Daio-Orengedokuto Inhibits HMG-CoA Reductase and Pancreatic Lipase

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To evaluate the antihyperlipidemic activities of Orengedokuto (OT) and Daio-Orengedokuto (DOT), the inhibitory effects of these polyprescriptions on HMG-CoA reductase and pancreatic lipase and on the rat hyperlipidemic model induced by Triton WR-1339 were measured. OT potently inhibited HMG-CoA reductase but did not inhibit lipase. Among their ingredients, Coptidis Rhizoma was the most potent inhibitor, followed by Rhei Rhizoma. The HMG-CoA reductase-inhibitory activity of 80% EtOH extract was superior to that of water extract. However, DOT potently inhibited HMG-CoA-reductase as well as pancreatic lipase. In the rat hyperlipidemic model induced by Triton WR-1339, OT and DOT decreased serum total cholesterol and low-density lipoprotein cholesterol levels. DOT also decreased serum triglyceride levels, but OT did not decrease it. These results suggest that the antihyperlipidemic activity of DOT may originate from the inhibition of pancreatic lipase as well as HMG-CoA reductase.

Key words Orengedokuto; Daio-Orengedokuto; HMG-CoA reductase; pancreatic lipase; inhibition

Lipid metabolism normally maintains an elegant balance between synthesis and degradation. When the balance is lost, hypercholesterolemia and hyperlipidemia may develop. This can cause variety of serious diseases, such as arteriosclerosis, hypertension, obesity, diabetes, functional depression of some organs, etc. 1) The rate-limiting enzyme for cholesterol biosynthesis from acetate is HMG-CoA reductase. 2,3) Pancreatic lipase is a key enzyme for lipid breakdown to absorb fatty acids. 4) Many researchers have developed inhibitors of HMG-CoA reductase or pancreatic lipase as an antihypercholesterolemic agents. 5,6)

Orengedokuto (OT, Whangryunhaedoktang in Korean), which consists of Scutellariae Radix, Coptidis Rhizoma, Phellodendri Cortex, and Gardeniae Fructus, is known to have antihyperlipidemic activity. 7) Furthermore, OT is known to increase cerebral blood flow, to reduce blood pressure, to exhibit antiinflammatory and vasorelaxant activity, etc. 8—12) However, most atherosclerotic patients also experience constipation, which is thought to be a factor in stroke. Therefore, when caring for atherosclerotic patients in an Oriental clinic, Daio-Orengedokuto (DOT, Daewhangwhangryunhaedoktang in Korean), Which is OT with purgative drugs such as Rhei Rhizoma added, has been used. However, the HMG-CoA reductase- and pancreatic lipase-inhibitory activities of herbal medicines, particularly OT and DOT, have not been studied.

Therefore we measured the HMG-CoA reductase and pancreatic lipase-inhibitory activity of OT and DOT, and their antihyperlipidemic activity in a rat hyperlipidemic model treated with Triton WR-1339.

MATERIALS AND METHODS

Materials Cholestyramine, Triton WR-1339, RS-HMG-CoA, NADPH, dl-dithiothreitol (DDT), triolein, tributyrin, pancreatic lipase, and EDTA-K were purchased from Sigma Chemical (U.S.A.). Total cholesterol and triglyceride assay kits were from Asan. Pharm. (Korea). Low-density lipoprotein (LDL) cholesterol assay kits were from BioMerieux (France). Lovastatin and orlistat were kindly donated by Dr. K. Kim of the Korea Food and Drug Administration.

Herbal medicines were purchased from Kyung Dong Marathon (Seoul, Korea) and identified by Dr. Sang-In Lee, Oriental Medicine College, Kyung Hee University. Voucher specimens are deposited at the Herbarium of the Kyung Hee East-West Medical Research Institute (Table 1).

DOT was consisted of 80% EtOH extract of Rhei Rhizoma 4 g and OT, of which the ingredients were 80% EtOH extracts of Coptidis Rhizoma 4 g, Phellodendri Cortex 4 g, Scutellariae Radix 4 g, and Gardeniae Fructus 4 g.

Extraction of Herbal Medicines Each herbal medicine (1 kg) was extracted twice with water or 80% ethanol in boiling water for 2 h. These extracts were filtered and evaporated in a rotary vacuum evaporator and then finally lyophilized with a freezing dryer. Dry weight yields (%) of extracts are shown in Table 1. To standardize the quality of these herbal medicines used in experiments, berberine in Coptidis Rhizoma and Phellodendri Cortex, baicalin in Scutellariae Radix, geniposide in Gardeniae Fructus, and sennoside A in Rhei Rhizoma were quantitatively assayed according to the previous methods. 13,14)

Animals Male Sprague–Dawley rats (240—260 g) were purchased from Jung-Ang Experimental Animal (Korea) and fed a commercial diet (Samyang, Korea). These animals were kept for at least 7 d prior to the experiments.

The rat hyperlipidemic model was prepared according to the method of Kusama et al. 15) Triton WR-1339 was injected at the end of the regular 16-h fasting period as a 10% solution in saline at a dose of 200 mg/kg body weight into the tail veins of rats under light ether anesthesia. Six rats were used per group. These rats were anesthetized with ether 18 h after Triton WR-1339 injection and 3 ml of blood was withdrawn by cardiac puncture. Serum was obtained by centrifugation (1500×g, 10 min). OT, DOT, orlistat, and lovastatin were administered orally once a day for 3 d. The final administrations of these samples were performed 1 h before Triton WR-1339 injection.

Determination of Total Serum Cholesterol, Triglyceride, and LDL Cholesterol Total cholesterol was mea-
sured by the enzyme method designed by Allain et al. Serum triglyceride was measured by the method designed by Sardesai et al. LDL Cholesterol was measured by the enzymatic method designed by Mainard et al.

**Partial Purification of HMG-CoA Reductase**

HMG-CoA reductase was partially purified according to the method of Edwards et al. Male Sprague–Dawley rats (250–300 g body weight) were housed one per cage in a room in which the lights were off from 07:00 to 19:00. Food and water were available ad libitum. For 3 d before killing, the rats were fed powdered rat chow containing 5% cholestyramine. Animals were killed at 13:00, at the peak of the HMG-CoA reductase cycle. Each rat liver was homogenized at 4 °C in 25 ml of buffer A (0.1 M sucrose, 0.05 M KCl, 0.04 M potassium phosphate, and 0.03 M potassium EDTA, pH 7.2) with a motor-driven, tight-fitting, glass-Teflon Potter–Elvehjem homogenizer, and the microsomes were prepared. Three-milliliter aliquots of the microsomal suspension were frozen in glass tubes at a rate of 6–8 °C per min unless otherwise stated, and stored at −20 °C for up to 2 months. For optimal solubilization of the reductase, the frozen microsomes were allowed to thaw at 37 °C before addition of an equal volume of 50% glycerol in buffer B (buffer A plus 10 mM DTT) preheated to 37 °C. The suspension was rehomogenized with 10 downward passes of the glass homogenizer and then incubated at 37 °C for 60 min. The suspension was diluted three-fold with buffer B heated to 37 °C to a final glycerol concentration of 8.3%, rehomogenized with 10 downward passes of the glass homogenizer pestle, and centrifuged at 100000 x g for 60 min at 25 °C. The supernatant containing solubilized HMG-CoA reductase was used for the enzyme inhibitory activity assay as the crude enzyme.

**Activity Assay of HMG-CoA Reductase**

The inhibitory activity assay of HMG-CoA reductase was performed according to the method of Edwards et al. Its activity was determined at 37 °C in a total volume of 0.5 ml using a Beckman spectrophotometer (Shimadzu). The cell path length was 1.0 cm. The oxidation rate of NADPH was initially determined in the absence of HMG-CoA and this blank value was subtracted from the rate obtained with both substrates. The activity assay reaction mixture contained 0.2 M KCl, 0.16 M potassium phosphate, 0.004 M EDTA, and 0.001 M DTT, pH 6.8, 0.2 mM NADPH, and 0.1 M RS-HMG-CoA.

**Activity Assay of Pancreatic Lipase**

The enzyme activity assay was performed according to the previous method. The reaction mixture (3.06 ml) containing 135 mM triolein (or tributyrin) emulsified in gum acacia, 2 mM sodium thioglycolate, and pancreatic lipase (0.6 unit using triacetin as a substrate) and the sample was adjusted to pH 8.8 with 0.1 M NaOH, incubated at 25 °C and titrated with 10 mM NaOH to adjust at the pH to 8.8. The inhibitory activity of the sample was calculated from the titrant volume.

**Statistical Analysis**

All the data from the in vivo experiments were expressed as mean ± standard deviation and statistical significance was determined using Student's t-test.

**RESULTS AND DISCUSSION**

To evaluate the inhibitory effects of OT and DOT on cholesterol biosynthesis, their inhibitory activities against HMG-CoA reductase were measured (Table 2). OT and DOT both potently inhibited it with IC50 values of 0.34 and 0.42 mg/ml, respectively. To investigate the inhibitors among the ingredients of these herbal formulae, the HMG-CoA reductase-inhibitory activities of their ingredients were measured. Coptidis Rhizoma was the most potent inhibitor, with IC50 value of 0.1 mg/ml, followed by Rheum palmatum. The HMG-CoA reductase-inhibitory activities of OT and DOT were more potent than those of their ingredients. This suggests that Coptidis Rhizoma synergistically inhibits HMG-CoA reductase by the other ingredients of the herbal formulae. The inhibitory activities of the 80% EtOH extract of OT, DOT, and ingredients were superior to those of the water extract.

We also measured the inhibitory activity of these herbal formulae against pancreatic lipase (Table 3). DOT potently inhibited it, although OT did not. To investigate its inhibitors, the inhibitory activity of DOT ingredients against pancreatic lipase was measured. Rheum palmatum was the most potent inhibitor, followed by Scutellariae Radix. The potent inhibitory activities of the 80% EtOH extract of OT, DOT, and ingredients were superior to those of the water extract.

To evaluate the antihyperlipidemic effects of OT and DOT, we measured their inhibitory activities in hyperlipidemic rats induced by Triton WR-1339 (Table 4). Triglyceride, total cholesterol, and LDL cholesterol levels in serum were increased by treatment with Triton WR-1339. This result was similar to those of the previous report (Olsson et al., 1956). However, compared with triglyceride, total cholesterol and LDL cholesterol levels in Triton WR-1339-alone group, those in the OT group were significantly decreased to 16.26.3, and 78.9% at the 200 mg/kg dose, respectively. In the DOT-treated group, serum triglyceride, total cholesterol, and LDL cholesterol levels were significantly decreased to 47.1, 35.7, and 67.0% those of the Triton-alone group at 200 mg/kg, respectively. The triglyceride level in the DOT-treated group was lower than in the 50 mg/kg lovastatin-treated group, and the total cholesterol level was similar to that in

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**Table 1. Extract Yields and Major Ingredient Component Contents of Five Constituent Herbs of Daio-Orendedokuto**

<table>
<thead>
<tr>
<th>Herbal medicine</th>
<th>Scientific name</th>
<th>Voucher number</th>
<th>Yield of extract (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>80% EtOH</td>
</tr>
<tr>
<td>Coptis japonica</td>
<td>Captis japonica MAKINO</td>
<td>KHUPVP01066</td>
<td>15.8 (27.8)(^a)</td>
</tr>
<tr>
<td>Phellodendron amurense</td>
<td>Phellodendron amurense RUPRECHT</td>
<td>KHUPVP01067</td>
<td>10.9 (4.3)(^a)</td>
</tr>
<tr>
<td>Scutellaria baicalensis</td>
<td>Scutellaria baicalensis GEORGI</td>
<td>KHUPVP01064</td>
<td>32.0 (17.2)(^a)</td>
</tr>
<tr>
<td>Gardeniae jasminoides</td>
<td>Gardenia jasminoides ELLIS</td>
<td>KHUPVP01059</td>
<td>28.3 (16.9)(^a)</td>
</tr>
<tr>
<td>Rheum palmatum</td>
<td>Rheum palmatum LINNE</td>
<td>KHUPVP01011</td>
<td>35.3 (0.76)(^a)</td>
</tr>
</tbody>
</table>

\(^a\)—d\) are contents of berberine, baicalin, geniposide, and sennoside A, respectively. Each result represents the mean of triplicate experiments.
the orlistat 10 mg/kg treated group.

The antihyperlipidemic activity of OT, which was a more potent HMG-CoA reductase inhibitor than DOT, was weaker than that of DOT. Accordingly, we believe that other factors, such as lipase-inhibitory activity, might be related to the antihyperlipidemic activity. We also measured the pancreatic lipase-inhibitory activity of OT, DOT, and their ingredients. Among them, Rhei Rhizoma and DOT exhibited the most potent inhibitory activity. DOT was more potent than OT. Accordingly, we believe that other factors, other than the oristat 10 mg/kg treated group.

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Judging from these results, we propose that the antihyperlipidemic activity of DOT may be due to the inhibition of pancreatic lipase as well as of HMG-CoA reductase.

REFERENCES