Corosolic Acid Induces GLUT4 Translocation in Genetically Type 2 Diabetic Mice

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The effect of corosolic acid (CA) on blood glucose was studied in KK-Ay mice, an animal model of type 2 diabetes. CA (10 mg/kg) reduced the blood glucose (p<0.05) of KK-Ay mice 4 h after single oral administration when compared with the control group. However, CA did not change the plasma insulin. The muscle facilitative glucose transporter isoform 4 (GLUT4) translocation from low-density microsomal membrane to plasma membrane was significantly increased in the orally CA-treated mice when compared with that of the controls (p<0.05). These results suggest that the hypoglycemic effect of CA is derived, at least in part, from an increase in GLUT4 translocation in muscle. Therefore, it may be that CA has beneficial effects on hyperglycemia in type 2 diabetes.

Key words corosolic acid; glucose transporter (GLUT) 4; translocation; hypoglycemic effect; type 2 diabetes

Insulin resistance in peripheral tissues, together with the impairment of glucose-induced insulin secretion from pancreatic beta cells, is known as one of the major pathogenic factors of type 2 diabetes. Although therapeutic agents to stimulate insulin secretion (for example, sulfonylureas) have been used for type 2 diabetic patients, drugs to directly increase muscle glucose transport are not yet available clinically, but they may become a new category of drugs which can be used in combination with sulfonylureas and insulin, because they involve different therapeutic mechanisms.

Glucose transport across the plasma membrane is mediated by carrier proteins known as glucose transporters.1,2) Recent cDNA cloning has demonstrated that the facilitative glucose transporters comprise a family of structurally related proteins with differing tissue distribution.3) The gene expression and protein content of glucose transporters have been found to be altered under pathological conditions such as diabetes mellitus.3–5) Skeletal muscles and adipocytes have been revealed to express glucose transporter (GLUT) 4, and the unique machinery required for the movement of GLUT4 from intracellular pools to the plasma membrane (PM) has been described by Baron et al.6,15) whose procedure was modified from that of Klip et al.16,17) The muscle translocation process is stimulated by insulin and AMP-activated protein kinase in muscle tissues.8) Defects in GLUT4 translocation contribute to characteristics of type 2 diabetes mellitus.3,10)

The leaf of Banaba (Lagerstroemia speciosa LINN.) has been used as an Oriental traditional medicine to treat diabetes (polypuria and polydipsia) in the Philippines.11) Banaba leaf has been reported to have an antidiabetic effect.12) We found a new antidiabetic compound, corosolic acid, of Banaba leaf. Therefore, we examined the hypoglycemic effect of corosolic acid in type 2 diabetes using an animal model.

MATERIALS AND METHODS

Materials Corosolic acid (CA) was a gift from Use Techno Corporation Co., Ltd. (Kyoto, Japan). CA was stored at room temperature until use.

Animals Male KK-Ay mice (Clea, Tokyo, Japan), 6—11 weeks, were used. Under non-fasting, those with blood glucose levels above 300 mg/dl were considered to be diabetic and were used in this study. They were housed individually in an air-conditioned room at an ambient temperature of 22±2°C with a 12 h light–dark cycle. The animals were kept in this experimental animal room for 7 d with free access to food and water.

To determine blood glucose and insulin levels, blood samples were taken from the cavernous sinus using a capillary before the administration (0 h) and at 2, 4 and 7 h after the administration. CA (10 mg/kg body weight) was administered orally.

Determination of Blood Glucose and Insulin Level Blood glucose levels in mice were determined using the glucose oxidase method,13) and plasma insulin was measured by the double antibody method.14)

Isolation of Hindlimb Muscle The effect of CA on GLUT4 translocation was studied at 4 h after the administration. The hindlimb muscle of KK-Ay mice was resected for the experiment.

Low Density Membrane (LDM) and Plasma Membrane (PM) Fraction of Skeletal Muscle The muscle tissue was placed in a buffer (5 mM sodium azide, 0.25 M sucrose, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM NaHCO3 (pH 7.0)) at 4°C. Subfractionation of the muscle membrane was as described by Baron et al.,15) whose procedure was modified from that of Klip et al. The muscle

Fig. 1. Structure of Corosolic Acid (CA)
was homogenized and was centrifuged at 1000 \( g \) for 10 min, and the supernatant was saved. The resulting pellet was resuspended in the buffer and rehomogenized with a glass homogenization tube. The supernatant was combined with the first supernatant, and centrifuged at 9000 \( g \) for 10 min. The resulting supernatant was then centrifuged at 190000 \( g \) for 60 min. These membranes were applied to a discontinuous sucrose gradient containing 25%, 30%, and 35% sucrose (w/v) solutions, and was centrifuged at 190000 \( g \) for 16 h. Plasma membranes were collected in 25% sucrose gradients (LDM 35%), resuspended in the buffer, pelleted by centrifugation at 190000 \( g \) for 60 min, and resuspended in the buffer.

**Western Blot Analysis** The antibody used in the Western blotting (East Acres, U.S.A.) was raised against a synthetic peptide corresponding to the COOH-terminal domain of mouse GLUT4 (12 amino acid peptide), as reported by James et al.\(^\text{18}\) (No reaction against brain, or liver. Does not cross-react with the GLUT1 or GLUT2 tested). The membrane fractions (30 \( \mu g \)) prepared were suspended in 1% SDS and 50 mM dithiothreitol, and subjected to SDS-polyacrylamide (9%) gel electrophoresis. Electrophoretic transfer to nitrocellulose paper and detection of the immunocomplex with enhanced chemiluminescence (Amersham, Buckinghamshire, U.K.) were carried out, as has been previously described.\(^\text{19}\) The sheet was exposed on RX X-ray film and an intensifying screen (Fuji, Tokyo, Japan). The prestained molecular weight standard (Bio-Rad, Richmond, VA, U.S.A.) was used for estimation of the molecular weight. The experiments were performed at least twice for each tissue, with similar results.

**Statistical Analysis** All data were expressed as means± S.E.M, and Student’s \( t \)-test and analysis of variance (ANOVA) were used for the statistical analysis. Values were considered to be significantly different when the \( p \) value was less than 0.05.

**RESULTS**

**Effect of CA on Blood Glucose and Insulin in KK-Ay Mice** The mean blood glucose levels in KK-Ay mice after oral administration of CA are shown in Fig. 2. CA (10 mg/kg) decreased the blood glucose level 4 h after the oral administration when compared with the controls (\( p<0.05 \)). However, CA did not affect the plasma insulin over the period of 2 to 4 h after administration in KK-Ay mice (Fig. 3).

**Muscle GLUT4 Translocation** Effects of CA on muscle PM and LDM fraction of GLUT4 protein levels in both control and CA-treated KK-Ay mice are demonstrated in Figs. 4 and 5. The quantitation of GLUT4 protein in membrane in muscle was assessed by Western blotting in the mice. Quantitation of the GLUT4 glucose transporter band isolated from nitrocellulose paper demonstrated that the relative amount of GLUT4 PM fraction in the muscle from CA-treated mice was 148% of that observed in the control mice (\( p<0.05 \)) (Fig. 5), but was not changed in the LDM fraction (Fig. 4).

**DISCUSSION**

The present results show that corosolic acid reduces blood glucose levels in KK-Ay diabetic mice. KK-Ay mice, which are known to express genetically induced diabetes, including
GLUT4 translocation in total muscle membrane. In a cose transport, presumably because of the increase of effect of CA is derived, at least in part, from increased glu.

ob/ob mice\(^{20}\) and KK mice,\(^{21}\) had hyperinsulinemia as a result of insulin resistance. CA decreased the blood glucose of KK-Ay mice. CA did not affect the blood glucose of normal mice (data not shown). These findings indicate that CA is useful for type 2 diabetes. It may be that CA has a beneficial effect on hyperglycemia in type 2 diabetics.

Furthermore, we examined the effect of CA on GLUT4 glucose transporter in mouse muscle, since it has been reported that GLUT4 plays a crucial role in the muscle process of glucose uptake. It is known that, in response to insulin secretion, GLUT4 translocates from a low-density microsome membrane (LDM) to the plasma membrane (PM) fraction, permitting the entry of glucose into myocytes. In a preliminary study, we examined dose-dependence (0.4, 2, 10 mg/kg) after treatment with CA, and found that the most effective dose was 10 mg/kg (data not shown). Therefore, we studied the effect of a glucose transporter of CA at the dosage of 10 mg/kg body weight. CA increased the PM GLUT4 protein content of muscle in KK-Ay mice. It is known that GLUT4 and GLUT1 are present in skeletal muscle. However, CA did not affect the GLUT1 protein content in skeletal muscle (data not shown).

From these findings, it is very likely that the hypoglycemic effect of CA is derived, at least in part, from increased glucose transport, presumably because of the increase of GLUT4 translocation in total muscle membrane. In a GLUT4 translocation mechanism, it is possible to activate AMP-protein kinase.

Typical therapeutic agents to stimulate insulin secretion (such as a glibenclamide) have been used for type 2 diabetic patients. However, in this study, CA did not affect the insulin level of KK-Ay mice, suggesting that the antidiabetic mechanism of CA is different from a glibenclamide.

The constituents of Banaba have been investigated chemically with some tannins.\(^{11}\) As is a well-known Tannin action, it is possible to inhibit glucose uptake in the small intestine. However, the structure of CA is different from tannins, as shown in Fig. 1.

Further investigations will be needed to elucidate the mechanism of these effects. These results suggest the validity of the clinical use of Banaba leaf in the treatment of diabetes mellitus.

REFERENCES