Effect of Mangiferin on muscle GLUT4 protein content in TSOD (Tsumura, Suzuki, Obese, Diabetes) mouse, a new type 2 diabetic mice

Toshihiro MIURA^{*1}, Naoki IWAMOTO¹, Motoshi KATO¹, Hiroyuki ICHIKI², Masayoshi KUBO², Yasuhiro KOMATSU², Hiroshi SASAKI², Torao ISHIDA³ and Keiichiro TANIGAWA¹

¹ Department of Clinical Nutrition, Suzuka University of Medical Science, 1001-1 Kishioka, Suzuka, Mie 510-0293, Japan. ² Tsumura Central Research Laboratories, Tsumura and Co., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-11, Japan. ³ Hi-tech Research Center, Suzuka University of Medical Science, 1001-1 Kishioka-cho, Suzuka, Mie, 510-0293, Japan.

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ABSTRACT

The effect of Mangiferin (MF) on muscle GLUT4 protein content was studied in TSOD (Tsumura, Suzuki, Obese, Diabetes) mice, a new animal model of type 2 diabetes with hyperinsulinemia. MF reduced the blood glucose of TSOD mice 7 h after oral administration, and also significantly lowered the serum insulin of TSOD mice under similar conditions. The muscle content of facilitative glucose transporter isoform 4 (GLUT4) protein content in the plasma membrane fraction of muscle significantly increased in the orally MF-treated mice when compared with that in the controls. These results suggest that the antidiabetic effect of MF is derived, at least in part, from the increase of GLUT4 protein content in the plasma membrane of the muscle.

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. Therapeutic agents to stimulate insulin secretion (for example, sulfonylureas) have been used for type 2 diabetic patients, whereas a limited number of drugs is available to decrease insulin resistance (13).

The rhizoma of *Anemarrhena asphodeloides* has been used in oriental as traditional medicine for diabetes (polyuria and polydipsia) (23) and contained xanthone compound, Mangiferin (MF) (14). Ichiki *et al.* reported that MF-treatment reduced the blood glucose of KK-Ay mice (8). It is reported that MF with exercise decreased the blood glucose (9) and lipid (15) of KK-Ay mice. However, detailed antidiabetic mechanism, remains still unclear.

The Tsumura Suzuki Obese Diabetes (TSOD) mouse strain, a new genetic type 2 diabetes model, develops moderate degrees of obesity and diabetes that are especially apparent in animals of more than

11 week old. Phenotypic characterization revealed that the TSOD mouse had reduced insulin sensitivity (24). Through a whole genome scan of quantitative trait loci (QTLs) affecting body weight, blood glucose, and insulin levels, Hirayama *et al.* (7) have identified three major loci meeting the rigorous criteria for linkage.

Glucose transport across the plasma membrane is mediated by carrier proteins termed glucose transporters (20, 25). 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, 4, 21).

In the present study, we have examined the effect of MF on blood glucose, and we also investigated the protein content of the muscle glucose transporter isoform 4 (GLUT4) in order to elucidate the mechanism of the antidiabetic effect using TSOD mouse.

Corresponding to: Dr Toshihiro Miura at above address.
Fax: +81-593-83-9666, E-mail: miura@suzuka-u.ac.jp

Materials. MF was isolated by conventional method as previously reported (6). The structure of MF was

confirmed by spectroscopic method (6). This was stored at room temperature until use.

Animals. TSOD mice (Tsumura, Ibaraki, Japan), 22 weeks old, were used. TSOD mice with blood glucose level above 300 mg/100 mL were considered to be diabetic in this study. The mice were housed in an air-conditioned room at $22 \pm 2^\circ\text{C}$ with a 12 h light-12 h dark cycle (light: 9:00 am to 9:00). The animals were kept in the experimental animal room for 7 days with free access to food (CE-2, Clea, Tokyo, Japan) and water (tap water). Blood samples were drawn from the cavernous sinus with a capillary to determine blood glucose levels under non-anesthesia and non-fasting. MF was dissolved in distilled water. The studies were started at 10:00-11:00 am. The oral administration of MF were given on a compulsory basis.

Determination of Blood Glucose and Insulin. Blood glucose levels in mice were determined by the glucose oxidase method (22), and serum insulin was measured by the double antibody method (1).

Isolation of Hindlimb Muscle. The mice were given insulin (0.5 U/kg) subcutaneously and, 0.5 h later, the hindlimb muscle was resected for the experiment.

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 NaHCO_3 (pH 7.0)) at 4°C . Subfractionation of muscle membrane was as described by Baron *et al* (2) whose procedure was modified from that of Klip *et al* (11, 12). The muscle was homogenized and was centrifuged at 1,000 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 9,000 g for 10 min. The resulting supernatant was then centrifuged at 190,000 g for 60 min. These membranes were applied to a discontinuous sucrose gradient containing 25%, 30%, and 35% sucrose (wt/vol) solutions and was centrifuged at 190,000 g for 16 h. Plasma membranes were collected in 25% sucrose gradients, resuspended in the buffer, pelleted by centrifugation at 190,000 g for 60 min, and resuspended in the buffer.

Western Blot Analysis. The antibody used in the Western blotting (East Acres, USA) 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.* (10) (No reaction against brain, or liver. Does not cross-react with GLUT1 or GLUT2 tested). To prepare the total membrane particulate fractions, the mice muscle were excised and 1-2 g of muscle slice was homogenized in 25 mL of 10 mM Tris-HCl, 1 mM phenylmethyl sulphonyl fluoride and 1000 units/mL of aprotinin (18). The homogenates were then centrifuged at 700 g for 10 min at 4°C to sediment the fraction containing mainly the nuclei and mitochondria. The resulting supernatant was centrifuged at 13,000 g for 20 min at 4°C to yield a pellet designated as the membrane fraction of the muscle in this study. The membrane fractions (30 μg) 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, UK) were carried out as has been previously described (19). The sheet was exposed on RX X-ray film and intensifying screen (Fuji, Tokyo, Japan). The prestained molecular weight standard (Bio-Rad, Richmond, VA, USA) was used for estimation of the molecular weight. The experiments were performed at least twice for each tissue with similar results.

Statistical Analysis. All the data were expressed as mean $\pm$ SEM, and Student's t-test was used for the statistical analysis. The values were considered to be significant when the *P* value was less than 0.05.

The effect of MF on Blood Glucose in TSOD. The effect of MF injected *p.o.* to TSOD mice is shown in Table 1. MF-treated animals (100 mg/kg body weight) showed lower blood glucose levels from 7 h after the administration ($P < 0.01$). The serum insulin level in MF-treated mice decreased 7 h after the administration ($P < 0.01$) (Table 1).

Muscle GLUT4 Protein

Effects of MF on GLUT4 protein levels in both control and MF-treated TSOD mice are demonstrated in Fig. 1. 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 relative amount of GLUT4 protein in the muscle from MF treated mice was 164% of that observed in the control mice ($P < 0.01$) (Fig. 1).

This study clearly showed that the water extract

Table 1 *Effect of MF on blood glucose and insulin in TSOD mice*

	Blood glucose (mg/dL) (7 h)	Insulin (μ U/mL) (7 h)
Control	522 $\pm$ 31	178 $\pm$ 4
MF	359 $\pm$ 30*	122 $\pm$ 4*

Each value represents the mean $\pm$ SEM from 5 mice.

Significantly different from control, * P <0.01.

Fig. 1 Effect of MF on GLUT4 Content in Mouse Skeletal Muscle Each value represents the mean $\pm$ SEM of 5 mice Significantly different from control, * P <0.01

of the fruits of Mangiferin produces a consistent hypoglycemic effect. In the preliminary study, we examined the dose-dependence (4, 20, 100 mg/kg body weight) 7 h after the single administration of MF, and found that the most effective dose was 100 mg/kg. It seems likely that MF display hypoglycemic activity after metabolic process, because MF lowered blood glucose 7 h after the administration. We examined the therapeutic effects of MF on hyperglycemia in TSOD mice, an animal model with type 2 diabetes mellitus. TSOD mice, which are known for genetically induced diabetes, including ob/ob mice (5) and KK mice (16), were hyperinsulinemia as a result of insulin resistance (17). After

treatment with MF, hypoglycemia resulted with reduced serum insulin. These results indicate that MF improves hyperinsulinemia in type 2 diabetes. The insulin resistance in peripheral tissues is known to be one of the major pathogenic factors of type 2 diabetes. The finding that MF decreases blood insulin level in TSOD mice, is important.

In addition, we examined the effect of MF 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. MF increased GLUT4 protein content of muscle in TSOD mice. It is known that GLUT4 and GLUT1 are present in skeletal muscle. However, MF did not affect GLUT1 protein content in skeletal muscle (data not shown). From these findings, it is likely that the hypoglycemic effect of MF is derived, at least in part, from the decrease in insulin resistance, due presumably to the increase of GLUT4 protein content in total membrane of muscle.

Further study would show how MF could become a useful drug in the treatment of diabetes through this unique therapeutic mechanism. The present results may give a therapeutic basis for the antidiabetic activity of MF on type 2 diabetes.

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