T-1095, an Inhibitor of Renal Na^+-Glucose Cotransporters, May Provide a Novel Approach to Treating Diabetes

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T-1095A and T-1095 are synthetic agents derived from phlorizin, a specific inhibitor of Na^+-glucose cotransporters (SGLTs). Unlike phlorizin, T-1095 is absorbed into the circulation via oral administration, is metabolized to the active form, T-1095A, and suppresses the activity of SGLTs in the kidney. Orally administered T-1095 increases urinary glucose excretion in diabetic animals, thereby decreasing blood glucose levels. Indeed, the postprandial hyperglycemia after a meal load was shown to be suppressed by this compound in streptozotocin (STZ)-induced diabetic rats. With long-term T-1095 treatment, both blood glucose and HbA_{1c} levels were reduced in STZ-induced diabetic rats and yellow KK mice. In addition, there was amelioration of abnormal carbohydrate metabolism, i.e., hyperinsulinemia and hypertriglyceridemia, and of the development of microalbuminuria, in yellow KK mice. Thus, T-1095 may be a useful antidiabetic drug, providing a novel therapeutic approach for diabetes. *Diabetes* 48:1794–1800, 1999

Diabetes is defined as a disorder exhibiting hyperglycemia caused by deficient insulin action, which is determined by both the capacity to secrete insulin from pancreatic β-cells and insulin action in peripheral insulin-sensitive tissues such as muscle and liver. According to its pathophysiology, diabetes is basically classified into two categories: type 1 and type 2. Impaired insulin secretion is the major cause of type 1 diabetes, while insulin resistance plays an important role in the pathophysiology of type 2 diabetes. Irrespective of the type of diabetes, chronic hyperglycemia reportedly leads to progressive impairment of insulin secretion and to insulin resistance of peripheral tissues in diabetes (so-called glucose toxicity [1,2]), which further worsens control of the blood glucose level. In addition, chronic hyperglycemia has been demonstrated to be a major risk factor for complications, including heart disease (3), retinopathy (4), nephropathy (5), and neuropathy (6).

The treatment of type 2 diabetes is based on caloric intake restriction and exercise. To date, several oral drugs and insulin have been developed for the treatment of diabetes. However, it is still difficult to maintain good glycemic control in most diabetic patients, although individual drugs may be highly effective for some patients.

Both intestinal absorption and renal reabsorption of glucose are mediated by Na^+-glucose cotransporters (SGLTs) (7–9). It has been reported that there are at least three isoforms of SGLTs: SGLT1 (10–13), SGLT2 (14–16), and SGLT3 (SAAT1-pSGLT2) (17–19). Phlorizin, a specific inhibitor of SGLTs, is reported to excrete glucose into urine and lower blood glucose levels in several diabetic animal models upon subcutaneous injection (20–24). However, to date, there have apparently been no attempts to use phlorizin or any of its derivatives as antidiabetic agents.

We introduce herein a novel agent that may be useful for the treatment of diabetic patients. This drug is a derivative of phlorizin, and when administered orally, it lowers blood glucose by inhibiting the function of SGLTs in the kidney and increasing urinary glucose excretion. This is a novel mechanism for an antidiabetic drug. We present the chemical structure of this agent, its in vitro characterization, as well as in vivo data obtained with diabetic animal models. Based on these results, we discuss how this drug can contribute to the treatment of diabetes.

RESEARCH DESIGN AND METHODS

Compounds. T-1095 (Fig. 1*A*) and T-1095A (Fig. 1*B*) were synthesized in Discovery Research Laboratory of Tanabe Seiyaku Company Ltd. Phlorizin (Fig. 1*C*) was purchased from Sigma (St. Louis, MO).

Inhibition of SGLT activity in brush-border membrane vesicles. Brush-border membrane vesicles (BBMVs) were prepared by the Ca^+ precipitation method (25) from kidney and small intestine of male Sprague-Dawley rats (7–13 weeks old, Japan SLC, Shizuoka, Japan) and from kidney of male ddY mice (10 weeks old, Japan SLC) and female mongrel dogs (21 months old, Yoshiki Farm, Gifu, Japan). We preincubated 50 µl of BBMV suspension (200-µg membrane protein) in assay buffer (10 mmol/l HEPES-Tris, pH 7.4, 100 mmol/l mannitol) at 37°C for 2 min and combined it with the test compounds (DMSO solution, final 0.5% DMSO): D-glucose (final 0.1 mmol/l), D-[6-^{3}H(N)]glucose (1 µCi; Du Pont-NEN, Boston, MA), and NaSCN or KSCN (final 100 mmol/l) in 150 µl of assay buffer. At 5 s later, the uptake reaction was terminated by the addition of 1.5 ml of ice-cold stop solution containing 150 mmol/l NaCl and 0.3 mmol/l phlorizin in 10 mmol/l

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AMG, α-methyl-D-glucopyranoside; BBMV, brush-border membrane vesicle; IC_{50}, 50% inhibitory concentration; OGTT, oral glucose tolerance test; PBS, phosphate-buffered saline; SCGTT, subcutaneous glucose tolerance test; SGLT, Na^+-glucose cotransporter; STZ, streptozotocin.

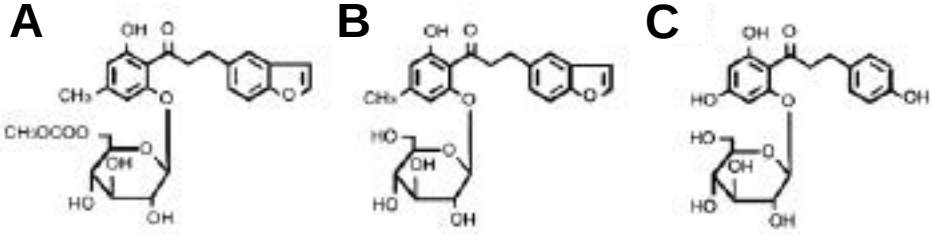

FIG. 1. Structures of T-1095 (*A*), T-1095A (*B*), and phlorizin (*C*).

HEPES-Tris (pH 7.4). Vesicles were immediately filtered through a nitrocellulose membrane filter (pore size 0.45 µm; Advantec, Tokyo) under light suction and then washed with 4.5 ml of ice-cold stop solution. Radioactivity on the membrane was measured with a liquid scintillation counter (Tricarb 4640; Packard, Meriden, CT).

Inhibition of human SGLT1 and SGLT2 expressed in Xenopus oocytes. cDNA clones of SGLT1 and SGLT2 were isolated from human small intestine and kidney cDNA libraries, respectively. Each clone was identified as the reported sequence (10,14). cRNA was transcribed in vitro from each of the clones as described by Lee et al. (11). Collagenase-treated and manually defolliculated Xenopus oocytes were injected with 50 nl of water or cRNA (50 ng/oocyte). The oocytes were incubated in ND96 medium (96 mmol/l NaCl, 2 mmol/l KCl, 1.8 mmol/l $CaCl_2$, 1.0 mmol/l $MgCl_2$, 10 mmol/l HEPES, pH 7.4) supplemented with pyruvate (2.5 mmol/l) and gentamicin (50 µg/ml). The uptake of α-methyl-D-glucopyranoside (AMG, Sigma) was measured 3 days after the injection. Groups of six to eight oocytes were incubated for 1 h in 0.5 ml of ND96 medium containing 0.25–0.5 µCi of ^{14}C-labeled AMG (Amersham, Amersham, U.K.) and nonlabeled AMG to provide the final concentrations (0.1–1.0 mmol/l). After the oocytes had been washed three times with ice-cold ND96 medium and solubilized with 10% sodium dodecyl sulfate solution, the radioactivity was measured with a liquid scintillation counter.

Effect on glucose uptake in human erythrocytes. Erythrocytes from healthy volunteers were suspended in a 4 × volume of phosphate-buffered saline (PBS). The suspension (100 µl) was preincubated at 4°C for 2 min and combined with the test compounds: D-glucose (final concentration, 0.5 mmol/l), D-[6-^{3}H(N)]glucose (0.5 µCi), and D-galactose (final 1.0 mmol/l) in 100 µl of PBS. At 20 s later, the uptake reaction was terminated by adding 1.0 ml of ice-cold PBS containing 0.3 mmol/l phloretin (stop solution). The erythrocytes were immediately collected by centrifugation and washed with 1.5 ml of ice-cold stop solution. Cells were then hemolyzed with distilled water and deproteinated with 5% trichloroacetic acid. Radioactivity in the supernatant was measured with a liquid scintillation counter.

Urinary glucose excretion in rats. Test compounds were administered orally or subcutaneously to male Sprague-Dawley rats (7 weeks old, Japan SLC). Urine was collected for 24 h using metabolic cages, and the glucose content was measured with a glucose analyzer (APEC, Danvers, MA).

Glucose tolerance test in mice. Male ddY mice (7 weeks old, Japan SLC) were given a 20% glucose solution (1 g/kg) orally (oral glucose tolerance test [OGTT]) or subcutaneously (subcutaneous glucose tolerance test [SCGTT]) after 16 h of fasting. One of the T-1095, phlorizin or control vehicle, was orally administered at 15 min before the glucose loading. Blood samples (10 µl) were collected from the tail tip, and the blood glucose level was measured using a glucose oxidase method (New Blood Sugar Test; Boehringer Mannheim, Mannheim, Germany). Urine was collected using metabolic cages. After the last blood sampling (3 h after the glucose load), mice were killed by vertebral dislocation, and urine in the bladder was combined with the collected urine. Urinary glucose contents were measured as described above.

Meal tolerance test in streptozotocin-induced diabetic rats. Male Sprague-Dawley rats (6 weeks old, Japan SLC) were intravenously injected with streptozotocin (STZ) (50 mg/kg, Sigma) to induce hyperglycemia. At 1 week later, STZ-treated rats and normal control rats were fasted for 16 h. T-1095 was administered orally, and 15 min later, rats were fed a liquid meal (20 ml/kg) containing 10% starch, 3.4% casein, 0.07% amino acids, and 3.5% oils (Oriental Yeast, Tokyo) via an intragastric catheter. Blood samples were collected from the tail tip. Urine was collected using metabolic cages. Glucose contents in blood and urine were measured as described above.

Chronic administration in STZ-induced diabetic rats and KK-A^y mice. Male Wistar rats (7 weeks old, Japan SLC), 1 week after the intravenous injection of STZ (50 mg/kg), and female KK-A^y mice (4 weeks old, Japan CLEA, Tokyo) were used. Age-matched male Wistar rats (Japan SLC) and female C57BL/6N mice (Japan CLEA) fed a normal diet were used as healthy control animals. Diabetic animals were fed a normal diet (CE-2, Japan CLEA) or a T-1095 mixed diet (low dose: 0.01% wt/wt [in STZ-treated rats] or 0.03% wt/wt [in KK-A^y mice]; high dose: 0.1% wt/wt, prepared by Japan CLEA) for 28 days. The calculated doses of T-1095 were 14–17 mg · kg^{-1} body wt · day^{-1} (low-dose group) and 127–172 mg · kg^{-1} body wt · day^{-1} (high-dose group) in STZ-treated rats, and 44–80 mg · kg^{-1} body wt · day^{-1} (low-dose group) and 148–244 mg · kg^{-1} body wt · day^{-1} (high-dose group) in KK-A^y mice. Blood glucose levels and urine glucose contents were determined as described above. HbA_{1c} levels were determined by an aminophenyl-boronate-agarose affinity chromatography method (Glyc-Affin. GHb; Seikagaku, Tokyo). Plasma triglyceride contents were determined with an enzymatic assay kit (Eiken Chemical, Tokyo). Plasma immunoreactive insulin and urinary albumin contents were measured with ELISA kits using rat insulin (Seikagaku Kogyo) and mouse albumin (Exocell, Philadelphia) as standards, respectively.

Statistical analyses. Data are expressed as means ± SE. Multiple comparisons were performed using Dunnett's tests. In experiments with STZ-treated rats and KK-A^y mice, statistical analyses were performed by closed testing procedures. In brief, initially, the untreated diabetic control group was compared with the normal group by unpaired Student's *t* test. When the difference between these two groups was significant, a multiple comparison was performed by Dunnett's test to compare each T-1095–treated group with the diabetic control group. Calculations of 50% inhibitory concentrations (IC_{50}) and 95% confidence intervals were performed by nonlinear least-squares analysis using a four-parameter logistic model.

RESULTS

Inhibition of SGLT activity by T-1095 and T-1095A in vitro. T-1095 (Fig. 1*A*) and T-1095A (Fig. 1*B*), derived from phlorizin (Fig. 1*C*), were developed as specific inhibitors of SGLT. The inhibitory effects of T-1095 and T-1095A on AMG uptake into oocytes overexpressing human SGLT1 and SGLT2 were investigated, in comparison with that of phlorizin (Fig. 2*A* and *B*). It was confirmed that these agents do indeed inhibit SGLT.

Furthermore, it was revealed that T-1095 and T-1095A inhibited Na^+-dependent glucose uptake in BBMVs prepared from rat kidney, rat small intestine, mouse kidney, and dog kidney. Thus, even assuming that there exists another type of SGLT, different from SGLT1 and SGLT2 (i.e., SGLT3), it appears that T-1095 and T-1095A inhibit the activities of these SGLTs, irrespective of their isoforms.

The K_i (inhibitor constant) values of T-1095A for the SGLT activities in rat kidney BBMVs (Fig. 2*C*) and human SGLT1 expressed in Xenopus oocytes (Fig. 2*D*) were calculated to be 0.33 and 0.07 µmol/l, respectively. Data showing the potencies of the inhibitory effects of T-1095, T-1095A, and phlorizin are summarized in Table 1. The inhibitory activity of T-1095A is nearly equal to that of phlorizin. Furthermore, T-1095A is 6–120 times more potent than T-1095, while the GLUT (GLUT1)-mediated uptake of glucose by human erythrocytes was inhibited by T-1095A only when a very high concentration was used.

In rats, mice, and dogs given T-1095 orally, T-1095A was detected in plasma as a metabolite (T. Okagaki, H. Yamakita, unpublished observations). Thus, T-1095 is considered to be a pro-drug of T-1095A, a potent and selective inhibitor of SGLTs, and it is likely that T-1095A primarily accounts for the in vivo effects of T-1095, which are described below.

Inhibition of SGLT activity by T-1095 and T-1095A in vivo. When T-1095 is administered orally, T-1095A is produced from T-1095 via metabolism and functions as an inhibitor of SGLTs in the kidney. It was demonstrated that oral administration of T-1095 to rats dose-dependently increased urinary glucose excretion (Fig. 3), via the inhibition of glucose

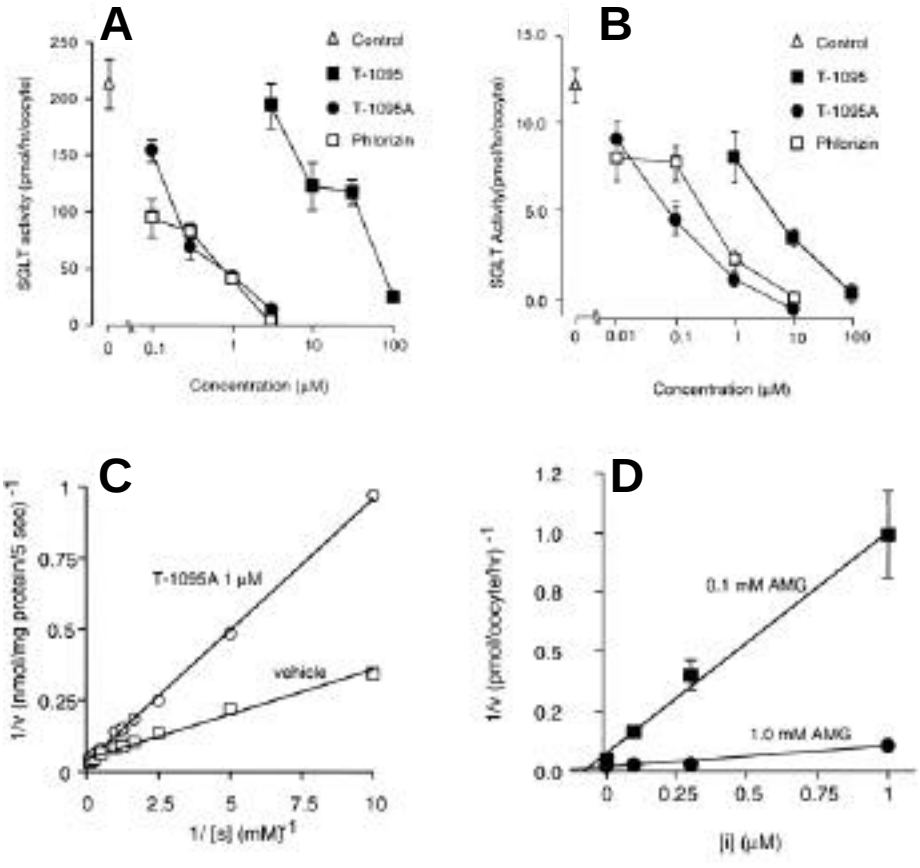

FIG. 2. Inhibition of SGLT activity by T-1095, T-1095A, and phlorizin. *A*, *B*: Dose-response curves of T-1095, T-1095A, and phlorizin on Na^+-dependent AMG uptake (1.0 mmol/l) by human SGLT1 (*A*) and SGLT2 (*B*) expressed in Xenopus oocytes. Uptake was measured as described in methods. The IC_{50} values are shown in Table 1. *C*: Competitive inhibition of SGLT activity of rat kidney BBMVs. BBMVs were incubated with (○) or without (□) T-1095A (1 μmol/l), and Na^+-dependent [^{3}H]glucose (0.1 mmol/l) uptake was determined as described in methods. The apparent Michaelis constants with inhibitor (K_{mi}) and without (K_m) were determined using a double reciprocal plot, and the inhibition constant (K_i = 0.33 μmol/l) was calculated from the formula $K_i = K_m[i]/(K_{mi} - K_m)$ where [i] is the concentration of the inhibitor. *D*: Inhibition of human SGLT1 activity expressed in Xenopus oocytes. [^{14}C]AMG uptakes at 0.1 mmol/l AMG (■) and 1.0 mmol/l AMG (●) in the presence of T-1095A at various concentrations were measured. The inhibition constant was determined using Dixon analysis. The intersection of the regression lines revealed a K_i value of 0.07 μmol/l. Data are the means ± SE of three to eight experiments. When not given, SE bars were smaller than the symbol used.

reabsorption in proximal tubules. In contrast, subcutaneously but not orally administered phlorizin induced glycosuria (Fig. 3). This is because phlorizin is not absorbed when administered orally.

Effect on blood glucose levels of single administration of T-1095. Having established that T-1095 can increase urinary glucose excretion, we next investigated the acute effect of T-1095 on the blood glucose level by performing glucose tolerance tests. Mice, fasted for 16 h, were loaded with glucose (1 g/kg) orally and subcutaneously in the OGTT and the SCGTT, respectively. Oral administration of T-1095 suppressed the elevation of blood glucose in both tests (Fig. 4*A* and *B*). Concurrently, urinary glucose excretion was increased by T-1095 (OGTT: 0.06 ± 0.01 [control] vs. 32.3 ± 3.2 [T-1095] mg · 3 h^{-1} · $head^{-1}$, $P < 0.01$; SCGTT: 0.08 ± 0.01 [control] vs. 37.9 ± 2.7 [T-1095] mg · 3 h^{-1} · $head^{-1}$, $P < 0.01$).

To clearly demonstrate that the blood glucose–lowering effect of T-1095 is due mainly to renal excretion, rather than the inhibition of the absorption of glucose from the gut, phlorizin was also administered orally. Phlorizin is a potent SGLT inhibitor, and its potency is comparable to that of T-1095A and much higher than that of T-1095 (Table 1). However, phlorizin is known to be not absorbed from the gut into blood as shown by the data in Fig. 3. Thus, by using phlorizin, we were able to investigate the effect of T-1095, which is limited in the gut, not via the mechanism operating after absorption

TABLE 1
IC_{50} values of T-1095, T-1095A, and phlorizin on SGLT activity

	IC_{50} (μmol/l)						
	Human SGLT1	Human SGLT2	Human erythrocytes	Rat kidney	Rat small intestine	Mouse kidney	Dog kidney
T-1095A	0.20 (0.17–0.25)	0.05 (0.03–0.08)	46.3 (40.0–53.6)	0.66 (0.63–0.70)	1.5 (0.6–3.8)	0.69 (0.57–0.81)	0.86 (0.78–0.94)
T-1095	22.8 (14.8–35.0)	2.3 (1.1–4.9)	26.8 (15.6–44.8)	5.9 (5.3–6.3)	12.4 (2.9–52.5)	6.7 (4.2–10.5)	5.5 (5.1–5.9)
Phlorizin	0.16 (0.12–0.21)	0.16 (0.09–0.30)	>100	0.96 (0.89–1.00)	4.7 (1.9–11.3)	1.2 (1.1–1.4)	1.4 (1.3–1.5)

Data are means (95% CI) from three to eight experiments. SGLT1 and SGLT2: inhibition of [^{14}C]AMG (1.0 mmol/l) uptake in Xenopus oocytes injected with human SGLT1 or SGLT2 cRNA. Erythrocytes: inhibition of [^{3}H]glucose (0.5 mmol/l) uptake. Rat kidney, rat small intestine, mouse kidney, dog kidney: inhibition of [^{3}H]glucose (0.1 mmol/l) uptake in isolated BBMVs.

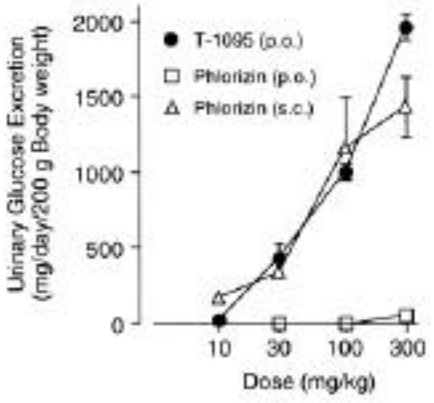

FIG. 3. Enhancement of urinary glucose excretion in normal rats by T-1095 and phlorizin. Urine was collected for 24 h after oral administration (p.o.) of T-1095 or phlorizin, or subcutaneous (s.c.) administration of phlorizin. Glucose contents in urine were measured as described in METHODS. Each point with bar represents the mean ± SE of three rats.

into the blood. Oral administration of phlorizin significantly delayed blood glucose elevation in the OGTT (Fig. 4*A*), but the maximal glucose level in the OGTT was only very slightly decreased. In contrast, essentially no effect was observed in the SCGTT with the oral administration of phlorizin (Fig. 4*B*).

We next investigated the effect of T-1095 on the hyperglycemia associated with meal tolerance tests using diabetic rats. Male Sprague-Dawley rats were intravenously injected with STZ (50 mg/kg) to induce hyperglycemia (type 1 diabetes). At 1 week later, STZ-treated and normal control rats were fasted for 16 h, and the liquid meal was given via an intragastric catheter. The blood glucose level was remarkably elevated in STZ-treated control rats as compared with normal control rats (Fig. 5*A*). T-1095 dose-dependently suppressed postprandial hyperglycemia (Fig. 5*A*), accompanied by a concurrent increase in urinary glucose excretion (Fig. 5*B*).

Antidiabetic effect of long-term treatment with an SGLT inhibitor. To study the effect of chronic administration, T-1095 mixed into the diet was given to STZ-treated rats for 28 days. There was no difference in food intake between the control and T-1095–treated STZ groups (data not shown). Both blood glucose and HbA_{1c} levels decreased significantly during T-1095 treatment (Fig. 6*A* and *B*). Next, we investigated the antidiabetic effects in genetically diabetic yellow KK (KK-A^y) mice, an obese type 2 diabetic model with typical insulin resistance (26). T-1095 mixed into the diet was given to female KK-A^y mice for 28 days. KK-A^y mice become increasingly hyperglycemic, hyperinsulinemic, hypertriglyceridemic, and obese with advancing age. In addition, there were increases in the contents of urinary glucose, urinary albumin, and HbA_{1c} levels in control KK-A^y mice on day 28 (Table 2). T-1095 suppressed the development of hyperglycemia and hypertriglyceridemia, and it reduced the elevated HbA_{1c} level. Furthermore, the obesity, hyperinsulinemia, and microalbuminuria were ameliorated by treatment with a high dose of T-1095 (Table 2). Interestingly, urinary glucose excretion in T-1095–treated KK-A^y groups was nearly equivalent to that of the control KK-A^y group at day 28, probably because of improvement of hyperglycemia with diminution of the glucose concentration in the glomerular filtrate.

DISCUSSION

Worldwide, diabetes is an important and increasingly prevalent disease. Type 2 diabetes accounts for up to 85% of the diabetic population in most countries. Environmental factors including excessive energy intake and physical inactivity contribute to the occurrence of type 2 diabetes. In fact, these environmental factors lead to obesity, which is present in >50% of men and 70% of women with type 2 diabetes. Because obesity reportedly induces insulin resistance, it is recommended that type 2 diabetes management start with caloric restriction and increased physical activity. However, it is difficult for patients to maintain an appropriate caloric intake, given their increased appetites. T-1095 was developed with the aim of normalizing blood glucose levels by eliminating excess blood glucose via the urine.

Glucose is absorbed in the intestines and distributed to blood and tissues. Both intestinal absorption and renal reabsorption are mediated by the coordinated functions of Na^+/K^+ ATPase, SGLTs, and facilitated glucose transporters (7–9). In the basolateral membrane of the epithelium, Na^+/K^+ ATPase pumps out Na^+, thereby decreasing intracellular Na^+ and creating an inward-oriented Na^+ gradient. In apical plasma membranes, SGLT cotransports glucose and Na^+ using the Na^+ gradient as

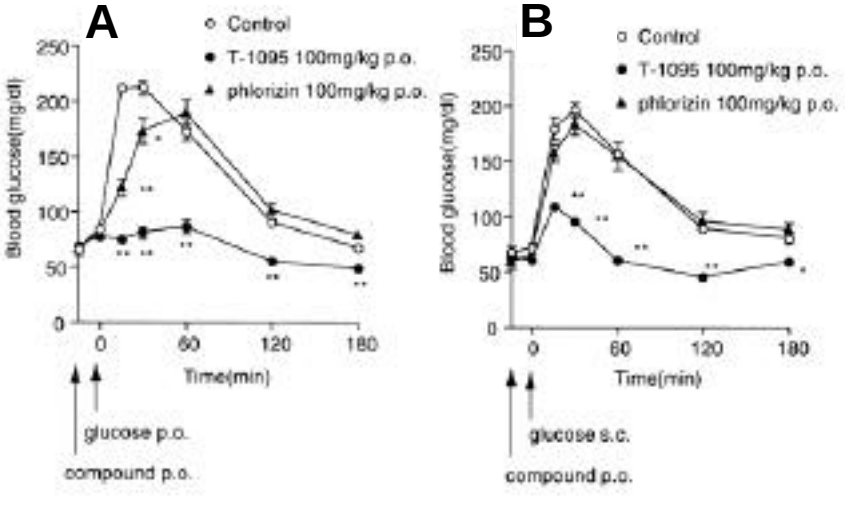

FIG. 4. Effect of T-1095 and phlorizin on blood glucose levels in the OGTT (*A*) and the SCGTT (*B*) in mice. Mice were loaded with glucose (1 g/kg) orally (p.o.) or subcutaneously (s.c.) after 16 h of fasting. Compounds were orally administered at 15 min before the glucose loading. Blood glucose level and urinary glucose content were measured as described in METHODS. *$P < 0.05$, **$P < 0.01$ vs. control group. Each point with bar represents the mean ± SE of five mice.

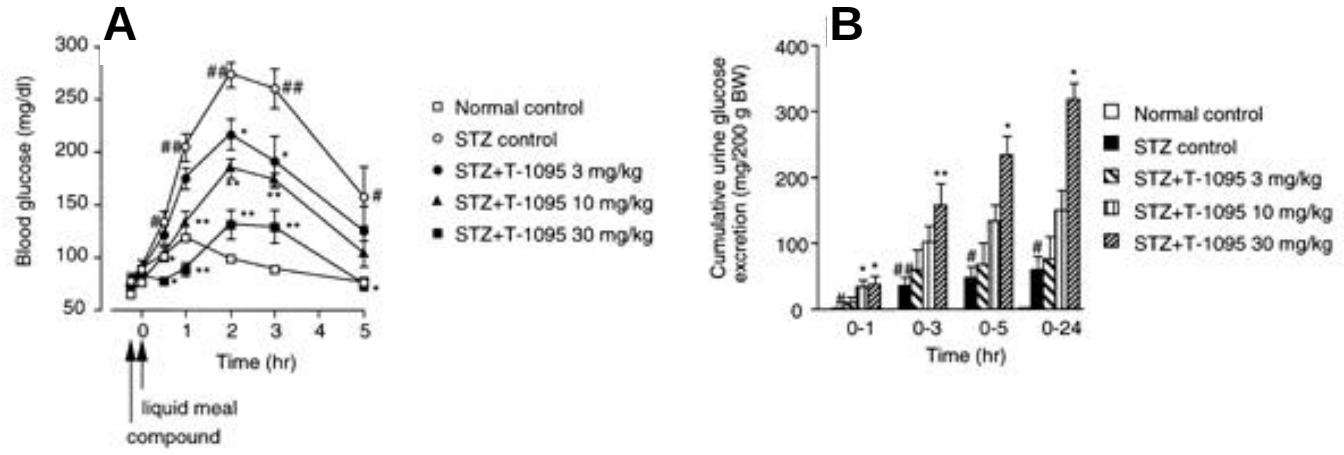

FIG. 5. Effect of T-1095 on blood glucose levels (*A*) and urinary glucose excretion (*B*) in the meal tolerance test in STZ-treated rats. Normal and STZ-treated rats were fasted for 16 h and then given a liquid meal (20 ml/kg, containing 10% starch) using an intragastric catheter. Blood glucose level and urinary glucose content were measured as described in METHODS. #*P* < 0.05, ##*P* < 0.01 vs. normal group; **P* < 0.05, ***P* < 0.01 vs. control STZ group. Each point with bar represents the mean ± SE of six rats.

the driving force. Cytosolic glucose, which is elevated, is then transported to the interstitial space by glucose transporters.

At least three SGLT isoforms have been cloned: SGLT1 (10–13), SGLT2 (14–16), and SGLT3 (SAAT1-pSGLT2) (17–19). In contrast to the ubiquitous expression of glucose transporters in all types of cells, SGLT1 is present in intestinal and renal epithelial cells (11), and SGLT2 is found only in the epithelium of the kidney (15). Expression of SGLT3 was reported to be strong in intestine, spleen, liver, and muscle and at a significantly lower level in kidney (17). SGLT1 mediates high-affinity low-capacity transport with a Na^+:glucose transport ratio of 2:1. SGLT2 and SGLT3 transport Na^+ and glucose at a ratio of 1:1 and function as low-affinity high-capacity transporters. Previous studies have shown that intraperitoneal or subcutaneous injection of phlorizin, a classic competitive inhibitor of SGLTs, induces glycosuria by inhibiting glucose reabsorption in the kidney (27). Because low oral bioavailability hampers the use of phlorizin as an oral antidiabetic agent, we have screened synthetic phlorizin analogs (28) and developed an SGLT inhibitor, T-1095, that can be taken orally.

The uptake of glucose (or AMG) in renal and intestinal BBMVs as well as reconstituted SGLTs was inhibited by T-1095A and T-1095, but the former agent is 6–120 times more potent than the latter (Table 1). After oral administration of T-1095 to mice, rats, and dogs, T-1095A was detected in blood, and further studies revealed that the metabolic conversion from T-1095 to T-1095A occurs in the hepatic S9 fractions of these animals (M. Tsuda, Y. Yamada, unpublished observations). In addition, we demonstrated that oral administration of T-1095 markedly increased the excretion of glucose into the urine (Fig. 3). There is still controversy about whether or not the SGLT2 clone used in this study is the "real" renal SGLT. However, even if another as yet unknown SGLT is present in the kidney and is the primary contributor to renal glucose reuptake, T-1095 and T-1095A would inhibit such a transporter, because SGLT activity in renal BBMVs was inhibited and urinary glucose was excreted in vivo. Taken together, these data indicate that orally administered T-1095 is converted to T-1095A in vivo, and that this metabolite suppresses the renal reabsorption of glucose through inhibition of SGLTs in proximal tubules (Fig. 7).

T-1095 blunted blood glucose elevation in both the OGTT and the SCGTT (Fig. 4). Although the blood glucose decrease after a subcutaneous glucose load is due to inhibition of renal

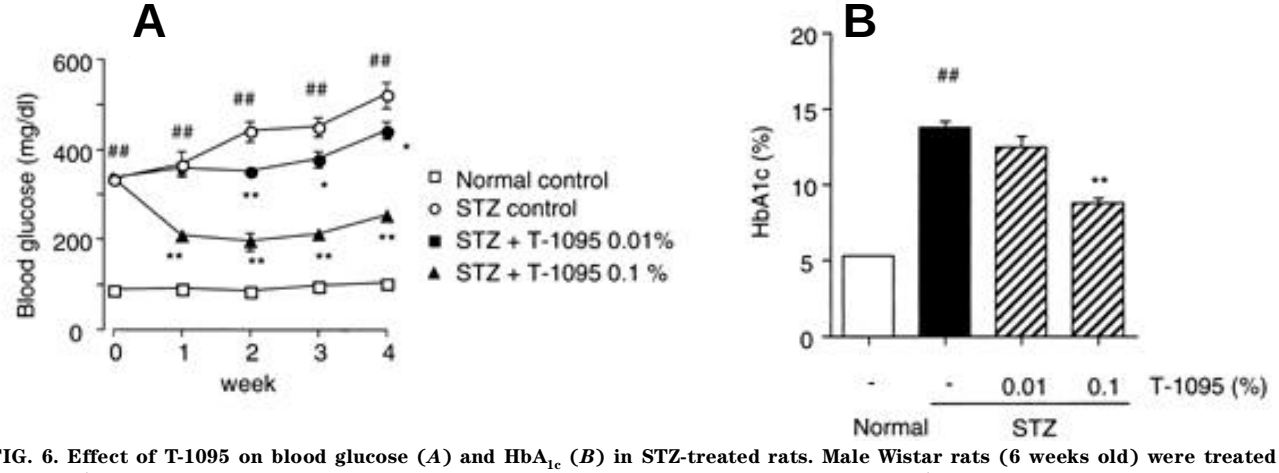

FIG. 6. Effect of T-1095 on blood glucose (*A*) and HbA_{1c} (*B*) in STZ-treated rats. Male Wistar rats (6 weeks old) were treated with STZ (50 mg/kg). At 1 week after the treatment, rats were given a normal diet or a T-1095 mixed diet (low dose: 0.01% wt/wt; high dose: 0.1% wt/wt) for 28 days. The blood glucose level and HbA_{1c} were determined as described in METHODS. ##*P* < 0.01 vs. normal group; **P* < 0.05, ***P* < 0.01 vs. control STZ group. Each point, column, and bar represent the mean ± SE of four to five rats.

TABLE 2
Effect of T-1095 on body weight, blood glucose, plasma triglyceride, plasma immunoreactive insulin, urine glucose, urine albumin, and HbA_{1c} levels in female KK-A^y mice

	T-1095 (% wt/wt)	Body weight (g)	Blood glucose (mg/dl)	Plasma TG (mg/dl)	Plasma IRI (ng/ml)	Urinary glucose (mg/day)	Urinary albumin (μg/day)	HbA_{1c} (%)
Before treatment								
C57BL/6N	—	14.4 ± 0.1	136 ± 2	29 ± 1	0.2 ± 0.0	0.2 ± 0.0		
KK-A^y	—	19.8 ± 0.3*	156 ± 2*	144 ± 10*	5.7 ± 0.9*	0.7 ± 0.1*		
	0.03	19.8 ± 0.3	156 ± 2	133 ± 9	3.9 ± 0.5	0.7 ± 0.1		
	0.1	20.1 ± 0.2	156 ± 2	126 ± 8	3.6 ± 0.3	0.5 ± 0.1		
Treated for 14 days								
C57BL/6N	—	18.6 ± 0.2	125 ± 2	59 ± 4	0.6 ± 0.1			
KK-A^y	—	33.5 ± 0.4*	315 ± 18*	360 ± 24*	28.2 ± 1.8*			
	0.03	32.0 ± 0.5†	217 ± 13‡	251 ± 13‡	27.8 ± 3.0			
	0.1	30.0 ± 0.4‡	168 ± 3‡	205 ± 12‡	9.0 ± 0.8‡			
Treated for 28 days								
C57BL/6N	—	20.8 ± 0.2	154 ± 8	49 ± 3	0.5 ± 0.0	0.6 ± 0.0	13 ± 4	4.4 ± 0.1
KK-A^y	—	40.8 ± 0.5*	366 ± 13*	589 ± 27*	57.3 ± 3.4*	512 ± 56*	1,795 ± 150*	9.1 ± 0.2*
	0.03	39.6 ± 0.7	252 ± 14‡	453 ± 24‡	61.3 ± 3.4	404 ± 51	1,679 ± 190	7.8 ± 0.1‡
	0.1	36.8 ± 0.5‡	147 ± 11‡	283 ± 24‡	28.6 ± 3.5‡	554 ± 47	1,135 ± 203†	6.4 ± 0.2‡

Data are means ± SE for 20 mice. Mice were given a normal diet or a T-1095 mixed diet for 28 days. TG, triglyceride; IRI, immunoreactive insulin; *$P < 0.01$ vs. normal group, †$P < 0.05$, ‡$P < 0.01$ vs. control KK-A^y group.

glucose reuptake, the effect in an OGTT may be regarded as the sum result of inhibiting both intestinal glucose absorption and renal reabsorption. However, T-1095A, an active metabolite of T-1095, is produced mainly in the liver after intestinal absorption; it therefore would not affect glucose absorption in the intestine. Taking into consideration that the oral administration of phlorizin delayed but failed to suppress maximal blood glucose elevation in the OGTT, it is reasonable to consider the acute antihyperglycemic effect of T-1095 to be primarily mediated by reduced renal glucose reabsorption.

In STZ-induced diabetic rats, marked postprandial hyperglycemia was effectively suppressed by T-1095 (Fig. 5*A*). Because SGLTs are abundantly expressed in proximal tubules, only part of SGLT function involves glucose reabsorption at normal blood glucose levels. However, when the blood glucose level rises >170 mg/dl, the reabsorption mechanism is saturated (i.e., SGLTs are functioning at full capacity), because glomerular filtration of glucose is a linear function of the plasma (blood) glucose concentration (7). Accordingly, inhibition of SGLTs is expected to increase urinary excretion of glucose more effectively under hyperglycemic than under normoglycemic conditions. Therefore, we speculate that T-1095 would effectively suppress postprandial hyperglycemia in diabetic patients, if administered orally before a meal.

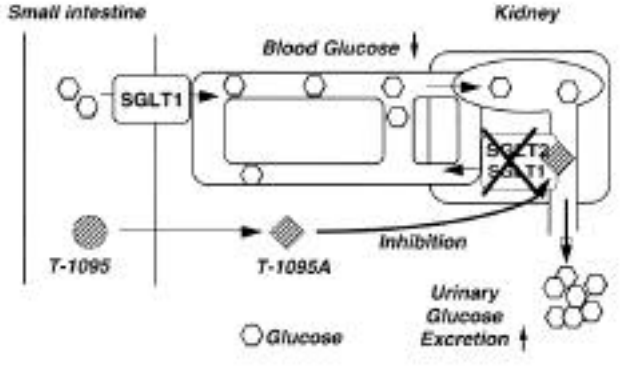

FIG. 7. Antidiabetic mechanism of T-1095 in vivo.

Long-term treatment with T-1095 reduced the fed blood glucose level as well as the HbA_{1c} level in both insulin-deficient STZ-treated rats (Fig. 6) and obese insulin-resistant KK-A^y mice (Table 2). Thus, T-1095 exhibits an antihyperglycemic effect, irrespective of the presence of endogenous insulin secretion. In addition, it was revealed that T-1095 treatment improved the hyperinsulinemia in KK-A^y mice (Table 2), which suggests amelioration of the insulin resistance in this murine model. It is well known that regardless of its underlying causes, hyperglycemia can itself impair both β-cell function and peripheral glucose metabolism (2). Thus, long-term elimination of excess serum glucose can contribute to the normalization of glucose metabolism through suppression of glucotoxicity. In fact, it was reported that insulin resistance is prevented if the concomitant hyperglycemia is suppressed by phlorizin (20,23).

Chronic hyperglycemia is also associated with a high incidence of complications including nephropathy (5), neuropathy (6), and retinopathy (4). The Diabetes Control and Complications Trial has demonstrated a reduced incidence of diabetic complications with strict control of blood glucose to near normal levels in patients with type 1 diabetes (4–6,29). KK-A^y mice used in this study showed microalbuminuria (Table 2), indicative of the development of diabetic nephropathy. T-1095 treatment markedly reduced the development of microalbuminuria in KK-A^y mice (Table 2), which agrees with evidence that normalization of glucose levels is important for the prevention of diabetic nephropathy.

The basis of therapy in type 2 diabetes is caloric restriction and exercise to promote the reduction of total body fat. In addition, several antidiabetic agents are available, including injectable insulin, sulfonylureas, biguanides, insulin sensitivity enhancers, and α-glucosidase inhibitors. However,

without restriction of caloric intake, it is difficult to maintain good glycemic control. It is also clear that the prevalence of type 2 diabetes is very low in developing societies where the food supply is inadequate. These observations suggest that the elimination of excess glucose is a potentially effective therapy for diabetes. At present, it is difficult to anticipate to what extent lowering high blood glucose with T-1095 would improve insulin resistance, and whether the incidence of diabetic complications would decrease. However, to date, no significant adverse effects other than a slight weight loss have been observed in diabetic animal models given the T-1095 doses used in this study. Thus, we suggest that the orally administered SGLT inhibitor T-1095 is a novel therapeutic approach to the management of type 2 diabetes.

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