Effect of corosolic acid on dietary hypercholesterolemia and hepatic steatosis in KK-Ay diabetic mice

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ABSTRACT

Corosolic acid (CA), contained in the leaves of the banaba plant (Lagerstroemia speciosa L.), is a pentacyclic triterpene, and has hypoglycemic effects. The effects of CA on dietary hypercholesterolemia and hepatic steatosis were assessed in KK-Ay mice, an animal model of type 2 diabetes. Two kinds of high cholesterol diet with or without 0.023% CA, were prepared for the study. KK-Ay mice were fed a normal diet (controls), the high cholesterol diet with CA (CA-mice) or that without CA (HC-mice) for 10 weeks. CA inhibited the mean blood cholesterol level by 32% (P < 0.05) and the liver cholesterol content by 46% (P < 0.05) compared with those of HC-mice 10 weeks after the start of dietary intake. Acutely, CA inhibited the mean blood cholesterol level 4 h after the administration of a high-cholesterol cocktail in an oral cholesterol-loading test, compared with that of control mice (P < 0.05). These results suggest that CA has some direct effects on the cholesterol absorption process in the small intestine. CA may inhibit the activity of cholesterol acyltransferase, which acts in the re-esterification of cholesterol in the small intestine, in type 2 diabetes.

The banaba leaf (Lagerstroemia speciosa L.) is a traditional oriental medicine used to treat diabetes in the Philippines. It contains corosolic acid (CA) (Fig. 1), a compound which consists of pentacyclic triterpene. The hypoglycemic effect of CA has been noted in various experiments (7, 14, 22, 25, 29). In addition to the hypoglycemic effect, Yamaguchi et al. reported its lowering effect on serum fatty acids in SHR/NDmcr-cp rats (31), while Yamada et al. observed that CA reduced fasting plasma and liver triglyceride contents in KK-Ay mice (30). The occurrence of hyperlipidemia is prevalent in more than 30% of all diabetics, two to three times more frequent than in nondiabetic patients (1). Diabetic dyslipidemia is a major factor contributing to accelerated atherosclerosis (16), while hypercholesterolemia especially is a major risk factor for atherosclerosis and coronary heart disease in type 2 diabetes (9, 20).

Whole-body cholesterol homeostasis is regulated by de novo synthesis of cholesterol in the liver, dietary cholesterol absorption, and biliary excretion and absorption. The daily diet of the average North American adult contains 350–500 mg of cholesterol, while bile contributes an additional 800–1200 mg of cholesterol to the intraluminal pool per day (8). About 50% of this is absorbed in the small intestine (2, 3). The rate at which the body synthesizes cholesterol can vary with changes in cholesterol intake (4, 21). As many type 2 diabetic patients tend to overeat and be obese, their diet may cause hypercholesterolemia and hepatic steatosis.

It is therefore very important for type 2 diabetics to improve their hypercholesterolemia by correcting
Determination of blood cholesterol levels. Blood cholesterol levels in the animals were determined using the cholesterol E-test Wako (Wako pure chemical industries, Osaka, Japan), based on the cholesterol oxidase method (23).

Measurement of liver cholesterol contents. The livers of the mice were removed ten weeks after the start of the diet. According to the procedure of the Folch method (5), the concentrated and exsiccated lipid was extracted using a chloroform-methanol mixed solution. This was dissolved in dissolution liquid, and then cholesterol concentrations (mg/dL) were measured using the cholesterol E-test Wako. The absolute quantity of total cholesterol included in 1 g of liver was calculated based on those concentrations.

Oral cholesterol-loading test. According to a previously used method (6), a high-cholesterol cocktail, which contained 10% cholesterol, 2% cholic acid, 25% sesame oil and 6% Tween 20, was prepared by homogenization with Potter-Elvehjem homogenizer. After an overnight (18 h) fasting, each of the mice in the experimental group was orally given CA (10 mg/kg body weight) suspended in distilled water. The mice in the control group received an equal volume of distilled water. Thirty minutes after this oral administration, all the mice were orally given 0.5 mL of the cocktail. Blood samples were collected immediately before, and 2, 4, 6 and 8 h after the oral administration of the cocktail.

Statistical analysis. All the data were expressed as mean ± standard error of the mean (S.E.M.). Student’s t test and analysis of variance (ANOVA) were used for the statistical analyses. The multiple comparisons between each pair of groups were performed by the least significant difference (LSD) method. Values were considered to be significantly different when the P value was less than 0.05.

RESULTS

Effect of CA on blood cholesterol levels in KK-Ay mice

Fig. 2 shows the effect of CA on blood cholesterol levels in KK-Ay mice 5 and 10 weeks after the start of the diet. The mean blood cholesterol level in the HC-mice was significantly higher compared with that of the control mice after 10 weeks (P < 0.05). CA significantly lowered the mean blood cholesterol level in the CA-mice compared with that of the

their lifestyle or diet. In the present study, we examined the inhibitory effect of CA on dietary hypercholesterolemia and hepatic steatosis in KK-Ay mice, an animal model of type 2 diabetes.

MATERIALS AND METHODS

Materials. CA was donated by Use Techno Corporation, Ltd. (Kyoto, Japan). The CA was stored at room temperature until use. Two kinds of specific diet were prepared for this study. One was a high cholesterol diet made of CE-2 (Clea, Japan), 1% cholesterol and 0.5% cholic acid, while the other was the same high cholesterol diet containing 0.023% CA, a dosage similar to that used in previous studies (22, 30).

Animals. Adult male KK-Ay mice (8 weeks old; Clea, Japan), weighing 35–40 g were used. Under non-fasting conditions, those with blood glucose levels above 300 mg/dL were considered to be diabetic and were used in this study. There were four to six animals in each group. The mice were housed individually in an air-conditioned experimental room at 22 ± 2°C with a 12-h light and 12-h dark cycle. They were kept in the room for 7 days with free access to food and water. Then one group was fed CE-2 (the controls), one group the high cholesterol diet with 0.023% CA (CA-mice), and one group the high cholesterol diet without CA (HC-mice) for 10 weeks. For the determination of blood cholesterol levels, blood samples were withdrawn from the cavernous sinus with a capillary before the start of the diet, and at 5 and 10 weeks thereafter. The animals were tested in accordance with the Guidelines for the Care and Use of Laboratory Animals (Prime Minister’s Office Directive no. 6, 1980).

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FIG. 1 Structure of corosolic acid. Corosolic acid consists of pentacyclic triterpene.
Corosolic acid and cholesterolemia

Effect of CA on oral cholesterol-loading test

Fig. 6 shows the effect of CA on blood cholesterol levels in KK-Ay mice after administration of the high-cholesterol cocktail. CA significantly inhibited the mean cholesterol level 4 h after the high-cholesterol cocktail administration, compared with the control mice ($P < 0.05$).

**DISCUSSION**

The purpose of this study was to research the effect of CA on dietary hypercholesterolemia and hepatic steatosis in type 2 diabetes. The mean blood cholesterol level of the HC-mice was significantly higher compared with its previous value, and different from that of the control mice after 10 weeks on their high cholesterol diet. Similarly, their mean liver cholesterol content after 10 weeks was also significantly higher than that of control mice. The results of these experiments show that dietary cholesterol causes hypercholesterolemia and hepatic steatosis in type 2 diabetes, as previous studies have also demonstrated (13, 16). In the animal model, CA showed an inhibitory effect on both conditions when compared with HC-mice which were not given CA.

**Effect of CA on body weights in KK-Ay mice**

Fig. 3 shows body weights of the KK-Ay mice during the course of the study. The body weights of both the HC-mice and the CA-mice were significantly different from that of the controls 10 weeks after the start of the diet ($P < 0.05$). There was no significant difference in body weight between the HC-mice and the CA-mice 5 and 10 weeks after the start of the diet.

**Effect of CA on liver cholesterol contents in KK-Ay mice**

Fig. 4 shows the mean liver cholesterol contents 10 weeks after the start of the diet. The value for the HC-mice was significantly higher than that of the control mice ($P < 0.05$). CA significantly inhibited the mean liver cholesterol content by 46% in the CA-mice compared to that in the HC-mice ($P < 0.05$) (3.2 ± 0.5 mg/g liver in the controls, 32.7 ± 5.4 mg/g liver in the HC-mice and 17.7 ± 2.4 mg/g liver in the CA-mice).

Fig. 5 shows the livers removed from the mice in each group. We can clearly recognize the hepatic steatosis in the livers of the HC-mice by contrasting their colors with those of the control mice or the CA-mice.
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The mean cholesterol level of CA-treated mice was significantly lower compared with control mice 4 h after administration of the cocktail in an oral cholesterol-loading test. This result suggests that CA has some direct actions on the cholesterol absorption process in the small intestine.

The cholesterol absorption pathway as a whole is essentially a triphasic process (26, 27). The initial intraluminal phase involves a constellation of physicochemical events that culminate in the delivery of sterols contained in mixed micelles up to the brush border membrane (BBM) of the enterocyte. The second, or BBM phase, entails the uptake of cholesterol and other sterols across the BBM. The third, or intracellular phase, encompasses multiple events including the re-esterification of much of the cholesterol after uptake by enterocytes, and the incorporation of cholesterol and other lipids, along with apolipoprotein B48 (apo B48), which is a marker protein of exogenous lipoprotein coming from dietary intake and specifically distributed in small intestine-derived chylomicrons, into nascent chylomicron particles (28). Various transporters or enzymes are associated with each of these processes.

The enzyme which acts on re-esterification of cholesterol in the third phase is acyl-coenzyme A: cholesterol acyltransferase (ACAT). The ACAT activity in the streptozotocin-induced diabetic rats which lack insulin is 9 times higher than that in normal rats (17). In Wistar fatty rats, a model for non-insulin-dependent diabetes mellitus, enhanced ACAT activity in the small intestine and hypercholesterolemia were observed when an atherogenic diet containing 1% cholesterol, 0.5% cholic acid,
and 5% lard was given (13). There are two isozymes in ACAT. In the mouse, ACAT-1 is distributed in many tissues, but ACAT-2 is the dominant esterifying enzyme in the liver and small intestine (15). ACAT-1 is responsible for foam cell formation in macrophages, whereas ACAT-2 is in charge of the cholesterol absorption process in intestinal mucosal cells (24). ACAT-2 is not required for cholesterol absorption when dietary cholesterol is low, but ACAT-2 deficiency limits the ability to increase cholesterol absorption capacity when dietary cholesterol intake is high (15). We confirmed that the administration of CA with cholesterol loading inhibited hypercholesterolemia and liver cholesterol content, although the administration of CA only, without cholesterol loading, did not affect the blood cholesterol levels of KK-Ay mice (data not shown). Taken together, these results suggest that CA has some effects on the enzyme which is active when dietary cholesterol is high. Fujinami et al. (6) reported that (1α, 2α)-2-[3-(2, 2-dimethylpropyl)-3-nonylureido]cyclohexane-1-y13-[(4R)-N-(2, 2, 5, 5-tetramethyl-1, 3-dioxane-4-carbonyl) amino] propionate (F1394), an ACAT inhibitor, decreased blood cholesterol level compared with controls 4 h after administration of the high-cholesterol cocktail in an oral cholesterol-loading test. They explained that this was due to the inhibition of cholesterol absorption in the small intestine by suppression of ACAT activity. The result of our oral cholesterol-loading test agrees with that from the experiment by Fujinami et al. (6).

Pentacyclic triterpenes, the group of compounds to which CA belongs, have been reported to exhibit various biological activities (12), and to inhibit ACAT-1 and ACAT-2 activities in an experiment using insect cells (Hi5 cells) containing baculovirally expressed ACAT-1 or ACAT-2 (19). In addition, ACAT inhibitors have been found to be effective in preventing hepatic cholesterol accumulation and hypercholesterolemia in animal models (11). Because CA reduced blood cholesterol levels and liver cholesterol contents when dietary cholesterol was loaded, it has been suggested that CA has the potential to inhibit the activity of ACAT-2 in the small intestine.

Our study has shown that CA inhibits hypercholesterolemia and hepatic steatosis caused by dietary cholesterol in KK-Ay mice. Further investigation will be required to clarify the detailed mechanisms.

REFERENCES


