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Anti-lipase and Lipolytic Activities of Ursolic Acid Isolated from the Roots of *Actinidia arguta*

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The aim of this study was to investigate the anti-obestic effects of ursolic acid isolated from the roots of *Actinidia arguta*, as well as the mechanism of action of this compound. This was conducted by testing whether ursolic acid inhibited the elevation of the rat plasma triacylglycerol levels after oral administration of a lipid emulsion containing corn oil in rats. Ursolic acid prevented the elevation of plasma triacylglycerol levels 2 h after oral administration of the lipid emulsion at a dose of 100 mg/kg. Furthermore, ursolic acid inhibited phosphodiesterase activity *in vitro* with an IC_{50} of 51.21 μM and enhanced lipolysis in rat fat cells. We suggest that the inhibitory effects of ursolic acid, isolated from the roots of *A. arguta*, on obesity, might be attributable to the inhibition of lipid absorption through the inhibition of pancreatic lipase and by enhancing lipolysis in fat cells.

Key words: Ursolic acid, Lipolysis, Obesity, Pancreatic lipase, Phosphodiesterase

INTRODUCTION

Obesity is a global health problem, resulting from an energy imbalance caused by an increased ratio of caloric intake to energy expenditure. Obesity is also known to be risk factor for the development of metabolic disorders, dyslipidemia, atherosclerosis and type 2 diabetes (Larsson et al., 1981; Hartz et al., 1983). In recent years, there has been a great increase in the use of herbal medicines for the treatment of obesity (Sharpe et al., 2007).

Actinidia arguta (family Actinidiaceae) is a smooth-skinned grape-sized kiwifruit native to Korea, Northern China, Siberia and Japan (Ferguson, 1991). It is commercially available in New Zealand as well as several other fruit-producing countries. This species has a long history of human consumption, and has been used in traditional Chinese medicine to improve general health (Kim and Xiao, 1995). *A. arguta* has recently been used as a complementary drug in the treatment of obesity in Korea. However, details of the mechanism of action and the active compound of the roots of *A. arguta* are still unclear. Previous phytochemical investigations on *A. arguta* resulted in the isolation of flavonoids (Webby and Markham, 1990), phenolic compounds (Lim et al., 2006), triterpenes (Whang et al., 2000) and lignans (Whang et al., 2000). Recently, a new pentacyclic triterpene, 3-*O*-*trans*-*p*-coumaroyl actinidic acid, was isolated from the roots of *A. arguta* by our group (Jang et al., 2008).

Ursolic acid, a pentacyclic triterpene acid, has been isolated from many kinds of medicinal plants (Fig. 1) (Huang et al., 1994, Ohigashi et al., 1986, Tokuda et al., 1986). There are a number of reports showing that ursolic acid exerts various biological activities including anti-tumor, anti-inflammatory, anti-microbial, anti-fungal and anti-hyperlipidemic properties (Huang et al., 1994; Lim et al., 2007; Manez et al., 1997; Min et al., 2008; Najid et al., 1992; Ohigashi et al., 1986). In a previous study, ursolic acid isolated from the roots of *A. arguta*, was shown to exert pancreatic lipase inhibition *in vitro* (Jang et al., 2008). Based on the inhibitory activity of ursolic acid on pancreatic lipase *in vitro*, we tested whether ursolic acid could

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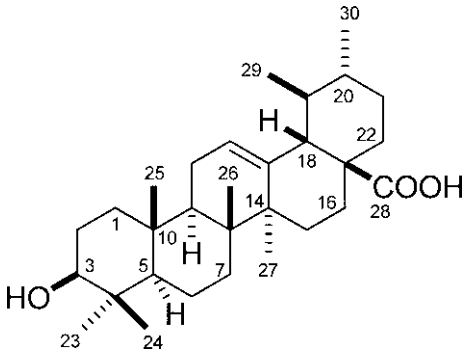

Fig. 1. A chemical structure of ursolic acid

inhibit fat absorption *in vivo*. We further investigated the mechanism by which ursolic acid modulated fat metabolism using a phosphodiesterase (PDE) activity assay and lipolytic assay in rat fat cells.

MATERIALS AND METHODS

Isolation of ursolic acid

We previously reported that ursolic acid was isolated from the roots of *A. arguta* (Jang et al., 2008).

Animals

Male Wistar rats (5 weeks old) were purchased from Koatech (Kyungkido, Korea), and housed for 1 week in a 12 h/12 h light/dark cycle in a temperature- and humidity-controlled room. The animals were given free access to food and water. After adaptation to the conditions for 1 week, the healthy animals were used in the present study. The Animal Studies Committee of Korea Institute of Orient Medicine approved the experimental protocol.

Estimation of plasma triacylglycerol after oral administration of lipid emulsion in rats

Rats (6 weeks old, body weight 200~250 g) that had fasted overnight were orally administered with 3 mL of lipid emulsion consisting of corn oil (6 mL), cholic acid (80 mg), cholesteryloleate (2 g) and saline solution (6 mL) with or without ursolic acid (50 and 100 mg/kg body weight). Blood was taken from the tail vein 0, 1, 2, 3 and 4 h after oral administration of the lipid emulsion and centrifuged at 5500 × g for 5 min to obtain the plasma. Triacylglycerol levels were determined using a triglyceride E-test Wako kit (Wako Chemicals).

Phosphodiesterase activity assay

PDE activities were assayed using the PDE-GloTM phosphodiesterase assay kit (Promega Corp., WI, USA) according to the manufacturer's instructions. 3-Isobutyl-1-methylxanthine (IBMX) was used as a positive control.

Lipolysis assay in fat cells

Young male Wistar rats were sacrificed by cervical dislocation, and their epididymal adipose tissue was quickly removed. Fat cells were isolated from the adipose tissue using a previously reported method (Rodbell, 1964). An aliquot of the fat cell fraction (50 μL packed volume) was incubated for 1 h at 37°C in 200 μL of Hanks balanced solution (pH 7.4) supplemented with 2.5% BSA (bovine serum albumin), isoproterenol (25 μL, final concentration: 10 μM) and the indicated amounts of test compounds (25 μL). The release of free fatty acid (FFA) was measured as described previously (Okuda et al., 1986). Briefly, the incubation mixture (250 μL) was mixed with 3 mL of chloroform/n-heptane (1:1, v/v) containing 2% methanol, and extracted by shaking the tube horizontally for 10 min in a shaker. The mixture was centrifuged at 2000 × g at 25°C for 5 min, and the upper aqueous phase was removed by suction, and copper reagent (1 mL) was added to the lower organic phase. The tube was then shaken for 10 min, the mixture was centrifuged at 2000 × g at 25°C for 10 min and 0.5 mL of the upper organic phase (which contained the copper salts of the extracted fatty acid) was treated with 0.5 mL of 0.1% (w/v) bathcurproine in chloroform containing 0.05% (w/v) 3-(2)-tertbutyl-4-hydroxyanisol. The absorbance of the solution was then measured at a wavelength of 480 nm using a microplate reader (Synergy HT, BioTek).

Data analysis

All experiments were repeated 3~4 times and representative data are shown. Data are expressed as mean ± S.E.M. of multiple experiments. Between-group differences were analyzed using a one-way ANOVA followed by a Tukey multiple comparison test (PRISM software, Graph Pad). Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

In the present study, in order to verify the anti-obestic effects of ursolic acid, we investigated whether ursolic acid exerted anti-lipase and lipolytic activity. Ursolic acid was first subjected to an *in vivo* experiment to evaluate its pancreatic lipase inhibitory activity. We examined the plasma triacylglycerol levels after oral administration of lipid emulsion in rats. Fig. 2 shows the time course of the plasma triacylglycerol level after oral administration of lipid emulsion with

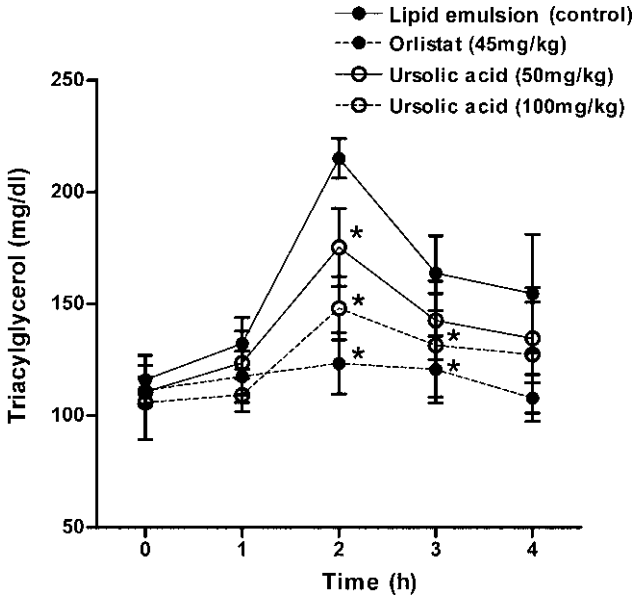

Fig. 2. Effects of ursolic acid and orlistat (a lipase inhibitor) on rat plasma triacylglycerol levels after oral administration of a lipid emulsion. Values are expressed as means ± S.E.M. (n = 4) *$p < 0.05$ vs. control.

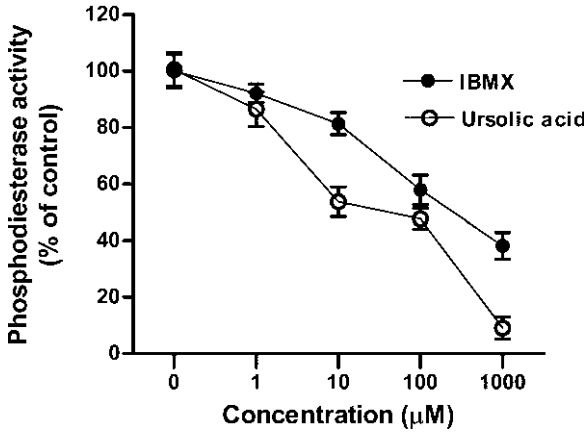

Fig. 3. Effects of ursolic acid and IBMX (a PDE inhibitor) in an *in vitro* PDE inhibition assay. Values are means ± S.E.M. (n = 4).

or without ursolic acid. At 2 h and 3 h after administration, ursolic acid dose-dependently (50 to 100 mg/kg body weight) reduced the plasma triacylglycerol levels, but this effect was weaker than that of the positive control, orlistat. Recently, we reported that ursolic acid, isolated from an EtOAc-soluble extract of the roots of *A. arguta*, also exhibited high anti-lipase activities (IC_{50} value of 15.83 μM) *in vitro* (Jang et al., 2008). Moreover, ursolic acid suppressed the time-dependent increase of plasma triacylglycerol concentration in rats that had been orally injected with corn oil. Considering its inhibition of lipase activity, as well as its suppressive effect on dietary triacylglycerol digestion, ursolic acid seems to be an active compound that exerts an inhibitory effect on lipase activity in the roots of *A. arguta.*

Obesity is associated with diabetes, cardiovascular disease and osteoarthritis (Kopelman, 2000). There are various therapeutic approaches to treating obesity, including suppression of food intake, increased thermogenesis, accelerated lipolysis and inhibition of lipogenesis. It has also been suggested that dietary fat promotes body fat storage more effectively than dietary carbohydrate. Thus, inhibition of the digestion and absorption of dietary fat is a key factor in treating obesity. Dietary fat is not directly absorbed from the intestine unless it has been subjected to the action of pancreatic lipase (Gargouri et al., 1997). Thus, the application of a pancreatic lipase inhibitor was tested as a treatment for high-fat diet-induced obesity in humans. Orlistat, a pancreatic lipase inhibitor, prevented obesity and hyperlipidemia through the enhancement of fat excretion in feces and the inhibition of pancreatic lipase (Drent et al., 1995). In the present study, we showed that ursolic acid prevented an increase in the plasma triacylglycerol levels after oral administration of lipid emulsion in rats. This may be attributable to the inhibition of intestinal absorption of dietary fat. This finding suggests that ursolic acid may inhibit the uptake of dietary fat.

Next, we evaluated the PDE inhibitory activity and lipolytic activity of ursolic acid. The results of inhibition of cAMP-PDE activity of ursolic acid are presented in Fig. 3. Ursolic acid is a potent inhibitor of PDE product (IC_{50} value of 51.21 μM). As shown in Fig. 4, ursolic acid induced the release of free fatty acid in fat cells by lipolysis at a concentration of 100 μM. FFA release was used as indicator of adipocyte lipolysis. Isoproterenol stimulated lipolysis via beta-adrenergic receptor activation and cAMP-dependent signaling (Robidoux et al., 2006), while IBMX induced lipolysis by inhibition of PDE. The analysis of the results (Fig. 4) confirmed that IBMX and isoproterenol stimulated lipolysis. Ursolic acid also significantly stimulated the lipolytic activity 1.5 fold higher than the IBMX. These results suggest that ursolic acid exerted potent lipolytic activity via PDE inhibition.

Lipolysis is a catabolic process leading to the breakdown of triglycerides stored in fat cells and the release of FFA and glycerol. Adipose tissue lipolysis is the major regulator of the body supply of lipid energy because it controls the release of fatty acids into plasma. The first step of this lipolytic process in adipocytes is regulated by a variety of hormones such as epinephrine, norepinephrine, glucagons and adrenocorticotropic hormone (Robidoux et al., 2006). The

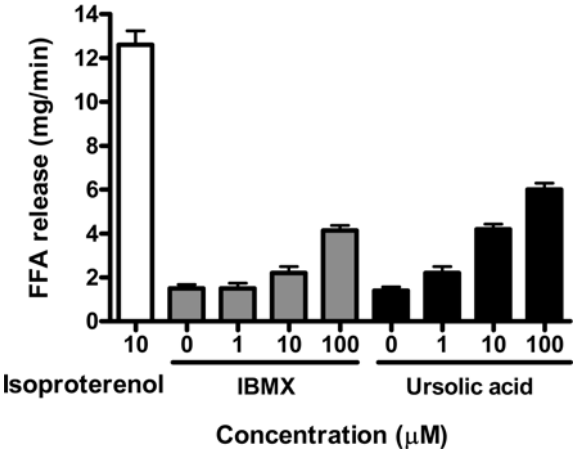

Fig. 4. Effect of ursolic acid on lipolysis in the rat fat cell. Free fatty acid release was measured in rat fat cells after treatment with isoproterenol, IBMX and ursolic acid. Values are means ± S.E.M. (n = 4).

mechanisms of action of these lipolytic hormones are believed to be mediated by the cAMP cascade. Lipolytic hormones activate adenylate cyclase, resulting in increased synthesis of cAMP. This leads to the activation of cAMP-dependant protein kinase and activation of hormone-sensitive lipase (Steinberg and Khoo, 1977). Activation of hormone-sensitive lipase results in the hydrolysis of stored triglycerides into FFA and glycerol. The lipolytic process is stimulated by beta adrenergic agonists, as well as the inhibition of c-AMP-dependent PDE (Girotti et al., 2005), which degrades cyclic cAMP and consequently inhibits the activation of hormone-sensitive lipase (HSL).

In conclusion, ursolic acid isolated from the roots of *A. arguta* may prevent lipid emersion-induced increases in plasma triacylglycerol by inhibiting intestinal absorption of dietary fat through the inhibition of pancreatic lipase activity. Moreover, ursolic acid may stimulate lipolysis in adipocytes by cAMP-PDE inhibition. The present study clearly indicated that ursolic acid isolated from the roots of *A. arguta* exerted significant anti-obestic effects.

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