We also investigated the latent mechanism of its possible biological function.

Materials and Methods

Preparation of Funoran

Funoran is a sulfated polysaccharide from Gloiopeltis tenax and the crude polysaccharide fraction was prepared as previously described.84 Crude funoran was extracted with hot water for 2h. The combined hot water extracts were concentrated to 1.0% solution, to which 2 volumes of 5% cetyl pyridinium chloride (CPC) were added. The acidic polysaccharide-CPC complex precipitated was washed with water and stirred in 2N KCl for 2h at 37°C. After removal of released CPC with ethanol, the polysaccharide was dissolved in 0.07 M phosphate buffer solution (pH 7.0) and treated with 2.0% DIFCO trypsin 1:250 (DIFCO Laboratories, Detroit, Michigan, U.S.A.) for 20h at 30°C. It was then dialyzed against distilled water, precipitated by adding of 5 volumes of ethanol, and lyophilized. The fraction was dissolved in a solution of 0.5N NaCl and applied to a Toyopearl HW-65F column (2.6 x 95 cm) and eluted with 0.5N NaCl. The eluate of a single peak fraction was concentrated by freeze-drying using a cellulose-acetate film developed in a 1.0M acetic acid/pyridine buffer (pH 3.5) and 0.5 M NaCl for 35 min.

The yield was 3.8%. The sugar composition was determined by gas liquid chromatography analysis using a Shimadzu GC-14A.44 The total sugar was measured by the phenol-sulfuric acid method and calculated as galactose after correction for the 3,6-anhydrogalactose content.77 Sulfate was estimated by the turbidimetric method of Dodgson–Price after hydrolysis of the polysaccharide specimen with 1N HCl at 100°C for 6h.81 The uronic acid was assayed by the carbazole-sulfuric acid method, using r-glucuronic acid as the standard.49 The protein content was determined by the Cu-Folin method of Lowry et al. with reference to bovine serum albumin.49 Cations were analyzed by a Shimadzu AA-680/680G atomic absorption spectrophotometer, and chloride49 and phosphate52 analyzed colorimetrically. The molecular weight was estimated by the viscosity method13 with a BM-Viscometer (Tokyo Keiki Co. Ltd., Japan). The value of specific rotation was determined with a DPH-370, JASCO automatic polarimeter. The data showed that the funoran consists of galactose, 3,6-anhydro-galactose, 6-O-methyl-galactose, 2-O-methyl-3,6-anhydro-galactose, xylose, arabinose, and sulfate with molar ratios of 31.3, 24.2, 18, 0.7, 0.9, 2.2, and 39.1%. Average molecular weight and specific rotation of the fraction were 90,500 and [α]D 25 = −31.8°, respectively. The protein and mineral contents were not detected in the fraction.

Animal and Feeding Tests

Four-week-old female Wistar strain rats were obtained from the Shizuoka Laboratory Animal Center, Hamamatsu, Japan. Clofibrate (CPIB) was purchased from Wako Pure Chemical Industries Ltd. as an antihyperlipidemic reference agent. Five-week Wistar rats were first held in a room at constant temperature (24 ± 2°C) and humidity (50 ± 10%). The rats were randomly divided into groups of eight animals each and were raised simultaneously under the same temperature and humidity for 20 days. The control group was fed with 1.5% saline and the artificial diet containing CPIB of 300 mg/kg while the experimental rats were fed with 1.5% saline and artificial diet containing funoran of 1000 mg/kg while the CPIB group was fed with an artificial diet containing CPIB of 300 mg/kg.

Determination of Blood Pressure

To determine the blood pressure, the experimental animals were maintained at 37°C for 10–15 min. The systolic blood pressure (SBP) was measured indirectly in the different groups of rats every 4 days by the indirect tail-cuff method using a BP recorder, MK-1000 (Muromachi Machine Co., Ltd., Tokyo).

Measurement of Urinary Volume and Urine Electrolyte Excretion

Sixteen days after the first measurement of SBP, a volume of 1.5% saline equivalent to 2.5% of the body weight was orally administered to the rats.
The animals were not fed for 8 h prior to this sodium load, however they were allowed to drink distilled water. Three hours after the saline loading, urine was collected using small isolation cages. Urinary volume, sodium, and potassium excretions were measured. Electrolyte concentrations in urine were analyzed by the electrode method using an automated electrolyte analyzer, EA 06 T/R (A&T Co., Ltd., Tokyo).

Measurement of Serum Electrolyte

The rats were kept at the above conditions. At the end of the experiment, the rats were starved for 12-14 h prior to collection of blood from the heart. Electrolyte concentrations in serum were analyzed by the electrode method using an automated electrolyte analyzer, EA 06 T/R (A&T Co., Ltd., Tokyo).

Determination of Serum Lipid Level

At the end of the experiment, the rats were starved for 12-14 h prior to collection of blood from the heart. Total cholesterol (TC), free cholesterol (FC), triglyceride (TG), and HDL-cholesterol (HDL) in serum were assayed by an enzymatic method,14,15 using a cholesterol E-Test Wako (Wako Pure Chemical Industries Ltd.) and a Spectrophotometer, ATAGO AT-60. LDL-cholesterol (LDL) was estimated using the following equation: LDL = TC-HDL. Atherogenic Index (AI) was referred to TC:HDL:HDL.

Determination of Liver Cholesterol Level

At the end of the experiment, the rats were starved for 12-14 h before extirpation of the liver. Liver cholesterol was extracted using Folch's method.16 Total cholesterol level was measured using a TCE-Test Wako.

Determination of Fecal Cholesterol Level

The feces of rats were collected on the last 3 days of the experiment. Fecal cholesterol was obtained using Hill and Aries's method.17 Total cholesterol level was measured by using a TC E-Test Wako.

Statistical Analysis

The data were expressed as a mean ± SD and statistical analysis was performed using the Student's t-test for unpaired groups and a statistical significance of p < 0.05.

Results

Change of Body Weight

Changes in body weight are shown in Fig. 1. The body weights of the funoran and CPIB groups were slightly higher than the control group after 16 days of feeding. During the experiment, the physical appearance of the rats was good.

Antihypertensive Effect of Funoran

The results of the antihypertensive effects of funoran are illustrated in Fig. 2. The animals fed with the algal polysaccharide diet were examined for changes in their SBP during 20 days of feeding. The SBP of the group fed with the funoran was markedly lower than that of the control group. The SBP of the funoran group was significantly (p < 0.05) lower than that of the control group at 8 days of feeding. The SBP of the funoran group significantly (p < 0.001) decreased to 86.0% compared with the control group on the last day of feeding.

Influence of Funoran on Urine Electrolytes

The results of the funoran on urine electrolytes are summarized in Table 1. The urinary excretion rate for 3 h urine samples was 139.0% higher in the funoran group than in the control group. Excretion of sodium and chloride was enhanced, and the Na⁺/K⁺ ratio was also 257.0% higher in the urine of the funoran group than in the control group. On the contrary, the excretion of potassium was significantly lower (62.0%) in the funoran than in the control groups.

Table 1. Influence of funoran on urine Na⁺, K⁺, and Cl⁻ levels in rats fed with funoran diet

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urinary output (ml/kg body weight)</th>
<th>Na⁺ (mg/kg body weight)</th>
<th>K⁺ (mg/kg body weight)</th>
<th>Na⁺/K⁺ (mg/kg body weight)</th>
<th>Cl⁻ (mg/kg body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.6 ± 1.8*</td>
<td>122.7 ± 15.2</td>
<td>129.4 ± 11.0</td>
<td>0.96 ± 0.03</td>
<td>163.8 ± 13.4</td>
</tr>
<tr>
<td>Funoran</td>
<td>18.9 ± 1.4*</td>
<td>198.9 ± 17.2*</td>
<td>79.8 ± 10.2*</td>
<td>2.47 ± 0.14*</td>
<td>220.1 ± 14.5*</td>
</tr>
</tbody>
</table>

*1: Results are expressed as mean ± SD of 8 rats. *p < 0.001, **p < 0.01.

*2: Numbers in parentheses show percent values to that of control group.
**Influence of Funoran on Serum Electrolytes**

The results of the funoran on serum electrolytes are shown in Table 2. After 20 days, the potassium level of the funoran group in serum increased to 114.0% compared with the control group. The ratio of sodium and potassium levels in the funoran group was significantly lower (87.0%) than in the control group. However, the amounts of sodium and chloride in serum were almost the same in the funoran and the control groups.

**Hypocholesterolemic Effect of Funoran and CPIB**

The effects of the diet containing funoran on the serum lipid level of rats with hypercholesterolemia are shown in Table 3. The funoran and CPIB groups showed a reduction in the Atherogenic Index of serum (61.0 and 70.0%, respectively) compared with the control group.

TC concentrations of the funoran group significantly decreased to 69.0% of the control group. The CPIB group also showed a similar result. The funoran and CPIB groups also showed remarkably lower levels of FC, TG, and LDL (52.0–66.0% of the control).

In contrast, the HDL concentration of the funoran group remained almost unchanged, while that of the CPIB group reduced remarkably (79.0%) compared with the control group.

**Effect of Funoran or CPIB on Liver Cholesterol Level**

The effects of the diet containing funoran on the liver cholesterol level of rats with hypercholesterolemia are shown in Table 4. The body weight of both the funoran and CPIB groups increased significantly during 20 days of feeding. In contrast, the total liver weight of the CPIB group markedly increased to as much as 135.0% of the control group, while no increase was found in the funoran group. In addition, the total liver cholesterol level of the CPIB group was enhanced significantly (127.0%) compared to the control group, although no difference was found in the liver cholesterol level between the funoran and control groups.

**Effect of Funoran and CPIB on Fecal Cholesterol Level**

The effects of the diet containing funoran on the fecal cholesterol level of rats with hypercholesterolemia are shown in Table 5. The food intake of the funoran and CPIB groups increased to 116.0 and 123.0% of the control group. The fecal excretion of cholesterol increased significantly in the funoran group to 142.0% higher than that of the control group. However, the CPIB group showed no such trend despite an increase of 134.0% in the fecal amount.

**Discussion**

The objective of the present research was to investigate the mechanism of the antihypertensive and cholesterol-lowering effect of funoran loading in saline and cholesterol-feeding. We found significant positive relations between urinary sodium excretion and SBP, and between urine sodium/potassium ratio and blood pressure in rats fed with a funoran diet. After 20 days of feeding, funoran reduced by nearly 14.0% the blood pressure of hypertensive rats. Concomitant with the antihypertensive effect of the funoran, sodium and urine excretion also increased significantly. For example, sodium excretions in the funoran and control groups were 198.9 ± 17.2 and 122.7 ± 15.2 mg/kg body weight, respectively, a remarkable difference. The potassium concentration of the funoran group in the serum increased to 114.0% (p<0.05) of the control group and the sodium/potassium ratio of funoran group in the serum became lower than that of the control group. Similar results were also reported with other algal polysaccharides.

The mechanisms by which the funoran decreases blood pressure are not clear, but the importance of the relationship between the volume of extracellular fluid (ECF) and arterial blood pressure is well known. Since homeostasis of the ECF is maintained by a balance between salt and water intake and urinary output, treatment with desoxycorticosterone acetate (DOCA) induces an increase in the ECF volume in early periods after a sodium load, leading to an increase in blood pressure. Moreover, studies on the antihypertensive effect of a diuretic have demonstrated that the fall in the blood pressure during diuresis is associated with a decrease in ECF. Further, according to some studies, the reduced ability of spontaneously hypertensive rats (SHR) and a stroke-prone strain of SHR (SHRSP) to excrete sodium improved with the elevation
feces. Absorption and enhancement of cholesterol excretion in data suggest that the mechanism of the hypolipidemic effect of funoran is not the same as that of CPIB. The present and excretion.36) Thus, the antihyperlipidemic mechanism triglycerides35) but it has no effect on cholesterol absorption drug32-34) that has been particularly effective in lowering opposite effect. CPIB is an extensively used hypolipidemic rats did not change. However, the CPIB group showed the cholesterol, but the liver cholesterol level in cholesterol-fed groups, the funoran markedly increased fecal excretion of its effects on the metabolism in liver and feces. In the test group.

Hypertension is a major risk factor for arteriosclerosis37-39) and the beneficial effects of lowering blood pressure on morbidity and mortality is well document-d.40-43) Previous studies also have emphasized the positive relationship of TC, LDL, and TG with the risk of coronary heart disease (CHD).44-47) The higher the concentration of any one of these blood lipids, the greater the risk of CHD. On the other hand, HDL appears to have an inverse relation on the risk of CHD.42,48-52) Low HDL concentrations lead to an increased risk of CHD. Moreover, many hypertensive therapies53,54) adversely interfere with the lipid profile by accompanying lowered HDL, raising TG and TC levels. Some investigators55) believe that these effects may reflect why the decline in CHD incidence has been so small in most hypertension intervention trials. Our data demonstrates that funoran has an antihypertensive effect and also inhibits the reduction of serum HDL levels and decreased TC, TG, LDL, and AI levels after 20 days of ingesting a high salt and cholesterol diet. Our studies therefore suggest the usefulness of funoran as an anti-hypertensive and antihyperlipidemic medicine.

of blood pressure. Finally, a direct effect of potassium concentration on the resistance vessels is another possible mechanism. There is convincing evidence that an increase in plasma potassium concentration exerts a direct vasodilator effect on vessels.30,31) Therefore, the urine level of K+ excretion and the increase in its serum level may be important factors in the antihypertensive effect of the funoran.

In the present study, the funoran and CPIB depressed TC, FC, TG, LDL, and AI levels in the sodium and cholesterol loaded rats. The funoran group increased HDL levels in the serum, whereas the CPIB significantly lowered HDL levels. Particularly low levels of FC and TG of 52.0 and 53.0% of control levels were observed in the funoran group.

The rats fed with the cholesterol diet were examined for its effects on the metabolism in liver and feces. In the test groups, the funoran markedly increased fecal excretion of cholesterol, but the liver cholesterol level in cholesterol-fed rats did not change. However, the CPIB group showed the opposite effect. CPIB is an extensively used hypolipidemic drug32-34) that has been particularly effective in lowering triglycerides35) but it has no effect on cholesterol absorption and excretion.36) Thus, the antihyperlipidemic mechanism of funoran is not the same as that of CPIB. The present data suggest that the mechanism of the hypolipidemic effect of funoran is due to the prevention of dietary cholesterol absorption and enhancement of cholesterol excretion in feces.

Table 4. Effect of funoran or clofibrate on liver cholesterol levels in rats fed with funoran or clofibrate diet

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight gain (g)</th>
<th>Liver weight (g)</th>
<th>Liver weight (g/100 g body weight)</th>
<th>TC (mg/liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.1±11.4*1</td>
<td>9.2±1.2</td>
<td>5.2±0.3</td>
<td>689±52.2</td>
</tr>
<tr>
<td></td>
<td>(100)*2</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Funoran</td>
<td>90.2±17.4</td>
<td>9.2±0.9</td>
<td>4.7±0.2*</td>
<td>667±76.4</td>
</tr>
<tr>
<td></td>
<td>(117)</td>
<td>(100)</td>
<td>(91)</td>
<td>(100)</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>91.3±12.8*</td>
<td>12.4±1.3*</td>
<td>6.5±0.4*</td>
<td>873±37.9*</td>
</tr>
<tr>
<td></td>
<td>(118)</td>
<td>(135)</td>
<td>(126)</td>
<td>(127)</td>
</tr>
</tbody>
</table>

*1: Results are expressed as mean±SD of 8 rats. *p<0.05, *p<0.01, *p<0.001.
*2: Numbers in parentheses show percent values to that of control group.

Table 5. Effect of funoran or clofibrate on fecal cholesterol in rats fed with funoran or clofibrate diet

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight gain (g)</th>
<th>Food intake (g/24 h)</th>
<th>Fecal weight (g)</th>
<th>TC (mg/g fecal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.1±11.4*1</td>
<td>11.3±1.6</td>
<td>0.38±0.03</td>
<td>18.4±1.2</td>
</tr>
<tr>
<td></td>
<td>(100)*2</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
</tr>
<tr>
<td>Funoran</td>
<td>90.2±17.4</td>
<td>13.1±0.9*</td>
<td>0.65±0.04*</td>
<td>26.9±2.7*</td>
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<td></td>
<td>(117)</td>
<td>(116)</td>
<td>(171)</td>
<td>(142)</td>
</tr>
<tr>
<td>Clofibrate</td>
<td>91.3±12.3*</td>
<td>13.9±2.5*</td>
<td>0.51±0.02*</td>
<td>19.1±3.0</td>
</tr>
<tr>
<td></td>
<td>(118)</td>
<td>(123)</td>
<td>(134)</td>
<td>(134)</td>
</tr>
</tbody>
</table>

*1: Results are expressed as mean±SD of 8 rats. *p<0.05, *p<0.01, *p<0.001.
*2: Numbers in parentheses show percent values to that of control group.

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