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Inhibitory effects of hydroxylated cinnamoyl esters on lipid absorption and accumulation

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ABSTRACT

Obesity is a risk factor associated with several lifestyle-related diseases, for example, diabetes, high blood pressure, hyperlipidemia and cancer. Caffeic acid 2-phenylethyl ester (CAPE, **1**), a naturally-occurring compound found in various plants and propolis, which exhibits anti-inflammatory, immunomodulatory and cytotoxic activities and inhibits 3T3-L1 differentiation to adipocytes. As part of our efforts to moderate lifestyle-related diseases, we synthesized analogs of **1** and studied their effects on pancreatic lipase activities, lipid absorption, and 3T3-L1 differentiation. We found that catechols **1**–**4** show inhibitory activities against pancreatic lipase in a dose-dependent manner in vitro. Compounds **1**–**3** proved to be more potent inhibitors of pancreatic lipase than **5**, **6**, **8**, and **9**, which have one hydroxyl group, respectively. Compound **7** has three aromatic hydroxyl groups and restrains greater lipase inhibitory activity than the other compounds. In addition, **7** and **3** significantly suppress a rise in blood triglyceride (TG) levels in mice given corn oil orally. Furthermore, **2** and **3** are more potent at preventing 3T3-L1 differentiation (lipid accumulation) than **1**, while **7** is more potent than **3**, **8**, and **9** in these assays. Compounds **2**, **3**, and **7** inhibit lipid absorption and accumulation, with new compound **7** being the most potent. These results indicate that **7** may have potential benefits as a health agent with anti-obesity properties.

1. Introduction

Obesity is an abnormal condition, in which excessive triglycerides (TGs) accumulate in the adipose tissue. Recently, the number of obese people has been increasing due to a lack of exercise, a Western diet, and irregular-eating habits. Obesity is the most important risk factor for lifestyle-related diseases, including hypertension, type 2 diabetes and hyperlipemia.[1–4] In particular, obesity causes an imbalance in the level of adipocytokines secreted from adipocytes, including leptin, adiponectin, resistin, and plasminogen activator inhibitor-1. This can result in a metabolic syndrome that includes an excessive accumulation of fat.[5,6] Furthermore, this metabolic syndrome can develop into atherosclerotic disease, which is associated with a high mortality rate.[5,7] Therefore, it is important to prevent or reduce obesity to achieve a better health-related quality of life.

Caffeic acid serves as a key intermediate in lignin biosynthesis. Caffeic acid 2-phenylethyl ester (CAPE) (**1**, Fig. 1), is a naturally occurring lipophilic derivative of caffeic acid, which is found in many plants, in particular propolis of the honeybee hive. Compound **1** has been known to exhibit numerous biological activities, including anti-oxidant,[8] anti-inflammatory,[9] anti-viral,[10] anti-bacterial,[11] anti-atherosclerotic,[12] immunostimulatory,[13] and anti-tumor[14] properties. Compound **1** can display antioxidant effects by blocking production of reactive oxygen species as well as xanthine/xanthine oxidase system.[15] It also exhibits anti-inflammatory properties through the down-regulation of prostaglandin and leukotriene synthesis by inhibiting cyclooxygenase.[16,17]

Abbreviations: **1**, caffeic acid 2-phenylethyl ester, 3,4-dihydroxycinnamic acid 2-phenylethyl ester, 3-(3,4-dihydroxyphenyl)propenoic acid 2-phenylethyl ester; **2**, 3,4-dihydroxycinnamic acid 6-phenylhexyl ester; **3**, 3,4-dihydroxycinnamic acid decyl ester; **4**, 3,4-dihydroxycinnamic acid 2-geranyl ester; **5**, 4-hydroxycinnamic acid 2-phenylethyl ester; **6**, 4-hydroxycinnamic acid 6-phenylhexyl ester; **7**, 3,4,5-trihydroxycinnamic acid decyl ester; **8**, 3-hydroxycinnamic acid decyl ester; **9**, 4-hydroxycinnamic acid decyl ester; AUC, the area under the curve; DIM, dexamethasone, insulin, methylisobutylxanthine; DMSO, dimethylsulfoxide; BSA, bovine serum albumin; PBS, phosphate-buffered saline (1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 136.9 mM NaCl, pH 7.2); EDTA, ethylenediaminetetraacetic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; SE, standard error.

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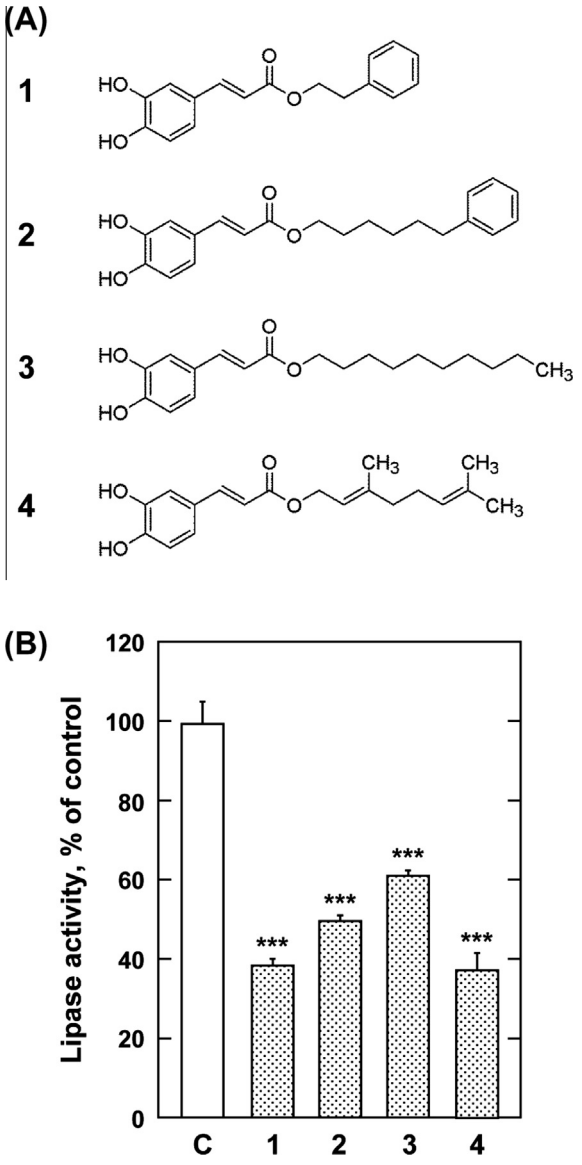

Figure 1. Inhibitory effects of compounds **1**–**4** on pancreatic lipase activity. (A) Chemical structures of compounds **1**–**4**, (B) pancreatic lipase activities measured in the presence of 30 μM concentration of **1**–**4** in vitro. Pancreatic lipase activities are shown as the relative activity (%) against control. Results are expressed as means ± SE of three independent experiments. $^{***}p$ <0.001 significant differences from the control.

Previous studies have shown that 25–50 μM concentrations of **1**, suppress 3T3-L1 differentiation to adipocytes by inhibiting peroxisome proliferator-activated receptor γ (PPARγ) and oxidative stress.[18–21] The oxidative stress-induced differentiation of 3T3-L1 cells to adipocytes is attenuated by treatment with **1**.[21] Since these results indicate that **1** may decrease accumulation of lipids synthesized during 3T3-L1 cell differentiation, it is possible that **1** might reduce obesity and thereby promote an improved health-related quality of life.

Fat absorption in the small intestine can be suppressed by mechanisms that include (1) inhibiting the activity of digestive lipases, which hydrolyze TGs to non-esterified fatty acids and monoglycerides or glycerol, and (2) decreasing the uptake of these hydrolysates by intestinal epithelial cells. These are several reports that fat absorption is suppressed by inhibiting pancreatic lipase activity.[22–25] In the current study, we synthesized new analogs of **1**; Compound **2** exhibits a phenylhexyl ester in which 4 carbons are added to the phenylethyl residue of **1**; Compound **3** is a decyl ester, which has high affinity to cell membranes; Compound **4** is geranyl ester, which is structurally more stable than either phenylhexyl or decyl esters. We examined their effects on pancreatic lipase activity, lipid absorption and lipid accumulation during 3T3-L1 cell differentiation.

2. Results

2.1. Effects of compounds 1–4 on pancreatic lipase activity

Pancreatic lipase hydrolyzes dietary TG substrates to 2-monoacylglycerol and two fatty acids. The resulting monomers are absorbed from the small intestine and resynthesized into TGs. Thus, pancreatic lipase is an enzyme essential for the absorption of TGs. We examined pancreatic lipase activities in vitro in the absence or in the presence of 30 μM of compounds **1**–**4** Fig. 1A). These compounds are all esters of caffeic acid having distinct ester groups. Pancreatic lipase activity was significantly inhibited by all four compounds (Fig. 1B). Compound **1** (2-phenylethyl ester) suppressed to the extent of approximately 39% as compared with control. In contrast, **2** (6-phenylhexyl ester), in which the side chain of **1** has been extended by four carbons, showed approximately 50% inhibition. On the other hand, **3** (decyl ester) and **4** (geranyl ester) showed approximately 38% and 62% inhibition, respectively. These results suggest that the side chain structure is critical for pancreatic lipase inhibitory activity, and that **4** is as potent as **1**.

2.2. Effects of hydroxyl substitution pattern on pancreatic lipase activity

In order to examine whether the number of hydroxyl residues in caffeic acid affects lipase inhibitory activities, we synthesized new analogs (**5**–**9**, Fig. 2A) and examined the effects of these compounds on pancreatic lipase activity at 30 μM concentration. While pancreatic lipase activity was significantly inhibited by the 3,4-dihydroxy-containing **1** and **2** (approximately 62% and 54%), compounds **5** and **6**, which have one hydroxyl at the 4-position, lost all inhibitory activity. Compound **3**, also which has 3,4-dihydroxy substituents, suppressed pancreatic lipase activity to approximately 67%. In contrast, **7**, which has a 3,4,5-trihydroxy pattern, was approximately 59% more potent than **3**, exhibiting an inhibitory potency of approximately 92%. In contrast, compounds **8** and **9**, which have single hydroxyls at the 3- and 4-positions, respectively, did not show inhibitory activity (Fig. 2B). Thus, **5**, **6**, **8**, and **9**, which have single phenolic hydroxyls, lost the pancreatic lipase inhibitory activity of **1**–**3**, which contain catechol motifs. Introduction of a third hydroxyl to **3** provided **7**, which was the most potent inhibitor among compounds examined. Compounds **1**–**3**, and **7** inhibited pancreatic lipase activity in dose-dependent manner (0.1–40 μM), with ED_{50} values being approximately 10 μM for **1**, 22 μM for **2**, >40 μM for **3**, and 0.9 μM for **7** (Fig. 3). The new compound **7** reduced pancreatic lipase activities more effectively than well-known **1**.

2.3. Effects of compounds on fat absorption

Because the above assays examined effects of compounds on pancreatic lipase activity in vitro, next we determined whether **7** and **3** (potent and weak inhibitors, respectively) affect fat absorption in vivo. Corn oil containing TGs was administered without or with **7** and **3**, and then blood TG levels were measured every 2 h. At 2–6 h after the administration of corn oil, the elevation of plasma TG levels was significantly lower in the groups treated with **7** as compared to the control group (Fig. 4A). The area under the