

Antidiabetic Effects of Corosolic Acid in KK-Ay Diabetic Mice

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The antidiabetic effects of corosolic acid (CA) were investigated in KK-Ay mice, an animal model of type 2 diabetes. CA (2 mg/kg body weight) reduced the blood glucose levels of KK-Ay mice 4 h after a single oral dose. CA (2 mg/kg) reduced the blood glucose levels in KK-Ay mice 2 weeks after a single oral dose and also significantly lowered plasma insulin levels were in KK-Ay mice under similar conditions. CA-treated KK-Ay mouse blood glucose significantly decreased in an insulin tolerance test. These results support the hypothesis that CA improves glucose metabolism by reducing insulin resistance. Therefore CA may be useful for the treatment of type 2 diabetes.

Key words corosolic acid; antidiabetic effect; KK-Ay mice; insulin resistance

Despite considerable progress in the management of diabetes mellitus with synthetic drugs, the search for indigenous natural antidiabetic agents is ongoing. The plant kingdom offers a wide field to search for effective oral hypoglycemics. More than 400 species have been reported to exhibit hypoglycemic effects, but only a few have been investigated.^{1–5)}

The leaf of Banaba (*Lagerstroemia speciosa* L.) has been used as a traditional Oriental medicine to treat diabetes (polyuria and polydipsia). It contains polyphenol compounds and corosolic acid (CA) (Fig. 1).⁶⁾ In a previous study, it was reported that polyphenol compounds have antidiabetic effects.⁷⁾ We found a new antidiabetic compound, CA of Banaba leaf, for single oral administration.⁸⁾ However, there is no experimental evidence detailing improved hyperglycemia after repeated administration. In the present study, we examined the antidiabetic effects of CA in KK-Ay diabetic mice, an animal model of type 2 diabetes.

MATERIALS AND METHODS

Materials CA was donated by Use Techno Corporation Co. Ltd. (Kyoto, Japan). CA was stored at room temperature until use. For oral administration, CA 0.4, 2 and 10 mg/kg was suspended in distilled water 20 ml. For subcutaneous administration, insulin 0.5 U/kg was dissolved in saline 10 ml.

Animals and Treatments KK-Ay strain male mice (6 weeks old, weighing 39–43 g) (Clea, Tokyo, Japan) with a blood glucose level greater than 300 mg/dl and considered to

be diabetic were used. The mice were housed in an air-conditioned room at 22±2 °C with a 12-h light and 12-h dark cycle. The animals were kept in the experimental animal room for 7 d with free access to food and water. Blood samples were withdrawn from the cavernous sinus with a capillary for glucose determinations. The experiments were started between 10:00 and 11:00 am.

Effects of a Single Dose of CA on Blood Glucose Levels in KK-Ay Mice CA (0.4, 2, 10 mg/kg) dissolved in 20 ml of distilled water was administered orally to the mice. The control group received an equal volume (20 ml/kg) of distilled water. Blood samples were taken for glucose determination 2, 4, and 7 h later. This experiment was performed under nonfasting conditions.

Effects of 2-Week Repeated Administration of CA on Blood Glucose Levels in KK-Ay Mice CA 2 mg/kg suspended in 20 ml of distilled water was administered orally once a day for 2 weeks to the mice. The control group received an equal volume (20 ml/kg) of distilled water. Blood samples were taken for glucose determination every week. This experiment was performed under nonfasting conditions.

Insulin Tolerance Test An insulin tolerance test was performed at the end of the repeated administration period. After overnight fasting, an insulin solution 0.5 U/kg was injected subcutaneously into the mice and blood samples were obtained for glucose determinations 0, 30, 60, and 120 min later.

Determination of Blood Glucose and Insulin Levels Blood glucose levels in both normal and diabetic animals were determined using the glucose oxidase method⁹⁾ and plasma insulin was measured with the GLAZYME Insulin-EIA TEST.¹⁰⁾

Statistical Analysis All data are expressed as mean±S.E.M. Student's *t*-test and ANOVA were used for statistical analyses. Values were considered to be significantly different when the *p* value was less than 0.05.

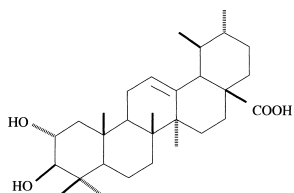


Fig. 1. Structure of CA

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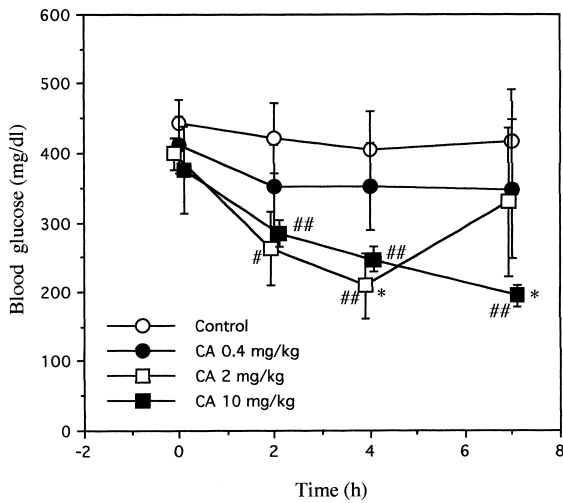


Fig. 2. Effects of a Single Dose of CA on Blood Glucose in KK-Ay Mice

CA 0.4, 2, and 10 mg/kg was administered orally to the mice. The control mice received the same volume of distilled water. Blood samples were taken for glucose determinations 2, 4, and 7 h later. Each value represents the mean \pm S.E.M. from 4 or 5 mice. Significantly different from control, * p <0.05 (by ANOVA). Significantly different from 0h, # p <0.05, ## p <0.01 (by ANOVA).

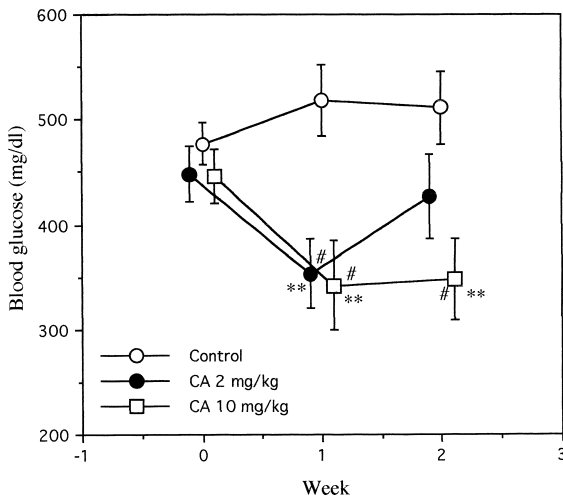


Fig. 3. Effects of Repeated Doses of CA on Blood Glucose Levels in KK-Ay Mice

CA 2 and 10 mg/kg was administered orally to the mice for 2 weeks. The control mice received the same volume of distilled water. Blood samples were taken for glucose determination every week. Each value represents the mean \pm S.E.M. from 5–7 mice. Significantly different from control, ** p <0.01 (by ANOVA). Significantly different from 0 week, # p <0.05 (by ANOVA).

RESULTS

Effects of CA on Blood Glucose Levels in KK-Ay Mice (Single Administration) The mean blood glucose levels of KK-Ay mice at various time intervals after a single oral administration of CA 0.4–10 mg/kg are shown in Fig. 2. CA 2 mg/kg *p.o.* lowered blood glucose levels 4 h after administration (p <0.05). CA 10 mg/kg-treated mice showed a significant decrease in plasma glucose levels 4 and 7 h compared with the control values (p <0.01) (Fig. 2).

Effects of Repeated Oral Dosing of CA on Blood Glucose Levels in KK-Ay Mice The mean blood glucose levels of KK-Ay mice at various weekly intervals after repeated oral dosing with CA 2 and 10 mg/kg are shown in Fig. 3. CA 2 mg/kg *p.o.* lowered blood glucose levels 1 week after ad-

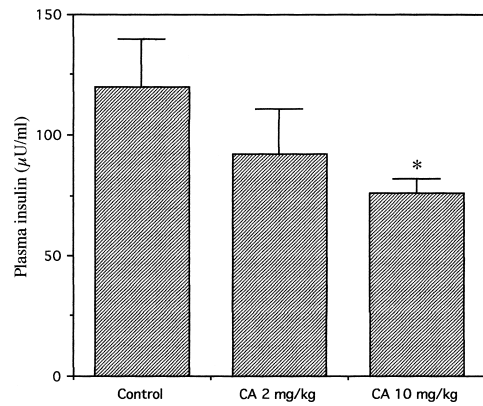


Fig. 4. Effects of CA on Plasma Insulin Levels in KK-Ay Mice (2 Weeks)

CA 2 and 10 mg/kg was orally administered to the mice for 2 weeks, and blood samples were taken for insulin levels determination. Each value represents the mean \pm S.E.M. from 5–7 mice. Significantly different from control, * p <0.05.

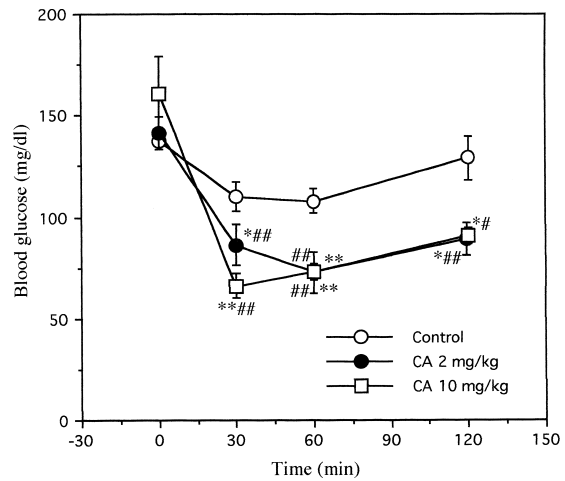


Fig. 5. Effects of CA on Blood Glucose Levels in the Insulin Tolerance Test

CA was administered to KK-Ay mice for 2 weeks and then, insulin 0.5 U/kg was injected subcutaneously. Blood samples were taken for glucose determinations 0, 30, 60 and 120 min later. Each value represents the mean \pm S.E.M. from 4–6 mice. Significantly different from control, * p <0.05, ** p <0.01 (by ANOVA). Significantly different from 0 min, # p <0.05, ## p <0.01 (by ANOVA).

ministration (p <0.05). CA 10 mg/kg *p.o.* lowered blood glucose levels 1 and 2 weeks after administration p <0.05. The body weights of the CA-treated mice were not significantly different from those of the control mice (data not shown).

Effects of CA on Plasma Insulin Levels in KK-Ay Mice The effects of CA on plasma insulin in KK-Ay mice are shown in Fig. 4. The plasma insulin levels in CA treated KK-Ay mice decreased 2 weeks after administration (p <0.05) (Fig. 4).

Insulin Tolerance Test The insulin tolerance test results for CA are shown in Fig. 5. CA 10 mg/kg-treated KK-Ay mice showed a significant decrease in blood glucose levels 30, 60, and 120 min after insulin administration compared with the controls (30 and 60 min, p <0.01; 120 min, p <0.05).

DISCUSSION

The results of this study clearly show that CA produces a consistent hypoglycemic effect. We examined the dose dependence (0.4, 2, 10 mg/kg) after CA treatment, and ob-

served antidiabetic activity at doses of 2 and 10 mg/kg after oral administration (Fig. 2). Therefore we examined the effects of repeated CA 2 and 10 mg/kg administration.

CA decreased the blood glucose levels of KK-Ay mice and had no effect on blood glucose levels in normal mice (data not shown), indicating that CA is useful in managing type 2 diabetes. It appears likely that CA exerts its hypoglycemic activity after the metabolic process because it lowered blood glucose levels 4 h after administration. Repeated administration of CA resulted in hypoglycemia with reduced plasma insulin levels. These results indicate that CA improves hyperinsulinemia in type 2 diabetes. Insulin resistance in peripheral tissues is known to be one of the major pathogenic factors in type 2 diabetes. The finding that CA decreases blood insulin levels in KK-Ay mice is important.

CA-treated KK-Ay mice also had lower blood glucose levels in the insulin tolerance test and hyperinsulinemia improved. Insulin 0.5 U/kg-treated KK-Ay mice did not have lower blood glucose levels because of insulin resistance in the peripheral tissues, suggesting that CA lessens insulin resistance. Previously, we reported that a single administration of CA induced GLUT4 translocation in KK-Ay mice.⁸⁾ From these findings, it is very likely that the hypoglycemic activity

of CA is derived, at least in part, from the decrease in insulin resistance, presumably because of the increase in GLUT4 translocation in total muscle membrane. These results suggest that the clinical use of CA in the treatment of diabetes mellitus, especially type 2 diabetes, may be appropriate.

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