Daisaikoto Inhibits Pancreatic Lipase Activity and Decreases Serum Triglyceride Levels in Mice

Yukiko Matsuo,* Kenya Matsumoto, Niro Inaba, and Yoshihiro Mimaki

Tokyo University of Pharmacy and Life Sciences, School of Pharmacy; 1432–1 Horinouchi, Hachioji, Tokyo 192–0392, Japan.

Received May 7, 2018; accepted July 3, 2018

Daisaikoto Extract, a Kampo medicine listed in the Japanese pharmacopoeia 17th edition, is clinically used to treat obesity and related symptoms. Lipid metabolism is closely related to obesity, and pancreatic lipase inhibitors are therefore regarded as effective for the treatment of obesity. Although Daisaikoto has shown promise in the treatment of obesity, its mechanism of action has yet to be elucidated. In the present study, we found that Daisaikoto extract inhibits pancreatic lipase activity in a dose-dependent manner and decreases serum triglyceride levels in mice. To determine the crude drugs responsible for lipase inhibition, 8 variants of Daisaikoto extract without one crude drug were prepared and evaluated for lipase inhibitory activity. The lipase inhibitory activity of the Daisaikoto extract was reduced by excluding Scutellariae Radix (SR), which was found to inhibit lipase activity with an IC_{50} value of 1.70 mg/mL. In conclusion, Daisaikoto represents a natural medicine, in particular SR, capable of inhibiting pancreatic lipase and lipid absorption.

Key words Daisaikoto; Kampo medicine; pancreatic lipase; Scutellariae Radix; triglyceride; obesity

Obesity is a lifestyle-related disease that leads to metabolic syndrome, which progresses to arteriosclerosis.3) Death can result from myocardial infarction, cerebral infarction, and hemorrhage. Pancreatic lipase is a digestive enzyme of dietary lipids. Inhibition leads to decreased triglyceride (TG) digestion and lipid absorption.3) Cetilistat, an anti-obesity medication, targets pancreatic lipase. However, cetilistat is known to cause gastrointestinal adverse effects such as oily and loose stool, fecal incontinence, frequent bowel movements, and flatulence.3) It has yet to be listed in the National Health Insurance (NHI) drug pricing standard. Currently, Kampo medicines are expected to produce promising treatments for obesity.4,5) Daisaikoto Extract is listed in the Japanese pharmacopoeia 17th edition and is clinically used for obesity, and related symptoms.6) Previous studies of mechanisms of Daisaikoto extract have resulted in the inhibition of TG and very low density lipoprotein (VLDL) synthesis in vitro and prevention of symptoms of metabolic syndrome in vivo.7,8) However, no examination has been carried out on the inhibitory potential of Daisaikoto against pancreatic lipase activity.

In the present study, Daisaikoto extract was revealed to inhibit pancreatic lipase activity, and decreases serum triglyceride levels in mice. Moreover, Scutellariae Radix (SR) was shown to contribute to the lipase inhibitory activity of Daisaikoto extract.

MATERIALS AND METHODS

Preparation of Test Samples Daisaikoto extract, its variant formulation extracts without one crude drug, and single crude drug extracts, were prepared. Daisaikoto consists of 8 crude drugs as listed in Table 1. The crude drugs were obtained from Uchida Wakan-Yaku Co., Ltd. (Tokyo, Japan). The formulation was immersed in 600 mL of distilled water and decocted to half the volume for 1 h. The decoction was filtered off, and filtrate was lyophilized. The yields of lyophilized extracts were 5.5 g from Daisaikoto, 1.7 g from Bupleuri Radix (6.0 g) (lot number; E2H0248), 0.67 g from Pinelliae Tuber (4.0 g) (lot number; E6T0528), 0.75 g from Scutellariae Radix (3.0 g) (lot number; AA30015), 0.86 g from Paeoniae Radix (3.0 g) (lot number; D330256), 2.2 g from Zizyphi Fructus (3.0 g) (lot number; D910328), 0.15 g from Aurantii Fructus Immatures (2.0 g) (lot number; E9F0118), 0.11 g from Zingiberis Rhizoma (1.0 g) (lot number; E360227), 0.38 g from Rhei Rhizoma (1.0 g) (lot number; E6E0301), respectively. The yields of Daisaikoto preparation without Bupleuri Radix was 4.5 g, the one without Pinelliae Tuber was 5.6 g, the one without Scutellariae Radix was 4.8 g, the one without Paeoniae Radix was 6.2 g, the one without Zizyphi Fructus was 6.0 g, the one without Aurantii Fructus Immatures was 5.4 g, the one without Zingiberis Rhizoma was 6.9 g, the one without Rhei Rhizoma was 6.1 g, respectively. In biological assay, the concentration of test samples of 8 variant Daisaikoto extracts without one crude drug were the same as that of Daisaikoto.

Animals Male ddY mice, 5 weeks old, were purchased from Japan Laboratory Animals (Tokyo, Japan) and maintained in the animal facility of the Tokyo University of Pharmacy and Life Sciences. The animals were housed in cages under a controlled temperature (23±2°C) and a 12 h light–dark cycle. The mice were allowed free access to breeding food and water. All experiments were approved by the Tokyo University of Pharmacy and Life Sciences Animal Use Committee (approval number: P16–03, P17–84).

Lipase-Inhibitory Activity The pancreatic lipase activity of each test sample was measured by a modified method using a commercially available kit (Lipase Kit S, Dainippon Pharmaceutical, Osaka, Japan). Lipase (lipase from porcine pancreas, PCode 1001926546) was purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Lipase activity was measured at 412 nm using a microplate reader. The inhibition rate was calculated by the following formula: Inhibition rate (%)=[1−(ΔA_{sample}−ΔA_{blank})/(ΔA_{control}−ΔA_{blank})]×100. The IC_{50} value was defined as the concentration of inhibitor required to inhibit 50% of the lipase activity.
Measurement of Triglyceride in Animals  After 5 weeks, mice were fasted overnight. Daisaikoto extract (1.70 g/kg), Daisaikoto variant formulation extract without Scutellariae Radix (1.70 g/kg), Scutellariae Radix extract (0.222 g/kg), 0.5% carboxymethyl cellulose (CMC), or cetilistat (0.240 g/kg) were orally administered to mice 15 min before intralipid (soybean oil 20% emulsion, 20 mL/kg, Sigma-Aldrich) loading, respectively. Blood samples were collected from the abdominal vena cava at 120 min after intralipid-loaded treatment, and centrifuged (700 × g for 10 min) to obtain serum samples. The TG levels in serum were measured using a commercially available kit (Wako Triglyceride E-Test kit, Wako Pure Chemical Industries, Ltd., Osaka, Japan). The dose of Daisaikoto extract to mice was 28 times higher than the usual human dosage.

Statistical Analysis  Data are represented as the mean±standard error of the mean (S.E.M.). Dunnett’s test was used for statistical analysis. The level of significance was indicated by p values. *** p<0.0001, ** p<0.001, * p<0.01.

RESULTS

The pancreatic lipase inhibitory activity of Daisaikoto extract was measured using a commercially available kit (Fig. 1). Cetilistat was used as a positive control, with 91.3% inhibition at 30.0 µg/mL. Daisaikoto extract was dissolved in H2O and diluted to concentrations of 6.25, 12.5, 25.0, and 50.0 mg/mL. Daisaikoto extract inhibited lipase activity in a dose-dependent manner. Daisaikoto extract at 12.5 mg/mL inhibited 47.0% of the lipase inhibitory activity, whereas 25.0 mg/mL showed 83.3% inhibition.

Next, the effects of Daisaikoto extract on TG levels in ddY mice fed a single dose administration of high-fat diet, intralipid, were examined (Fig. 2). Serum TG levels were determined following a single-dose of Daisaikoto extract and intralipid oral treatment. In the control group, serum TG levels were increased from 92.0±13.1 to 355±44.8 mg/dL following administration of intralipid compared to those of the untreated group. Treatment of the mice with cetilistat reduced intestinal fat absorption by inhibiting pancreatic lipase activity. Serum TG levels were held at 160±20.6 mg/dL. Daisaikoto significantly suppressed the elevation in serum TG levels at 206±43.0 mg/dL in mice.

To identify which ingredients of Daisaikoto are involved in pancreatic lipase inhibition, 8 variant Daisaikoto extracts without one crude drug were prepared, and the lipase activity at 6.25 and 12.5 mg/mL was evaluated. The Daisaikoto variant extract without SR showed no lipase inhibitory activity. In contrast, the other 7 variant extracts maintained lipase inhibitory activity in a dose-dependent manner (Fig. 3).

Extracts of the 8 crude drugs were examined to confirm that SR plays an important role in pancreatic lipase inhibition (Table 2). Daisaikoto extract showed lipase inhibitory activity, with an IC50 value of 13.4±1.0 mg/mL. Among the 8 crude drug extracts, SR was the most potent, with an IC50 value of 1.70±0.05 mg/mL. Although the inhibitory potency was weaker than that of SR extract, Rhei Rhizoma extract also acted as a lipase inhibitor with IC50 value of 7.11±0.23 mg/mL. The remaining extracts did not inhibit lipase activity at 20.0 mg/mL (IC50>20.0 mg/mL).

Finally, to clarify the effect of SR extract on serum TG levels in mice fed intralipid, a single oral dose of either SR extract or Daisaikoto variant extract without SR was administered to mice (Fig. 4). The SR group exhibited significantly lower TG levels at 160±35.0 mg/dL, relative to the control group. The Daisaikoto without SR group tended to have reduced serum TG levels at 308±47.0 mg/dL, but the difference compared to the control group was not statistically significant.

DISCUSSION

Pancreatic lipase plays a key role in the hydrolysis of dietary fats. In the present study, we investigated the pancreatic lipase inhibitory activity of Daisaikoto extract. Daisaikoto extract was shown to inhibit lipase activity in a dose-dependent manner, as shown in Fig. 1. Daisaikoto extract was evaluated...
for its effect on serum TG levels using ddY normal mice fed a high-fat diet. A single oral dose of Daisaikoto extract efficiently reduced serum TG levels in mice loaded with intralipid (Fig. 2). These data indicate that Daisaikoto extract suppress elevated serum TG levels via lipase inhibition, which partially accounts for the anti-obesity effects of Daisaikoto.

Kampo medicines are combinations of several crude drugs. The pharmacological activity of Kampo medicines is explained by the additive effects of ingredient crude drugs.\textsuperscript{9,10} To identify the ingredients contained in Daisaikoto involved in pancreatic lipase inhibition, Daisaikoto extract and variant extracts without one crude drug were tested. SR was revealed to be the primary ingredient of Daisaikoto contributing to lipase inhibition, as shown in Fig. 3.

SR inhibited pancreatic lipase with an IC\textsubscript{50} value of 1.7±0.05 mg/mL, as depicted in Table 2. A single oral dose of Daisaikoto variant extract without SR did not significantly decrease serum TG levels, whereas treatment with the SR extract showed an anti-hyperlipidemic effect. These results indicate that SR plays an important role in the lipase inhibitory and anti-hyperlipidemic effects of Daisaikoto (Figs. 3, 4). The single crude drug extract of Rhei Rhizoma also inhibited lipase activity (Table 2); however, the Daisaikoto variant extract without Rhei Rhizoma maintained lipase inhibitory activity (Fig. 3). Conversely, the lipase inhibitory effect decreased when Zingiberis Rhizoma was excluded from Daisaikoto, although Zingiberis Rhizoma did not inhibit lipase activity as a single agent (IC\textsubscript{50} >20.0 mg/mL). This phenomenon, which may be a characteristic of Kampo medicine, remains to be determined.

In a previous study, spontaneous obese type II diabetes (TSOD) mice were fed diets containing 1.0 or 3.0% Daisaikoto extract for 8 weeks.\textsuperscript{11} TG levels were significantly lower in both groups relative to those of the control group. In ddY normal mice, treatment with Daisaikoto extract did not induce any changes in TG levels under the same conditions. These results suggest that Daisaikoto improves lipid metabolism in diabetes models, when mice are administered Daisaikoto for a long term. In another study, Daisaikoto decreased plasma cholesterol levels in female heritable heterozygous hypercholesterolemic rabbits, whereas there was no effect on TG levels.\textsuperscript{12} This phenomenon, which may be notable that a single-dose of Daisaikoto extract in normal mice reduces serum TG levels directly through inhibition of lipase activity and lipid absorption. Therefore, Daisaikoto could be a possible alternative medicine to treat obesity.

In conclusion, Daisaikoto is a promising natural medicine for obesity. One of the mechanisms underlying the action of Daisaikoto is inhibition of pancreatic lipase and lipid absorption, resulting in reduced serum TG levels. SR contained in Daisaikoto is mainly responsible for the lipase inhibitory activity. Recently, baicalin, wogonin, and oroxylin A isolated from SR have been reported to inhibit lipase activity.\textsuperscript{13} Further studies are required to identify additional active compounds in Daisaikoto that are capable of inhibiting pancreatic lipase.

\textbf{Conflict of Interest} The authors declare no conflict of interest.

---

\begin{table}[h]
\centering
\begin{tabular}{ll}
\hline
\textbf{Extract} & \textbf{IC\textsubscript{50} (mg/mL)} \\
\hline
Daisaikoto & 13.4±1.01 \\
Bupleuri Radix & >20.0 \\
Pinelliae Tuber & >20.0 \\
Scutellariae Radix & 1.70±0.0500 \\
Paonieiae Radix & >20.0 \\
Zizyphi Fructus & >20.0 \\
Aurantii Fructus Immaturus & >20.0 \\
Zingiberis Rhizoma & >20.0 \\
Rhei Rhizoma & 7.11±0.225
\hline
\end{tabular}
\caption{The Pancreatic Lipase Inhibitory Activity (IC\textsubscript{50} mg/mL) of the Extracts of Daisaikoto and 8 Crude Drugs Composed of Daisaikoto}
\end{table}

---

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{The Pancreatic Lipase Activity (% of Control) of the Daisaikoto Variant Extracts without One Crude Drug and Daisaikoto Extract (6.25 and 12.5 mg/mL) Data are represented as the mean±S.E.M. in triplicate.}
\end{figure}

---

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Effects of Daisaikoto Variant Extract without SR and SR Extract on Serum TG Levels of Mice Fed Intralipid Daisaikoto variant extract without SR (1.70 g/kg), SR extract (0.222 g/kg), and cetilistat (0.240 g/kg). Data are represented as the mean±S.E.M. (n=5); ***p<0.0001 vs. control, **p<0.001 vs. control, *p<0.01 vs. control.}
\end{figure}
interest.

Supplementary Materials  The online version of this article contains supplementary materials.

REFERENCES


