Mechanism for the Antihypertensive Effect of a Polysaccharide-glycopeptide Complex from Lactobacillus casei in Spontaneously Hypertensive Rats (SHR)

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Pharmacological studies on the antihypertensive effect of a polysaccharide-glycopeptide complex (SG-1) isolated from Lactobacillus casei were carried out by using spontaneously hypertensive rats (SHR). An antihypertensive effect of SG-1 was observed by oral, but not by intravenous or intraperitoneal administration, and the effect was attenuated by orally pre-treating with indomethacin. A single oral administration of SG-1 (20 mg/kg) decreased the peripheral vascular resistance (PR). The daily oral administration of SG-1 (10 mg/kg) for 14 days had no effect on either the urine volume or urinary electrolytes (Na⁺, K⁺, and Cl⁻), but it did increase the excretion of 6-keto-PGF₁α, a metabolite of PGI₂, in the urine. Moreover, a single oral administration of SG-1 (20 mg/kg) also increased the biliary 6-keto-PGF₁α excretion. These results suggest that the antihypertensive effect of orally administered SG-1 resulted from an enhancement of PGI₂ biosynthesis and the subsequent decrease in PR.

We have previously reported that extracts of the autologous cell lysate of Lactobacillus casei contained antihypertensive compounds, the active substance of which was a polysaccharide-glycopeptide complex, designated as SG-1. Oral administration of SG-1 to spontaneously hypertensive rats (SHR) or to renal hypertensive rats induced an antihypertensive effect. In the present study, we investigated the influence of the oral administration of SG-1 on the metabolism of water electrolytes and prostaglandins (PGs), and on the peripheral blood vessels in SHR to clarify the mechanism for this antihypertensive effect.

Materials and Methods

Preparation of SG-1. SG-1 was prepared from Lactobacillus casei YIT9018 as reported in the previous paper.

Animals. Male SHR purchased from Charles River Japan were fed on a standard laboratory diet (MF, Oriental Yeast Industry Co.) and tap water ad libitum. All the animals were housed in individual cages with a 12-h light/dark cycle. The temperature and humidity were controlled to 24 ± 1°C and 60 ± 5%, respectively.

Administration. SG-1 was given to SHR intravenously, intraperitoneally or orally, after being dissolved in saline (for intravenous and intraperitoneal administration) or distilled water (for oral administration).

Indomethacin (IND), purchased from Sigma Chemical Company, was dissolved in 0.2% Tween 80 and administered orally to SHR at a dose of 5 mg/kg.

Measurement of blood pressure and blood flow.

Indirect measurement of blood pressure. After SHR had been placed in a box kept at 38°C for a few minutes, the systolic blood pressure and heart rate were measured by the tail cuff method with a programmable sphygmomanometer (type PS-100, Riken Kikai Co.).

Direct measurement of blood pressure. SHR at 18 to 21 weeks old and weighing 320-370 g were used. The rats were anesthetized with ether, and the abdominal aorta was cannulated with a polyethylene tube (PE-10 fused to PE-50) via the femoral artery. A catheter was passed subcutaneously to emerge on the neck, and filled with saline containing heparin. One day was allowed to recover from the surgery, before the blood pressure was measured by a Statham transducer (type P23ID, Nihon Kohden Co.).

Measurement of blood flow. The contralateral artery at the site for blood pressure measurement was isolated, and a probe of 0.5 mm in diameter was inserted into the artery to measure the blood flow. The probe cable was guided to the neck, where a connector was fixed to the skin. On the morning of the experiment, the implanted measuring cable was connected to a pulse-doppler flow meter (type VF-1, Crystal Biotech.), and the signal from the meter was recorded with an RTA-1300 M instrument (Nihon Kohden Co.). The change in peripheral vascular resistance (ΔPR) was calculated as described by Smits and Struyker-Boudier from changes in the femoral blood flow (ΔFBF, ml/min) and mean blood pressure (ΔMBP, mmHg) as follows: ΔPR (%) = [(100 × ΔMBP)/(100 + ΔFBF)] × 100.

Measurement of the urine volume, urinary electrolytes, and urinary PG content. SHR of 18 weeks age (body weight (B.W.), 320-350 g) were used. One group of SHR was administered with SG-1 (10 mg/kg B.W./day) and another with distilled water (5 ml/kg B.W./day) once for a day 14 days. After administering SG-1 on the 1st, 3rd, 5th, 7th, 10th, and 14th days, urine was collected over 24 h in metabolic cages, in which the animals had free access to food and water. The urine volume for 24 h was measured, and samples were stored at -20°C until biochemical determinations were performed.

Urinary electrolytes: Urinary Na⁺, K⁺, and Cl⁻ concentrations were determined by the ion-electrode method with a NAKL-2 instrument (Olympus Co.) after diluting with deionized water.

Urinary PG: Urinary 6-keto-PGF₁α and PGE₂ were determined by an enzyme immunoassay (EIA), using a kit from Cayman Chemical Co.

Collection of bile and measurement of the biliary concentration of 6-keto-PGF₁α. Nineteen-week-old SHR weighing 320-250 g were used. A catheter was implanted into the bile duct under ether anesthesia, and each animal was kept in a Boliman cage (type KN-236, Natsume). On the next day, SG-1 (20 mg/kg) was administered orally to conscious SHR, and bile was collected for 24 h through the catheter. Measurement of the biliary concentration of 6-keto-PGF₁α was carried out by using the same method as that for urinary 6-keto-PGF₁α measurement.

Statistical analyses. All results are expressed as the mean ± S.E.M. In the evaluation of the antihypertensive effect, significant differences between

Abbreviations: SHR, spontaneously hypertensive rats; SG-1, polysaccharide-glycopeptide complex; PR, peripheral vascular resistance; PG, prostaglandin; IND, indomethacin.
Results

Antihypertensive effect of SG-1 on SHR

SG-1 was administered to SHR intravenously (i.v.), intraperitoneally (i.p.) or orally (p.o.) at a dose of 1 or 5 mg/kg B.W. A significant antihypertensive effect was only observed in the group receiving oral administration (Fig. 1).

Antihypertensive effect of SG-1 on SHR pre-treated with IND

A single oral administration of SG-1 (10 mg/kg B.W.) reduced the blood pressure at 6 (p < 0.01), 12 (p < 0.01), and 24 h (p < 0.01) in SHR pre-treated with 0.2% Tween 80 one hour previously. However, no antihypertensive effect of SG-1 was observed in SHR pre-treated with IND (5 mg/kg B.W.). Oral administration of IND (5 mg/kg B.W.) had no influence on the blood pressure either (Fig. 2). The administration of distilled water after pre-treating with 0.2% Tween 80 did not influence the blood pressure of SHR (data not shown).

Effect of SG-1 on PR

A significant decrease in the mean blood pressure (MBP) was observed at 3 and 6 h after orally administering SG-1 (20 mg/kg B.W.). The average percentage (%) decrease of MBP from the start was 7.5 ± 1.3 (p < 0.001) at 3 h, and 8.8 ± 1.1 (p < 0.001) at 6 h. The femoral blood flow (FBF) tended to increase compared to that of the control, but the difference was not statistically significant. From the changes in MBP and FBF, the average percentage decrease in PR from the start was 9.6 ± 5.0 (p < 0.05) at 3 h, and

![Graph showing blood pressure changes](image)

**Fig. 2.** Effect of an IND Pre-treatment on the Antihypertensive Effect of SG-1.

IND (5 mg/kg) dissolved in 0.2% Tween 80 was administered orally, and 1 hour after, SG-1 (10 mg/kg) was administered orally to SHR. Each point indicates the mean for 6 animals, and vertical bars represent S.E.M. —○—, control (distilled water after pre-treating with IND); —△—, SG-1 after pre-treating with IND; —●—, SG-1 after pre-treating with 0.2% Tween 80. Significantly different from the control, **p<0.01 (Dunnett's test).**

**Table** Effect of SG-1 on the Peripheral Vascular Resistance

<table>
<thead>
<tr>
<th>Time after administration (h)</th>
<th>3</th>
<th>6</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.8 ± 0.9</td>
<td>-2.2 ± 1.5</td>
<td>5.0 ± 4.4</td>
</tr>
<tr>
<td>dMBP (%)</td>
<td>-2.5 ± 2.5</td>
<td>-6.9 ± 2.5</td>
<td>2.4 ± 8.1</td>
</tr>
<tr>
<td>dFBF (%)</td>
<td>3.8 ± 2.6</td>
<td>5.5 ± 3.3</td>
<td>12.2 ± 7.0</td>
</tr>
</tbody>
</table>

SG-1 (20 mg/kg) was administered orally to SHR, and the mean blood pressure (MBP) and femoral blood flow (FBF) were measured at 3, 6, and 24 h after administration. The change in peripheral vessel resistance (dPR) was calculated as follows: 

\[ dPR (%) = \frac{[(100 + dMBP) / (100 + dFBF)] - 100}{100} \times 100 \]

Values are the mean ± S.E.M. (n = 7-8). *p < 0.05, **p < 0.01, and ***p < 0.001, compared to the control group. (Student's t-test)
6.0 ± 3.5 (p < 0.05) at 6 h after the oral administration of SG-1 (20 mg/kg B.W.) as shown in the table.

Effect of SG-1 on the urine volume, urinary electrolytes, and urinary PG excretion

The urine volume and urinary electrolytes during the administration period for SG-1 (10 mg/kg/day) are shown in Fig. 3. No significant difference was observed compared to the control group on the 1st, 3rd, 5th, 7th, 10th, or 14th day.

SG-1 had no effect on the urinary excretion of PGE₂. However, the excretion of 6-keto-PGF₁α increased, significant differences being observed on the 1st (p < 0.05), 10th (p < 0.01), and 14th (p < 0.01) day after administering SG-1 (Fig. 4).

Effect of SG-1 on the biliary 6-keto-PGF₁α excretion

The bile of SHR administered with SG-1 (20 mg/kg B.W.) was collected for 24 h, and the amount of 6-keto-PGF₁α was determined by the ELISA system. In comparison with the control, the cumulative biliary excretion of 6-keto-PGF₁α was increased by a single oral administration of SG-1 (Fig. 5).

Discussion

In this study, SG-1 (an extract from *Lactobacillus casei*) reduced the blood pressure in SHR when administered orally, and this effect was attenuated by pre-treating with IND. IND or nonsteroidal anti-inflammatory drugs that interfere with the activity of cyclooxygenase have been reported to decrease the effectiveness of antihypertensive therapy. Furthermore, many studies have reported that PGs may be involved in regulating blood pressure, and that the urinary excretion of PGE₂ or 6-keto-PGF₁α, a
Although it is unknown why SG-1 was effective only when orally administered, it is presumed that SG-1 with or without digestion by the enzyme(s) of a host and/or gastrointestinal microorganisms would increase the biosynthesis of PGI₂ in vessel walls, and that PGI₂ would reduce PR and blood pressure subsequently.

References


major metabolite of PGI₂, increased after administering antihypertensive drugs to essential hypertensive patients⁹–¹¹ or SHR.¹² Studies on the hemodynamic and physiological effects of PGs have shown that PGI₂ synthesized in various vessel walls was a more potent vasodilator than PGE₂. Additionally, PGI₂-induced decreases in PR and MBP after intravenous administration,¹³,¹⁴ while PGE₂ derived from the kidney was involved in the antihypertensive mechanism by influencing water-electrolyte metabolism in the kidney.¹⁵,¹⁶ In the present study, SG-1 significantly increased the urinary excretion of 6-keto-PGF₁α, but not of PGE₂, and had no effect on the urine volume or excretion of urinary electrolytes. Therefore, the antihypertensive effect of SG-1 probably did not involve a renal hypotensive mechanism. Moreover, the increase of 6-keto-PGF₁α content in bile after orally administering SG-1 suggests that SG-1 decreased PR by augmenting the PGI₂ synthesis in various vessel walls, and by subsequent reductions in PR and blood pressure in SHR.