Correction of Hyperglycemia and Insulin Sensitivity by T-1095, an Inhibitor of Renal Na\(^+\)-Glucose Cotransporters, in Streptozotocin-Induced Diabetic Rats

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ABSTRACT—We investigated the effects of T-1095 (3-(benzo[b]furan-5-yl)-2',6'-dihydroxy-4'-methylpropioophenone 2'-O-(6-O-methoxy-carbonyl)-β-D-glucopyranoside), an orally active inhibitor of Na\(^+\)-glucose cotransporter, on hyperglycemia and insulin resistance in skeletal muscle of streptozotocin (STZ)-induced diabetic rats. Chronic (4 weeks) administration of T-1095 as food admixture (0.01 – 0.1% wt/wt) suppressed the blood glucose level without affecting the food intake and body weight. In addition, the reduced 2-deoxyglucose uptake and lactate release in the soleus muscle of STZ rat was ameliorated by chronic treatment of T-1095. These data suggest that T-1095 improves insulin sensitivity in skeletal muscle through correction of hyperglycemia and has novel therapeutic potential for treatment of diabetes mellitus through removing glucose toxicity.

Keywords: Diabetes-mellitus, Streptozotocin, Na\(^+\)-glucose cotransporter

Chronic hyperglycemia in diabetes mellitus not only is a major risk factor for diabetic complications but also leads to progressive impairment of insulin secretion and to insulin resistance of peripheral tissues (1). The latter mechanism further worsens the control of the blood glucose level and is referred to as glucose toxicity (1). Therefore, the current therapy of diabetes mellitus is primarily aimed to control blood glucose levels to near normal levels.

Plasma glucose is filtered in the glomerulus of kidney and reabsorbed via Na\(^+\)-glucose cotransporter (SGLT) in proximal tubules (2). Previous studies have shown that injection of phlorizin, a competitive inhibitor of SGLT, induces glycosuria by inhibiting renal glucose reabsorption and ameliorates hyperglycemia in diabetic animal models (3 – 7). Since low bioavailability hampers use of phlorizin as an oral antidiabetic agent, we have recently developed a novel SGLT inhibitor, T-1095 (3-(benzo[b]furan-5-yl)-2',6'-dihydroxy-4'-methylpropioophenone 2'-O-(6-O-methoxy-carbonyl)-β-D-glucopyranoside) (8). After being absorbed from the gut into the blood, T-1095 is metabolized to T-1095A (3-(benzo[b]furan-5-yl)-2',6'-dihydroxy-4'-methylpropioophenone 2'-O-β-D-glucopyranoside) and inhibits specifically and competitively renal SGLT (9). Oral administration of T-1095 increases urinary glucose excretion and lowers blood glucose levels in various diabetic animal models (8 – 13) independent of insulin secretion. SGLT also mediates glucose absorption in the small intestine, but our previous study suggests that the effect of T-1095 on intestinal glucose absorption is negligible and that the antihyperglycemic effect of T-1095 is primarily mediated by reduced renal glucose reabsorption (9). Thus, T-1095 lowers blood glucose by a novel mechanism as an antidiabetic drug.

Skeletal muscles are major peripheral tissues that incorporate plasma glucose in an insulin-dependent manner (1). Hyperglycemia causes the development of insulin resistance in peripheral tissues including the skeletal muscles (1). In the present study, we determined the antidiabetic effect of T-1095 in streptozotocin (STZ)-induced diabetic rats by chronic administration. After the treatment, we assessed the effect of T-1095 on peripheral insulin sensitivity by insulin-stimulated 2-deoxyglucose (2-DG) uptake and lactate release in isolated soleus muscle.

The animal experiments had the approval of the ethics committee of Tanabe Seiyaku (Osaka). T-1095 was synthe-
sized in the Discovery Research Laboratory of Tanabe Selenaku. Male Wistar rats (6- or 7-week-old; Japan SLC, Shizuoka) were once injected with STZ (50 mg/kg, i.v.; Sigma, St. Louis, MO, USA) in 0.05 M sodium citrate buffer (pH 4.5) (STZ rat). Normal control animals were injected with buffer only. From the 7th day after the STZ injection, animals were fed with a normal diet (CE-2; Japan Clea, Tokyo) or T-1095-mixed diet (0.01 and 0.1% w/w) for 4 weeks. Blood samples were taken from the tail vein and blood glucose was determined by a glucose oxidase method (New Blood Sugar Test; Boehringer Mannheim GmbH, Mannheim, Germany). Hemoglobin A1c (HbA1c) was determined by an affinity column method (Glyc-Affin-GHb®; Seikagaku, Inc., Tokyo).

After the 4-week treatment, 2-DG uptake and lactate release were measured by a modification of the method as previously described (14, 15). Under anesthesia with pentobarbital sodium (50 mg/kg, i.p.), the soleus muscle was isolated and split into two equal longitudinal portions. The muscles were preincubated for 60 min and stimulated with insulin (0, 0.04, 0.2 mU/ml) for 30 min at 35°C in 2 ml of oxygenated Krebs-Henselei buffer (KRB) supplemented with 8 mM glucose, 32 mM mannitol and 0.1% bovine serum albumin (BSA, Sigma). The medium of the first incubation was sampled for determination of lactate concentration by an enzymatic assay kit (Determiner LA; Kyowa Medics, Tokyo). The muscles were rinsed for 12 min at 29°C in 2 ml of KRB containing 40 mM mannitol 0.1% BSA and incubated for 20 min at 29°C with 8 mM 2-deoxy-6-[1,2-3H]glucose (2.25 μCi/ml; American Radiolabeled Chemicals, St. Louis, MO, USA), 30 mM [U,14C]mannitol (0.3 μCi/ml, American Radiolabeled Chemicals), 2 mM pyruvate and 0.1% BSA with or without insulin. The tissue was then blotted on a paper towel and dissolved in 1 ml of Soluene 350 (Packard, Meriden, CT, USA). The radioactivity was counted by a liquid scintillation counter (Tricarb 4640, Packard). The data are expressed as the mean ± S.E.M. For statistical analyses, the diabetic control

![Figure 1](image_url)

**Fig. 1.** Effect of chronic treatment (4 weeks) with T-1095 on blood glucose (A), HbA1c (B) body weight (C) and food intake (D) in STZ-treated rats. A, C, D: Open square, normal control; open circle, STZ control; closed circle, STZ + 0.01% T-1095; closed triangle, STZ + 0.1% T-1095. **: P<0.01 vs normal group. ##: P<0.01 vs STZ control group. Each point, column and bar represents the mean ± S.E.M. of 5–6 rats.
group was initially compared with the normal group by the unpaired Student's t-test. When the difference between these two groups was significant, Dunnett's test (for multiple comparison) or Student's t-test was performed between the treated group and control groups (closed testing procedure).

STZ rats exhibited severe hyperglycemia, decrease of body weight and hyperphagia (Fig. 1), as compared with normal rats. Plasma insulin was not detected in STZ rats (data not shown). Chronic administration (4 weeks) of T-1095 reduced fed blood glucose (Fig. 1A) and HbA1c levels in STZ rats (Fig. 1B), without affecting body weight (Fig. 1C) and food intake (Fig. 1D). The calculated doses of the drug for 0.01% and 0.1% were 14.3 ± 0.3 and 137.1 ± 2.8 mg·kg⁻¹·day⁻¹, (mean ± S.E.M.), respectively.

Insulin elicited a concentration-dependent increase in 2-DG transport and lactate release in soleus muscles (Fig. 2: A and B). Basal and insulin-stimulated 2-DG transport and lactate release were reduced in STZ rats and restored to normal levels after treatment with T-1095 (Fig. 2: A and B).

As STZ-treated rats are deficient in insulin and develop severe hyperglycemia, it has been partly possible but difficult to control blood glucose levels with oral antidiabetics including sulfonyureas and insulin sensitizers. We have previously reported that T-1095 increases the urinary glucose excretion through inhibition of renal glucose reabsorption and thus reduces hyperglycemia in diabetic animal models (8–13) independent of endogenous insulin action. In this study, the blood glucose and HbA1c levels were corrected by T-1095 in STZ rats. In addition, in spite of the improvement of hyperglycemia, there was no effect on weight gain, suggesting that a normal glucose level is adequate to maintain glucose metabolism even in the insulin-deficient diabetic rats.

Chronic hyperglycemia impairs both insulin secretion and insulin sensitivity of peripheral tissues (1). Thus, hyperglycemia not only represents the manifestation of diabetes mellitus but also is a self-perpetuating factor responsible for the diabetic state (glucose toxicity). Phlorizin reportedly reverses peripheral insulin sensitivity via normalizing blood glucose levels in diabetic animals (3–7), but is not effective by oral administration because of its poor oral bioavailability. Our previous study using the glucose clamp technique has shown that long-term oral administration of T-1095 as a food admixture improves whole-body insulin sensitivity of diabetic animals without affecting that of normal animals (12). In this study, insulin-induced 2-DG uptake and lactate release were extensively impaired in the soleus muscle of STZ-treated rats. The impaired insulin sensitivity of skeletal muscles was ameliorated by chronic administration (4 weeks) of T-1095 in accord with the correction of blood glucose control. From these results, we consider that T-1095 reduces insulin resistance in skeletal muscle via removing glucose toxicity.

In conclusion, the present study has shown the long-term anti-hyperglycemic effect of T-1095 in insulin-deficient diabetic rats and amelioration of hyperglycemia-induced insulin resistance in skeletal muscle via improvement of blood glucose levels. Therefore, we suggest that T-1095 has novel

![Fig. 2. Effect of chronic treatment (4 weeks) with T-1095 on 2-deoxyglucose uptake (A) and lactate release (B) in isolated soleus muscles of STZ-treated rats. Open bar, normal control; closed bar, STZ control, dotted bar, STZ + 0.1% T-1095. °: P<0.05, **: P<0.01 vs normal group. **: P<0.01 vs STZ control group. Each column and bar represents the mean ± S.E.M. of 4 experiments.](image-url)
therapeutic potential for treatment of diabetes mellitus through removing glucose toxicity.

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