Antihyperlipidemic Effect of Crocin Isolated from the Fructus of Gardenia jasminoides and Its Metabolite Crocetin

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The pancreatic lipase inhibitors were isolated from the fructus of Gardenia jasminoides Ellis, and their antihyperlipidemic activities were measured. Gardeniae fructus (GF) water extract inhibited pancreatic lipase activity. Crocetin and crocin were isolated from GF water extract as inhibitors of pancreatic lipase with an IC50 value of 2.1 and 2.6 mg/ml (triolein as a substrate). Crocin and crocetin significantly inhibited the increase of serum TG level in corn oil feeding-induced triglyceridemic mice, as well as that of serum triglyceride and total and LDL cholesterol levels in Triton WR-1339-induced hyperlipidemic mice. These compounds also showed hypolipidemic activity in hyperlipidemic mice induced by high cholesterol, high fat or high carbohydrate diets for 5 weeks. The results suggest that the hypolipidemic activity of GF and its component crocin may be due to the inhibition of pancreatic lipase and crocin, and its metabolite, crocetin, can improve hyperlipidemia.

Key words Gardeniae fructus; crocin; crocetin; pancreatic lipase; hypolipidemic activity

Lipid metabolism normally maintains an elegant balance between synthesis and degradation. When the balance is lost, hyperlipidemia, such as hypertriglyceridemia and hypercholesterolemia, may develop. This can cause variety of serious diseases, such as arteriosclerosis, hypertension, obesity, diabetes, functional depression of some organs, etc.1,2

Pancreatic lipase (PL) is a key enzyme for lipid breakdown required to absorb fatty acids.3,4 Pancreatic lipase, one of the exocrine enzymes of pancreatic juice, catalyzes the hydrolysis of emulsified esters of glycerol and long-chain fatty acids. Short-chain fatty acids can be directly absorbed into the blood, while long-chain fatty acids and monoglycerides combine with bile salts to form water soluble micelles.5,6 The micelles are absorbed into the mucosal cells of the intestine, and the fatty acids and monoglycerides are resynthesized into triglycerides. These triglycerides are formed into small particles known as chylomicrons, which consist of triglycerides, the sterol lipid cholesterol, and apoproteins. These chylomicrons are transported to the muscle and adipose tissue. Dietary triglyceride is usually stored in the adipose tissue. Pharmacological agents that reduce the absorption of dietary triglyceride, thereby reducing the probability of the formation of atherosclerotic plaque, have been developed, such as orlistat and clofibrate.7,8 However, the PL-inhibitory activities of natural herbal products have not been studied.

As part of our continuing search for biologically active antitumor agents from natural herbal resources, we selected PL-inhibitors from the fructus of Gardenia jasminoides (Gardeniae fructus, Family Rubiaceae).

Gardeniae fructus (GF) is widely used in Asian countries as a natural colorant, and as a Chinese traditional medicine for its homeostatic, antiphlogistic, analgesic and antipyretic effects. It contains geniposide and crocin as main components.5,6 These components exhibit antioxidant, cytotoxic, antitumor and neuroprotective effects.7,8—12 However, antihyperlipidemic effects of GF and its components have not been thoroughly studied.

Therefore, we isolated the main components from GF and their metabolites by human intestinal bacteria, and measured their PL-inhibitory activity and their antihyperlipidemic activities on hypertriglyceridemic and hypercholesterolemic mouse models.

MATERIALS AND METHODS

Materials Triton WR-1339, triolein, PL and lovastatin were purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Total cholesterol and triglyceride assay kits were from Asan Pharmaceutical Co. Ltd. (Korea). Low-density lipoprotein (LDL) cholesterol assay kits were from BioMerieux (France). Orlistat (Xenical) was kindly donated by Dr. B.W. Song of Kyung Hee Medical Center, Kyung Hee University (Seoul, Korea).

A high fat diet containing 25% beef tallow [American Institute of Nutrition (AIN)-76 fat-diet #180337], a high cholesterol diet containing 1% cholesterol and 0.25% choline chloride (AIN-76 fat-diet #180338), and a high carbohydrate diet containing 50% sucrose (AIN-76 fat-diet #100000) were purchased from Dyets, Inc. (Bethlehem, PA, U.S.A.).

GF was purchased from Kyung Dong Market (Seoul, Republic of Korea) and identified by Dr. Nam-Je Kim, East-West Medical Research Institute, Kyung Hee Medical Center, Kyung Hee University. A voucher specimen (KHUV0-01059) was deposited at the Herbarium of the College of Pharmacy, Kyung Hee University.

Extraction of GF and Isolation of Crocin from GF and Its Metabolite Crocetin GF (500 g) was extracted twice with 51 of boiling water, filtered and evaporated in a rotary vacuum evaporator. The extract (98 g) was suspended with 500 ml water. The suspended extract was extracted with butanol. The butanol fraction (56 g) was applied to silica gel column chromatography and eluted with CHCl3:MeOH (10:1→4:1). Two major compounds, G-1 (2.1 g) and G-2 (0.76 g), were isolated. These isolated compounds (Fig. 1) were identified to be geniposide and crocin by comparison of their physicochemical data in the literature, respectively. Each component (0.5 g) was incubated with Bacteroides JY-6 (wt weight 5 g) for 12 h at 37 °C in 11 PBS, and then extracted with ethyl acetate. The extract was subjected to silica gel column chromatography and eluted with
CHCl₃ : MeOH (10 : 1→4 : 1). Genipin (55 mg, purity>90%) and crocetin (68 mg, purity>90%) were isolated.

Crocin: Pale yellowish amorphous powder. mp 127—128 °C. FAB-MS: 977 [M+H]⁺. ¹H-NMR (DMSO) δ: 7.39 (3H, 3' H, d, J=10.8 Hz), 6.84 (8H, 8' H, m), 6.81 (5H, 5' H, d, J=14.8 Hz), 6.67 (4H, 4' H, d, J=15.2, 11.2 Hz), 6.51 (7H, 7' H, m), 1.98 (10H, 10' H, s), 1.96 (9H, 9' H, s).


Geniposide: Colorless needles. mp 159—161 °C. FAB-MS ([M+H]⁺)=387. ¹H-NMR (CD3OD) δ: 3.72 (3H, s, OCH₃), 4.37 (2H, br. s, H₂-10), 5.82 (1H, m, H-7), 7.51 (1H, s, H-3).

Genipin: Colorless needles. mp 120—121 °C. FAB-MS: 227 [M+H]⁺.

Activity Assay of PL

The enzyme activity assay was performed according to the previously reported method.¹³ The reaction mixture (3.06 ml) containing 135 mM triolein emulsified in gum acacia, 2 mM sodium thioglycolate, and PL-Kusama anesthesia. Two hours after the administration of corn oil, blood samples were drawn by cardiac puncture under ether anesthesia. Six mice were used per group.

RESULTS

**In Vitro Inhibitory Effect of GF on Pancreatic Lipase Activity**

As part of our continuing search for biologically active antiarteriosclerosis agents from natural herbal resources, the water extract of GF potentely inhibited pancreatic lipase activity. Therefore, two main components, geniposide and crocin, and their metabolites by human intestinal bacteria were isolated, and their pancreatic lipase-inhibitory activity was determined (Table 1). Among the isolated components, crocin and its metabolite crocetin potently inhibited pancreatic lipase. The most potent inhibitor was crocin, with an IC₅₀ value of 2.1 mg/ml (triolein as a substrate).

**In Vivo Antiarteriosclerosis Activity of GF and Its Components**

To evaluate the hypolipidemic effects of GF, we measured its inhibitory activities in Triton WR-1339-induced hyperlipidemic mice (Table 2). Triglyceride, total cholesterol, and LDL cholesterol levels in serum were increased by treatment with Triton WR-1339. These results were simi-
lar to those in a previous report.\(^{19}\) Compared with the triglyceride, total cholesterol and LDL cholesterol levels in the Triton WR-1339-alone group, those in the GF, crocin and crocetin-treated groups were increased. However, HDL-cholesterol levels in the crocin and crocetin-treated groups were increased. The triglyceride level in the crocetin-treated group (0.05 g/kg) was decreased to less than that in the normal control group. We also measured the inhibitory effects in corn oil feeding-induced hyperlipidemic mice (Table 3). The serum level of triglyceride, not cholesterol, was increased by treatment with corn oil. In the crocin and crocetin-treated groups, serum triglyceride levels were significantly decreased to less than those in the control group. Crocetin exhibited a more potent effect than crocin.

**Effect of Long-Term Feeding of Crocin and Crocetin on Hyperlipidemic and Hypercholesterolemic Mouse Models** The hypolipidemic effect of long-term feeding of crocin and crocetin in high fat diet-induced hyperlipidemic mice was measured (Table 4). Triglyceride, total cholesterol, and LDL cholesterol levels in serum were increased by treatment with a high fat diet for 5 weeks. Crocetin- and crocetin-treated groups significantly decreased these levels. Crocetin exhibited the most potent effect.

We also measured antihyperlipidemic effect of crocin and crocetin in high cholesterol diet-induced hypercholesterolemic mice (Table 5). Triglyceride, total cholesterol, and LDL cholesterol levels in serum were increased by treatment with a high cholesterol diet. Compared with the control group, these levels in the crocin or crocetin-treated groups were decreased to less than those in the normal control group. Crocetin exhibited the most potent effect.

The antihyperlipidemic effect of crocin and crocetin in high carbohydrate diet-induced hyperlipidemic mice was measured (Table 6). The triglyceride, total cholesterol, and LDL cholesterol levels in serum were increased by treatment with a high carbohydrate diet. Compared with the control levels.
Table 5. The Effect of Crocin and Crocetin on Serum Triglyceride, Total Cholesterol, High Density Lipoprotein (HDL) Cholesterol and LDL Cholesterol Levels in High Cholesterol Diet-Induced Hyperlipidemic Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/d)</th>
<th>Serum level (mg/dl)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Triglyceride</td>
</tr>
<tr>
<td>Normal</td>
<td>—</td>
<td>74.92±4.48&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>109.15±4.44&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crocin</td>
<td>50</td>
<td>52.62±2.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crocetin</td>
<td>50</td>
<td>47.54±3.01&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>10</td>
<td>56.31±2.43&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values of serum levels are means±S.D. (n=6). <sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup>, <sup>e</sup> Those with the same letter are not significantly different at p<0.05.

Table 6. The Effect of Crocin and Crocetin on Serum Triglyceride, Total Cholesterol, High Density Lipoprotein (HDL) Cholesterol and LDL Cholesterol Levels in High Carbohydrate Diet-Induced Hyperlipidemic Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/d)</th>
<th>Serum level (mg/dl)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Triglyceride</td>
</tr>
<tr>
<td>Normal</td>
<td>—</td>
<td>94.12±3.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>158.02±1.93&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>Crocin</td>
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<td>Crocetin</td>
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</tr>
<tr>
<td>Xenical</td>
<td>10</td>
<td>117.17±1.53&lt;sup&gt;c&lt;/sup&gt;</td>
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</tbody>
</table>

Values of serum levels are means±S.D. (n=6). <sup>a</sup>, <sup>b</sup>, <sup>c</sup>, <sup>d</sup>, <sup>e</sup> Those with the same letter are not significantly different at p<0.05.

Fig. 2. Effect of Crocin and Crocetin on Body (A) and Epididymal Fat Pad (B) Weights of Hyperlipidemic Mice Induced by High Fat (HFD), High Cholesterol (HCD) or High Carbohydrate (HCD) Diets

Mice were classified into 15 groups. Each group contained 6 mice and their body weights were 24.3±0.93 g (mean±standard deviation): 1, normal; 2, control; 3, crocin; 4, crocetin; 5, Xenical for high fat and high carbohydrate diets or Lovastatin for group treated with high cholesterol diet. High cholesterol, high fat and high carbohydrate control groups were fed high-cholesterol, high fat and high carbohydrate diets for 5 weeks, respectively. The normal group received a solid normal diet alone. Crocin or crocetin at a dose of 50 mg/kg/d and Xenical at a dose of 10 mg/kg/d for the high fat and high carbohydrate diet-treated groups and Lovastatin at a dose of 10 mg/kg/d for the high cholesterol group were orally administered for 5 weeks. Body weight was measured before the final administration of the samples. The epididymal fats were taken under ether anesthesia and its weight was measured. *Significantly different, compared with normal group (p<0.05). †Significantly different, compared with control group (p<0.05).

DISCUSSION

Most herbal medicines are orally administered and their components inevitably come into contact with intestinal microflora in the alimentary tract. These components may be transformed before they are absorbed from the gastrointestinal tract. Crocin is easily transformed to crocetin by the intestinal bacteria of human or mouse (data not shown). Therefore, we isolated the main components geniposide and genipin and crocetin, and measured their pancreatic lipase-inhibitory and hypolipidemic effect. Crocin and crocetin inhibited pancreatic lipase-inhibitory activity, and had potent hypolipidemic activity in Triton WR-1339 or corn oil-induced hyperlipidemic mice. The hypolipidemic effect of GF was similar in the mode of inhibition of pancreatic lipase. Crocin and crocetin also significantly lowered serum cholesterol and triglyceride levels in hypercholesterolemic or hyperlipidemic mice induced by long-term feeding of high cholesterol, high fat or high carbohydrate diets. Crocetin and crocin inhibited the incremental increase in body weight in these animal models compared with that of control group (Fig. 2). These compounds also significantly reduced epididymal fat pad mass increased by a high fat diet. Their po-
tencies at a dose of 50 mg/kg are comparable with that of xenical at a dose of 10 mg/kg. These results suggest that crocin and crocetin can inhibit biosynthesis of triglycerides as well as cholesterol and/or their absorptions from the intestine into blood. PL is a key enzyme for lipid breakdown that enables the absorption of fatty acids. Short-chain fatty acids hydrolyzed by PL can be directly absorbed into the blood, while long-chain fatty acids and monoglycerides combine with bile salts to form water soluble micelles. The micelles are absorbed into the mucosal cells of the intestine. The fatty acids are resynthesized into triglycerides and then form chylomicrons, which consist of triglycerides, the sterol lipid cholesterol, and apoproteins. The chylomicrons are transported to the muscle and adipose tissue. Therefore, the inhibition of pancreatic lipase should not only be caused the hypolipidemic effect, but can also reduce the fat weight of epididymis.

However, crocin and crocetin should inhibit pancreatic lipase in the intestine and may exhibit a hypolipidemic effect. If absorbed, these compounds may similarly affect serum levels of triglyceride and cholesterol, and decrease body weight. The present results suggest that crocin can inhibit pancreatic lipase activity and improve hyperlipidemia more potently than crocin. Finally, we propose that GF, crocin and crocetin may be effective as hypolipidemic agents.

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REFERENCES