Full Paper

Hypolipidemic Mechanisms of *Ananas comosus* L. Leaves in Mice: Different From Fibrates but Similar to StatinsWeidong Xie¹, Wei Wang², Hui Su², Dongming Xing², Guoping Cai¹, and Lijun Du^{2,*}¹Laboratory of Life Sciences & Marine Biology, Life Sciences Division, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China²Laboratory of Pharmaceutical Sciences, Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, China

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Abstract. In this study, we investigated hypolipidemic mechanisms of the ethanolic extract of *Ananas comosus* L. leaves (AC) in mice and then determined its activities in related enzymes. The results showed that AC (0.40 g/kg) significantly inhibited the increase in serum triglycerides by 40% in fructose-fed mice. In mice induced by alloxan and high-fat diets, serum total cholesterol remained at a high level (180–220 mg/dl) within 7 days of removing high-fat diets but reached normal level (120–140 mg/dl) after AC (0.40 g/kg per day) treatment. Also, AC (0.40 and 0.80 g/kg) significantly inhibited serum lipids from the increase in Triton WR-1339-induced hyperlipidemic mice. AC (0.01–100 µg/ml) selectively activated lipoprotein lipase (LPL) activity by 200%–400% and significantly inhibited 3-hydroxyl-methyl glutaryl coenzyme A (HMGCoA) reductase activity by 20%–49% in vitro. Furthermore, 2 months of fenofibrate (0.20 g/kg) administration particularly increased mice liver weights (0.0760 ± 0.0110 g/g) while AC (0.40 g/kg) had no effect (0.0403 ± 0.0047). Taken together, these results suggest that AC will be a new potential natural product for the treatment of hyperlipidemia that exerts its actions through mechanisms of inhibiting HMGCoA reductase and activating LPL activities. Its action mechanisms differentiate from those with fibrates but may be partly similar to those with statins. It is hopeful that AC may serve as the adjuvant for fibrates.

Keywords: *Ananas comosus* L., hyperlipidemia, lipoprotein lipase, phenol, 3-hydroxyl-methyl glutaryl coenzyme A (HMGCoA) reductase

Introduction

Hyperlipidemia is one of important risk factors involved in the development of cardiovascular disease (1). Treatment of hyperlipidemia involves diet control, exercise, and the use of lipid-lowering diets and drugs (2). However, some patients cannot tolerate the adverse effects from these oral drugs (3). As a consequence, there continues to be a high demand for new oral anti-hyperlipidemic drugs. Management of hyperlipidemia without any side effects is still a challenge to the medical system. Plant products are frequently considered to be less toxic and more free from side effects than synthetic

ones. Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemics, and hypolipidemics.

Ananas comosus (L.) MERRILL. (family Bromeliaceae), also, named pineapple, has long been one of the most popular of tropical and subtropical fruits. It is grown extensively in Hawaii, Philippines, Caribbean area, Malaysia, Taiwan, Thailand, Australia, Mexico, Kenya, South Africa, Hainan province of China and so on. Besides agricultural utilities such as being a fruit with nutritional value, some folk medicinal uses have been found. In Thailand, *A. comosus* was used as an indigenous medicinal plant (4, 5) for the treatment of dysuria. In China, *A. comosus* cortexes served as alexipharmic, antitussive, and antidiarrheal agents; and *A. comosus* leaves were usually used as an antidyspepsia

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or antidiarrheal agent in Chinese Traditional Medicine (6). In Australia, some molecules from *A. comosus* leaves are claimed to have an antitumor effect.

In our preliminary study, the ethanolic extract of *A. comosus* leaves (AC) enriched with phenols has anti-diabetic, anti-hyperlipidemic, and anti-oxidative effects (7). Recently, we showed that the hypoglycemic effect of AC is related to improvement of insulin sensitivity (8). However, its hypolipidemic mechanism remains undetermined. Considering the complexity of both hypolipidemic mechanisms and components of AC, we investigated the activities of AC in multi-models of mice and some enzymes related to lipid metabolism to elucidate its hypolipidemic mechanisms.

Materials and Methods

Materials

Blood glucose, total cholesterol, and triglyceride diagnosis kits were purchased from Zhongsheng Beikong High-tech Bio-engineering Co. (Beijing, China). Lipoprotein lipase (LPL) kits were obtained from Nanjing Jiancheng Bioengineering Co. (Nanjing, China). Fenofibrate was purchased from Beijing Medicinal Company (Beijing, China). Commassier bright blue (G250) was purchased from Xijingke Co. (Beijing, China). Simvastatin was provided by Hangzhou MSD Pharmaceutical Co., Ltd (Hangzhou, Zhejiang, China). Alloxan, 3-hydroxyl-methyl glutaryl coenzyme A (HMGCoA), and pancreatic lipase were purchased from Sigma (St. Louis, MO, USA). Lipid emulsion (Intralipos®) was provided by Guangzhou Green Cross Pharmaceutical Co., Ltd. (Guangzhou, Guangdong, China). The other chemicals used in this study were all reagent grade.

Collection of plant material

A. comosus leaves were collected from Boao, Hainan province of China and authenticated by Dr. Shouquan Lin, the Institute of Medicinal Plants, Chinese Academy of Medical Sciences. A voucher specimen (No. 020501) was deposited in the herbarium of Laboratory of Pharmaceutical Sciences, Department of Biological Sciences and Biotechnology, Tsinghua University. This plant was dried in the shade, powdered, and the powder was used for the ethanolic extraction.

Plant extract

Dried powder of *A. comosus* leaves was refluxed with 70% ethanol three times for 1 h. Each extracted solution was condensed to a final concentration of 0.20 g/ml (in terms of dried starting material). Then, the extracted suspension was allowed to stand for 10 h in the dark or

Fig. 1. HPLC of the ethanolic extract of *Ananas comosus* L. leaves: 1) 1-*O*-caffeoylglycerol, 2) caffeic acid, 3) 1-*O*-*p*-coumaroylglycerol, and 4) *p*-coumaric acid.

shade, and then the supernatant was eluted through a resin column (HPD-100; Cangzhou Bon Chemical Co., Ltd., Cangzhou, Hebei, China). The column for first eluted with distilled water until no sugar was detected (the eluate was more than 5 times the volume of the column). Subsequently, 80% ethanol was used to elute the column and this eluate was collected, condensed, and dried to the extract for use in the experiments. The yield of the ethanolic extract of *A. comosus* (AC) was 3.3% (w/w in terms of dried starting material). Phytochemical screenings of AC were conducted by Dr. Wei Wang, Department of Biological Sciences and Biotechnology, Tsinghua University. AC contained total phenols (60%, w/w in terms of the extract). Known phenols included *p*-coumaric acid (1.5%), 1-*O*-*p*-coumaroylglycerol (0.3%), caffeic acid (1.0%), and 1-*O*-caffeoylglycerol (0.2%) (See Fig. 1). Also, some sterols were found in the plant extract. The composition of the AC preparation was well-defined from the present results and allowed guaranteed a high standard of quality control for use in future studies.

Animals

Male ICR mice (18–20 g) and Wistar rats (180–200 g) were obtained from the Laboratory Animal Institute of Chinese Academy of Medical Science (Beijing, China). Animals were kept in an environmentally controlled breeding room (temperature: $20 \pm 2^\circ\text{C}$, humidity: $60 \pm 5\%$, 12 h dark/light cycle). They were fed standard laboratory chow with water ad libitum and fasted overnight before the experiments. Experimental animals were maintained in accordance with internationally accepted principles for laboratory animal use. Our research was conducted in accordance with the Declaration of Helsinki and/or with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes

of Health.

Diabetes was induced in mice by the intraperitoneal (i.p.) administration of alloxan (150 mg/kg body weight) as previously prescribed (9). Induction of diabetic hypercholesterolemia in mice was according to the previous method (10) with a slight modification, that is, diabetic mice were allowed free access to a high-fat diet (84.3% standard laboratory chow, 5% lard, 10% yolk powder, 0.2% cholesterol, 0.5% bile salt). In addition, normal rats were used to supply ample plasma containing LPL and livers for separation and purification of HMGCoA reductase, which were used to assay activities of the tested samples for LPL and HMGCoA reductase in vitro, respectively.

Fructose load test

Acute administration of fructose leads to a sharp increase in plasma triglyceride level in mice according to the previous method (11). Based on that, we made some modifications in the method. In brief, animals were divided into four groups of 10 mice each. Groups 1–3 were fed fructose to induce hypertriglyceridemia and group 4 was fed water as a normal control. Group 1 served as a fructose-fed hypertriglyceridemic control and received distilled water. The fructose-fed mice of group 2 were orally administered AC suspension in distilled water at a dose of 0.40 g/kg. The fructose-fed mice of Group 3 served as positive control treated with fenofibrate (0.20 g/kg). Group 4 served as normal control mice treated with distilled water only. The administration of AC was continued for 3 days, once daily. The time to the last administration of the tested samples is at 5 h prior to the oral administration of fructose. Following that, mice were received a 20% W/V fructose solution by gavage at a dose of 0.3 ml/10 g body weight at the start and then had free access to the fructose solution for 6 h after supplying neither diet nor water overnight. Following that, blood samples were collected for triglyceride assaying through tail veins. This trial was used to evaluate if AC could affect endogenous synthesis of triglycerides or not.

Hypercholesterolemia progression test

Diabetic mice were selected and well matched according to blood glucose and body weights in each group ($n = 7$). Simultaneously, they had free access to a high-fat diet for the induction of hypercholesterolemia, which was well developed in our previous study (10). Subsequently, AC (0.40 g/kg) was orally administered in diabetic mice (Group 2). Diabetic (Group 1) and normal controls (Group 4) received distilled water. Positive controls (Group 3) were treated with fenofibrate suspension in distilled water at the oral dose of

0.20 g/kg. After 4–5 h of fast, blood samples were collected for measuring total cholesterol level through tail veins on days 0, 1, 3, 5, and 7 of AC administration. This trial was used to evaluate if AC could inhibit dietary lipid or cholesterol absorption or not.

Hypercholesterolemia regression test

Diabetic mice were selected and fed a high-fat diet for the induction of the diabetic hypercholesterolemic animal model. Following feeding for two weeks, diabetic hypercholesterolemic mice were divided into three groups according to fasting blood glucose and total cholesterol levels. Subsequently, the fed high-fat diet was replaced with the low-fat regular chow in the diabetic hypercholesterolemic mice. Group 1 served as diabetic hypercholesterolemic controls treated with distilled water. Group 2 served as diabetic hypercholesterolemic mice treated with AC at the oral dose of 0.40 g/kg. Group 3 served as positive controls treated with a fenofibrate suspension in distilled water at the oral dose of 0.20 g/kg and normal controls (Group 4) received distilled water only. After 4–5 h of fasting, blood samples were collected for measuring serum total cholesterol levels through tail veins on days 0, 1, 3, 5, and 7 of AC administration. This trial was used to evaluate if AC could accelerate cholesterol metabolism and/or inhibit cholesterol synthesis or not.

Trial of Triton WR-1339-induced hyperlipidemic mice

Animals were divided into five groups of 10 mice each. Group 1 served as normal control mice treated with distilled water only. Groups 2–5 received an i.p. injection of Triton WR-1339 at a dose of 500 mg/kg (12). Group 2 served as control and orally received distilled water. Group 3 served as positive control treated with fenofibrate (0.20 g/kg). The mice of groups 4–5 were orally administered AC suspension in distilled water at doses of 0.40 and 0.80 g/kg, respectively. The administration of AC was continued for 2 days, once daily, which was conducted prior to 5 h of blood sample collection. Blood samples were collected for serum triglyceride and total cholesterol assaying at 24 and 48 h after injection of Triton WR-1339. This trial was used to evaluate if AC could activate inactive serum lipase and/or inhibit endogenous lipid synthesis and/or accelerate lipid metabolism or not.

Trial of hepatomegaly in mice

Animals were divided into three groups of 10 mice each. Group 1 served as control and orally received distilled water. The mice of group 2 were orally administered AC suspension in distilled water at a dose of 0.40 g/kg. Group 3 served as positive controls

treated with fenofibrate (0.20 g/kg). After 2 months of treatment, mice were sacrificed by cervical dislocation and livers were removed and weighed. Since fenofibrate can activate peroxisome proliferators-activated receptor (PPAR) α and result in hepatomegaly in rodents, this trial was used to determine if AC shared the same mechanism as fenofibrate or not.

Measurements of pancreatic lipase, lipoprotein lipase, and HMGCoA reductase activities in vitro

Pancreatic lipase activity in vitro was assayed as prescribed previously (13) with a slight modification. In brief, the reaction mixture solution consisted of 0.1 ml of (final concentration, 10 U/ml) pancreatic lipase, 0.01 ml of 20% lipid emulsion (Intralipos®), 1 ml of 0.1 mol/l Tris-HCl buffer (pH 8.2), and 0.79 ml of distilled water, which was incubated at 37°C for 60 min. Subsequently, free fatty acids produced by pancreatic lipase were extracted by 5 ml of fatty acid extraction solution (chloroform/heptane/methanol, 49/49/2, v/v/v). Following that, 2 ml of extracted organic phase was combined with 2 ml of 0.05 mol/l $\text{Cu}(\text{NO}_3)_2$ reagent (contained supersaturated NaCl and 0.1 mol/l triethanolamine) and formed complexes of free fatty acid and Cu^{2+} . The complexes entered the water phase of the solution after extraction. Then, 0.5 ml of extracted water phase containing the complexes was combined with 0.1 ml of 4 mg/ml diphenyl carbazide (contained 0.1 mol/l triethanolamine), and the solution immediately appeared red. Optical density of the final reaction solution was assayed at 550 nm within 30 min. Free fatty acids produced by pancreatic lipase were quantified according to the standard curve plotted by different concentrations of palmitic acid solution (0.1–1 mmol/l stock solution) and matched optical density values after palmitic acid exposed to similar treatment. Pancreatic lipase activity was determined as mmol fatty acid released per hour in 1 ml of the reaction mixture at the start. The final concentrations of the tested samples incubated with the tested enzymes ranged from 0.01 to 100 $\mu\text{g}/\text{ml}$.

LPL was prepared from the blood in rats 15 min after intravenous of heparin (50 U/kg); its activity in vitro was measured by the use of LPL kits according to the previous method (14) with a slight modification. Plasma total lipase activity was assayed and then hepatic lipase activity was measured after LPL activity was inhibited by high-concentration NaCl (0.79 mol/l, final concentration). LPL activity was calculated according to the formula: LPL activity = (serum total lipase activity) – (serum hepatic lipase activity). In brief, the reaction mixture solution consisted of 0.1 ml of plasma, 0.01 ml of lipid emulsion, 1 ml of 0.1 mol/l Tris-HCl buffer

(pH 8.2), 0.79 ml of distilled water (for total lipase activity assay), or high-concentration NaCl (0.79 mol/l, final concentration, for hepatic lipase assay) and incubated at 37°C for 60 min. The following procedure performed according to the pancreatic lipase method described above. Lipoprotein lipase activity was expressed as mmol free fatty acid produced by plasma LPL per hour in 1 ml of the reaction mixture solution at the start. The final concentrations of the tested samples incubated with the tested enzymes ranged from 0.01 to 100 $\mu\text{g}/\text{ml}$.

The activity of HMGCoA reductase prepared from rat liver microsomes was assayed by the previously described colorimetric method (15) with a slight modification. In brief, the reaction mixture solution consisted of 0.1 ml of prepared enzyme (final concentration, about 4 U/ml), 0.1 ml of HMGCoA (final concentration, 0.15 $\mu\text{mol}/\text{l}$), 0.1 ml of the tested samples, 1.6 ml of 0.1 mol/l potassium phosphate buffer (pH 7.0) (containing 3.5 mmol/l EDTA, 10 mmol/l dithiothreitol, and 0.1 g/l bovine serum albumin) and incubated at 37°C for 5 min. Following that, 0.1 ml of NADPH solution (final concentration, 0.30 $\mu\text{mol}/\text{l}$) was added to the reaction solution. The solution was mixed immediately and assayed for changes of the optical density at 340 nm in 1–2 min. HMGCoA reductase activity was expressed as U/mg protein. The final concentrations of the tested samples incubated with the tested enzymes ranged from 0.01 to 100 $\mu\text{g}/\text{ml}$. Simvastatin at the final of concentrations of 1, 10, and 100 $\mu\text{g}/\text{ml}$ served as the positive control.

Statistical analyses

All values were expressed as the mean $\pm$ S.D. Data were statistically analysed by ANOVA. The Newman-Keuls comparisons were used to determine the source of significant differences where appropriate. *P* values less than 0.05 were considered statistically significant.

Results

Effect of AC on serum triglyceride level in fructose-induced hypertriglyceridemic mice

After acute administration of fructose, a significant increase in serum triglyceride level (47%, $P < 0.05$) was induced in fructose-induced control mice (1.44 ± 0.62 , $n = 10$) compared with normal controls (0.98 ± 0.22 , $n = 10$). However, AC (0.40 g/kg) significantly prevented the increase (0.86 ± 0.27 , $n = 10$) by 40% ($P < 0.05$) in the fructose-induced mice. Also, fenofibrate (0.20 g/kg) significantly inhibited the increase (0.64 ± 0.15 , $n = 10$) by 56% ($P < 0.01$).

Effect of AC on hypercholesterolemia progression and regression in diabetic hypercholesterolemic mice

In the trial of hypercholesterolemia progression, untreated hypercholesterolemic mice exposed to high-fat diets for 7 days progressively developed hypercholesterolemia compared with normal mice fed normal

Fig. 2. Effect of the ethanolic extracts of *Ananas comosus* L. leaves (AC) on the regression of hypercholesterolemia in diabetic animals induced by alloxan and a high-fat diet at different days after sub-acute treatment. Data are each expressed as a mean $\pm$ S.D., $n = 7$. $^{##}P < 0.01$: compared with the normal control group. $^{*}P < 0.05$, $^{**}P < 0.01$: compared with the hypercholesterolemic control group. Serum total cholesterol level remained at high levels in the untreated hypercholesterolemic mice even at the 7th day when high-fat diets that the mice had been fed were changed to normal chow but decreased to normal after 7 days of treatment of AC at a dose of 0.40 g/kg and fenofibrate at a dose of 0.20 g/kg.

diets (data were not shown). However, no significant inhibition activity in hypercholesterolemia progression was observed in hypercholesterolemic mice treated with AC (0.40 g/kg) or fenofibrate (0.20 g/kg) compared with untreated hypercholesterolemic controls. In the trial of hypercholesterolemia regression, serum total cholesterol in untreated hypercholesterolemic mice remained at a significantly high level (180–220 mg/dl) compared with normal controls even at the 7th day when high-fat diets that the mice were fed had been changed to normal chow. However, significant hypocholesterolemic activity was observed in hypercholesterolemic mice after the treatment of AC (0.40 g/kg) or fenofibrate (0.20 g/kg) (Fig. 2). After 7 days of treatment, fasting serum total cholesterol level in AC or fenofibrate-treated groups almost reached a normal level (120–140 mg/dl).

Effect of AC on acute hyperlipidemia in Triton WR-1339-induced hyperlipidemic mice

At 24 h after the i.p. injection of Triton WR-1339, the treated mice showed a significant increase in serum triglyceride and total cholesterol compared with normal controls without the injection of Triton WR-1339 (Fig. 3). However, this increase was significantly attenuated by AC (0.40 and 0.80 g/kg, respectively) and fenofibrate (0.20 g/kg). At 48 h, hypolipidemic effects were still seen in AC or fenofibrate-treated mice, although serum lipid levels were almost decreased to normal levels in all Triton WR-1339-treated mice.

Fig. 3. Effect of the ethanolic extract of leaves of *Ananas comosus* L. (AC) on serum triglyceride and total cholesterol levels in Triton WR-1339-induced hyperlipidemic mice. Data are each expressed as a mean $\pm$ S.D., $n = 10$. $^{##}P < 0.01$: compared with the Triton WR-1339-untreated normal control group. $^{*}P < 0.05$, $^{**}P < 0.01$: compared with Triton WR-1339-treated hyperlipidemic control group. Fenofibrate at a dose of 0.20 g/kg and AC at doses of 0.40 and 0.80 g/kg significantly inhibited the increase in serum total cholesterol and triglycerides in Triton WR-1339-treated hyperlipidemic mice at 24 and 48 h.

Effect of fenofibrate and AC on hepatomegaly in normal mice

Fenofibrate caused a significant increase in liver/body weights (g/g) in mice (0.0760 ± 0.0110 , $P < 0.01$), but AC did not cause any change (0.0403 ± 0.0047) compared with those in untreated normal controls (0.0402 ± 0.0012) after two months of the administration.

Effect of AC on pancreatic lipase, LPL, and HMGCoA reductase activities in vitro

AC at the final concentrations of $0.01 - 1 \mu\text{g/ml}$ significantly inhibited the activity of pancreatic lipase in vitro. However, higher doses ($10 - 100 \mu\text{g/ml}$) had no significant effect (Fig. 4); this remains to be further investigated in future studies. Interestingly, AC at the

final concentrations of $0.01 - 100 \mu\text{g/ml}$ significantly activated LPL activity by 200%–400% in vitro (Fig. 4). AC at the final concentrations of $0.01 - 100 \mu\text{g/ml}$ and simvastatin ($1 - 100 \mu\text{g/ml}$) significantly inhibited HMGCoA reductase activity by 20%–49% and 25%–65% in vitro, respectively (Table 1).

Discussion

Plasma lipid levels are determined by exogenous lipid absorption and endogenous lipid synthesis and metabolism in body, which usually involves targets for lipid-regulating drugs. Considering the complexity of both hypolipidemic mechanisms and components of the tested samples, mice may be more popular for investigation into mechanisms because they are easy to control

Fig. 4. Effect of the ethanolic extracts of *Ananas comosus* L. leaves (AC) on both pancreatic and lipoprotein lipase in vitro at the final concentrations of $0.01 - 100 \mu\text{g/ml}$. Data are each expressed as a mean $\pm$ S.D., $n = 6$. ** $P < 0.01$ vs control. AC at low concentrations of $0.01 - 1 \mu\text{g/ml}$ significantly inhibited pancreatic lipase by 18%–22% but did not show any effect at high-concentrations of $10 - 100 \mu\text{g/ml}$ compared with the control. AC at concentrations of $0.01 - 100 \mu\text{g/ml}$ significantly activated lipoprotein lipase activity by 200%–400% compared with the control.

Table 1. Effect of the ethanolic extracts of *Ananas comosus* L. leaves (AC) on HMGCoA reductase activity in vitro

Groups	Final concentration ($\mu\text{g/ml}$)	Enzyme activity (U/mg)	Inhibition percentage
Control		3.76 ± 0.11	
AC	0.01	$3.02 \pm 0.34^{**}$	19.7
	0.1	$2.63 \pm 0.12^{**}$	30.1
	1	$1.92 \pm 0.79^{**}$	48.9
	10	$2.00 \pm 0.32^{**}$	46.8
	100	$2.00 \pm 0.82^{**}$	46.8
Simvastatin	1	$2.78 \pm 0.22^{**}$	25.9
	10	$2.35 \pm 0.94^{**}$	37.4
	100	$1.33 \pm 0.30^{**}$	64.5

Mean $\pm$ S.D., $n = 6$. ** $P < 0.01$ vs control.

and cost less than other animals such as rats or rabbits. It should be noted that a special mouse model involved in the specific processes involved in lipid metabolism may be helpful for elucidating hypolipidemic mechanisms of unknown components.

In the trial of hypercholesterolemia progression, cholesterol-enriched diets dominated the increase in serum total cholesterol. No significant effect was observed in AC- or fenofibrate-treated mice in the trial of hypercholesterolemia progression, which suggested that AC or fenofibrate did not effectively inhibit the absorption of intestinal dietary cholesterol. Fenofibrate that has a potent hypolipidemic effect did not effectively inhibit the increase in serum total cholesterol in the trial of hypercholesterolemia progression, which indirectly suggested that serum total cholesterol level might be determined by dietary cholesterol in this trial. In the trial of hypercholesterolemia regression, serum total cholesterol level was determined mainly by endogenous cholesterol metabolism when high-fat diets were replaced with normal chow. AC accelerated the fall in serum total cholesterol level, which suggested that AC inhibited the synthesis of endogenous cholesterol. Furthermore, in the *in vitro* trial, AC inhibited the activity of HMGCoA reductase. These results established the idea that the hypolipidemic effect of AC *in vivo* was correlated with inhibition of the activity of HMGCoA reductase, which might be associated with flavonoids enriched in AC since flavonoids could inhibit HMGCoA reductase activity (16). This trial suggested AC might share the same mechanism as statins.

AC attenuated the increase in serum triglyceride in the fructose-induced mice, indicating that AC played a role in inhibiting endogenous triglyceride synthesis (17). Triton WR-1339-treated mouse is a useful acute hyperlipidemic animal model (12) associated with inactive LPL. Triton WR-1339-induced hyperlipidemic mice treated with AC showed no increase in serum lipids at 24 h, indicating that AC might activate LPL activity. Interestingly, AC activated plasma LPL activity *in vitro* at the appropriate ranges of dosage. LPL is a key enzyme related to plasma triglyceride metabolism. Furthermore, no activating effect was observed in intestinal pancreatic lipase exposed to AC, which indicated that AC had specificity in activating lipase. In the following trial, we determined if this effect worked by the mechanism of PPAR α agonists.

When mice and rats are given PPAR α agonists, fibrates, they exhibit peroxisome proliferation, hypertrophy, and hyperplasia (18). In the trial of hepatomegaly, fenofibrate can activate PPAR α receptors, which results in hepatomegaly in rodents after a long-term administration. We studied this special phenomenon to

determine whether AC has a different mechanism from fenofibrate or not. AC caused no hepatomegaly in mice, different from fenofibrate, which suggested that AC might have different action mechanism than fenofibrate. This simple and effective trial supplied significant scientific evidence and also could be a method to assess the effects of other PPAR α agonists. It might be particularly useful for those researchers or labs that are unable to conduct trials using the expensive techniques of molecular biology.

In conclusion, the hypolipidemic effect of AC may be related to inhibition of the activity of HMGCoA reductase and thereby selectively activate the activity of plasma LPL. AC may partly share an action mechanism with statins, but acts differently than fibrates. Therefore, AC may be able to serve as an adjuvant for fibrates that can be used in those patients who cannot tolerate the side-effect of fibrates. In addition, some simple animal model might be useful for determining the molecular action mechanisms of drugs even if no molecular biological methods are adopted.

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