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Review Article

SGLT as a therapeutic target

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Abstract

The expression of the sodium/glucose cotransporter (SGLT), a membrane protein, is limited to renal tubules and the intestinal basolateral membrane. A classic competitive inhibitor of SGLT, phlorizin, given by injection reportedly exerts a hypoglycemic effect in diabetic rodents via induction of glycosuria. Unlike phlorizin, the recently developed phlorizin derivative T-1095 is effectively absorbed in the gut and enters the circulation after oral administration. Similar to injected phlorizin, orally administered T-1095 lowers blood glucose in diabetic animals by enhancing urinary glucose excretion via suppression of renal SGLT function. In addition to this acute hypoglycemic action, chronic T-1095 treatment induces other effects favoring the restoration of impaired insulin secretion from pancreatic β-cells and normalization of hyperglycemia-induced insulin resistance in muscle and liver. In addition, it is possible that correcting hyperglycemia with this SGLT inhibitor may contribute to the prevention and treatment of diabetic complications. Thus, oral SGLT inhibitors are a promising new class of antidiabetic drugs.

Introduction

Diabetes mellitus is on the rise in nearly all countries, making it an increasingly important social and health problem. Insulin resistance and deficient insulin secretion have been regarded as the two major factors underlying the development and severity of diabetes mellitus. Initially, insulin resistance plays the leading role in the development of type 2 diabetes mellitus, which accounts for over 85% of all cases of diabetes worldwide. To compensate for this insulin resistance, pancreatic β-cells must secrete more insulin. However, eventually, the capacity of β-cells to produce adequate amounts of insulin becomes exhausted and blood glucose rises. Since the magnitude of the hypoglycemic effect is determined by both the capacity of pancreatic β-cells to secrete insulin and insulin sensitivity in peripheral tissues, the molecular mechanisms of currently available antidiabetic drugs can be classified as either: 1) compensating for or inducing insulin secretion; or 2) normalizing insulin resistance.

However, it has recently been widely recognized that hyperglycemia is not merely a consequence of diabetes mellitus, but also an important factor which accelerates worsening of this disease. This is the so-called “glucotoxicity” phenomenon (1, 2), which impairs the limited insulin-secretory capacity of pancreatic β-cells and also worsens insulin resistance in peripheral tissues. Thus, improving hyperglycemia itself is a potential strategy for the treatment of diabetes mellitus.

Phlorizin has been widely used to achieve experimental normalization of hyperglycemia in animals (3, 4). Phlorizin is a potent inhibitor of the sodium/glucose cotransporter (SGLT), but does not inhibit facilitative glucose transporter (GLUT) function. Injected phlorizin suppresses renal SGLT transport activity, resulting in a rise in glycosuria and a drop in blood glucose. Unfortunately, phlorizin is not absorbed from the gut, and thus can not

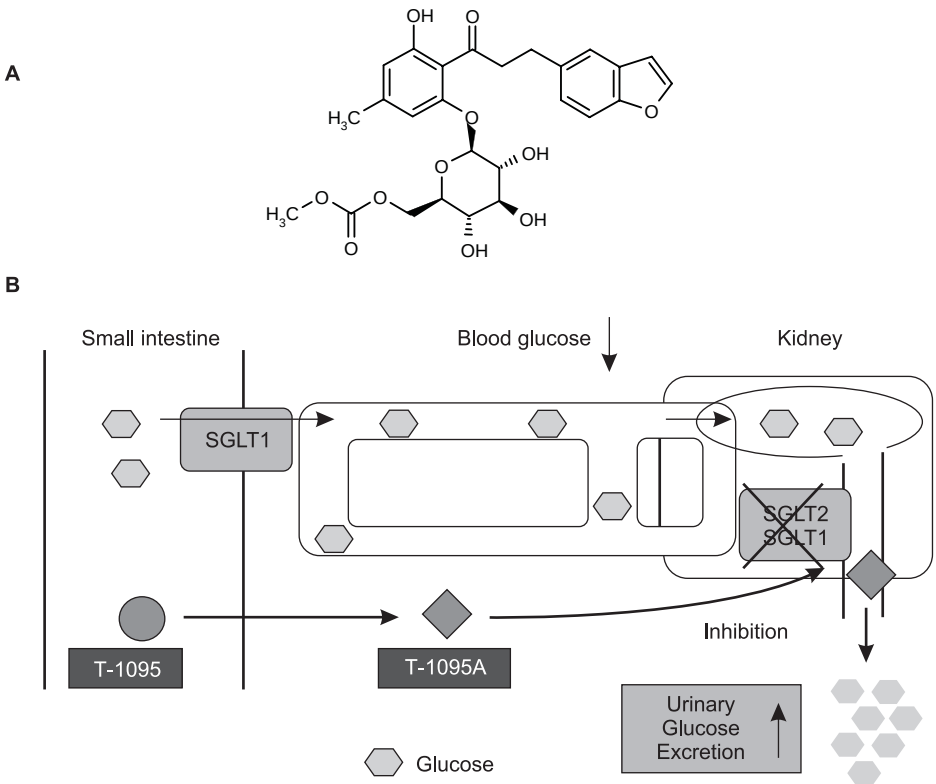

Fig. 1. Chemical structure of T-1095 and mechanism of its hypoglycemic effect.

be given orally. Recently, a phlorizin derivative that is absorbed intestinally and enters the circulation was developed by the Discovery Research Laboratory of Tanabe Seiyaku (5). To date, several studies have assessed the potential of this SGLT inhibitor as an antidiabetic drug. This review examines the possible use of SGLT inhibitors, based on these reports, as therapeutics for diabetes.

Structure and pharmacological actions of the SGLT inhibitor T-1095

The orally active phlorizin derivatives T-1095 and T-1095A were developed at Tanabe Seiyaku and the mechanism by which orally administered T-1095 exerts its *in vivo* hypoglycemic effect was elucidated (Fig. 1). T-1095 is efficiently absorbed in the intestine, thereby entering the circulation. The compound is then metabolically converted to T-1095A in the liver. Thus, T-1095A is the major compound detectable in blood after oral administration of T-1095.

The inhibitory effects of T-1095 and T-1095A on SGLT transport activity have been investigated using brush border membrane vesicles prepared from rat kidney and oocytes overexpressing human SGLT1 and SGLT2. In these experiments, the inhibitory potencies of T-1095, T-1095A and phlorizin were compared (5). Table I summarizes the oocyte data and shows T-1095A to be 6-120 times more potent than T-1095, without significant inhibition of GLUT-mediated glucose uptake. Thus, T-1095 is assumed to be a prodrug of T-1095A.

Hypoglycemic effect related to elimination of excess plasma glucose

The SGLT family consists of three isoforms, which are distributed in the intestine and kidney (6). Glucose, absorbed in the intestines, is distributed to blood and tissues. SGLT is essential to the intestinal absorption of glucose from food (7), while the renal role of SGLT is reabsorption of urinary glucose (8).

Phlorizin, a specific inhibitor of SGLTs, reportedly induces urinary glucose excretion and lowers blood glucose in several diabetic animal models following subcutaneous or intraperitoneal injection (3, 4). Although low oral bioavailability hampers the application of phlorizin as an antidiabetic drug, the novel SGLT inhibitor T-1095 can be taken orally (5). Oral T-1095 is metabolized in the liver to T-1095A, and the functions of intestinal and renal SGLT are inhibited by T-1095 and T-1095A, respectively (Fig. 1B). Since the inhibitory potency of T-1095A on SGLT far exceeds that of T-1095 (5), it appears that oral T-1095 exerts its hypoglycemic effect mainly by inhibiting renal SGLT function, resulting in a rise in urinary glucose.

Table I: HbA1c and parameters from a glucose clamp study in normal neonatally streptozotocin (nSTZ)-treated rats following 4 weeks' treatment with or without T-1095.

	T-1095 (in food)	HbA1c (%)	GIR (mg/kg/min)	GUR (mg/kg/min)	Triglycerides (mg/dl)	Free fatty acids (mEq/l)
Normal	-	4.5 ± 0.1	16.7 ± 0.9	19.0 ± 1.8	159.2 ± 11.7	302.6 ± 23.5
nSTZ	-	12.6 ± 0.2##	9.3 ± 0.8##	13.4 ± 0.8##	161.9 ± 22.7##	421.4 ± 54.2##
nSTZ	0.03%	270.2 ± 9.6	N.A.	N.A.	153.3 ± 11.2	320.7 ± 27.9
nSTZ	0.1%	271.6 ± 8.8*	14.0 ± 0.8**	16.6 ± 0.9**	120.3 ± 5.6**	255.2 ± 13.6**

T-1095 (0.1% w/w) was mixed with food and fed to the rats. Data are means ± SE (n=6-12). ##$p < 0.01$ *vs.* normal rats; *$p < 0.05$, **$p < 0.01$ *vs.* nSTZ rats. GIR: glucose infusion rate; GUR: glucose utilization rate; N.A.: not assayed.

The inhibitory effect on intestinal SGLT, which might delay glucose transfer from food to blood, would only be additive, if present at all. Furthermore, glucose uptake via GLUT is not inhibited, indicating that glucose delivery to the brain, liver, muscle, etc., remains unaffected.

The hypoglycemic effects of T-1095 have been clearly demonstrated in several studies using diabetic rodent models (5, 9-11). The hypoglycemic effect is seen immediately after T-1095 administration, as confirmed by an oral glucose tolerance test (OGTT). Figure 2 shows representative data obtained with single and chronic T-1095 administration to Zucker diabetic fatty (ZDF) rats (12). T-1095 dose-dependently suppresses postprandial hyperglycemia (Fig. 2A), associated with an increase in urinary glucose excretion (Fig. 2B). These observations suggest that postprandial hyperglycemia improves when T-1095 is taken just before a meal.

In addition, continuous administration of T-1095 effectively lowers both blood glucose and HbA1c levels. Figure 2C depicts the effects of chronic administration of T-1095 mixed with the food. Although food intake did not differ between the control and T-1095-treated diabetic groups, both blood glucose and HbA1c levels decreased to a significant extent during T-1095 treatment (Fig. 2A, C, Table I). Similar results were consistently obtained regardless of the diabetic rodent model used.

The hypoglycemic effect of T-1095 was concluded to manifest even after a single dose and to persist for the duration of treatment. This was the case for both type 1 and type 2 diabetes mellitus. In addition, since the only mechanism by which T-1095 increases urinary glucose excretion is by lowering the urinary secretion threshold (normal blood glucose concentration: 180 mg/dl), T-1095 does not cause blood glucose to fall below the normal fasting level (approximately 100 mg/dl). Thus, T-1095 would not produce hypoglycemia, a common problem with insulin and sulfonylurea use.

Caloric restriction is essential for virtually all type 2 diabetic patients, even those who are not obese. Appropriate caloric intake is also important in the treatment of type 1 diabetes. Although some type 2 and most type 1 diabetic patients are not obese, this is only because of their low capacity to secrete insulin from pancreatic β-cells. Thus, hyperglycemia persists despite the absence of obesity. Even for these nonobese patients,

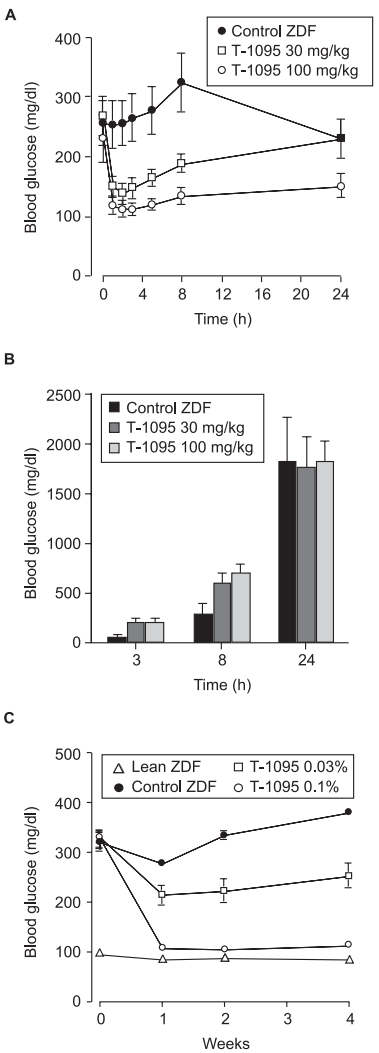

Fig. 2. Effects of a single oral dose of T-1095 on blood glucose (A) and urinary glucose excretion (B) in Zucker diabetic fatty (ZDF) rats and effects of continuous oral T-1095 administration on blood glucose in ZDF and lean rats (C). A, B: T-1095 was administered using an intragastic catheter; changes in blood glucose and urinary glucose excretion were monitored for 24 h. Each value indicates the mean ± SEM (n=6). *$p < 0.05$, **$p < 0.01$ *vs.* the corresponding control. C: T-1095 was administered as a dietary admixture. Each value indicates the mean ± SEM (n=6). #$p < 0.01$ *vs.* lean rats; *$p < 0.05$, **$p < 0.01$ *vs.* ZDF control rats.

reducing caloric intake is essential for improving hyperglycemia, as well as preventing hyperglycemia-induced damage to pancreatic β-cells and insulin resistance in peripheral tissues. Without appropriate caloric intake, none of the currently available drugs, including injectable insulin, sulfonylureas, biguanides and insulin sensitivity enhancers, can maintain adequate glycemic control. However, most diabetic patients have great difficulty adhering to strict caloric restriction. Therefore, T-1095, considered to be highly effective in maintaining the balance between caloric intake and calories utilized, via excretion of excess blood glucose, appears to be a very promising treatment option for both type 1 and type 2 diabetic patients.

Protection from hyperglycemia-induced damage

Chronic hyperglycemia reportedly produces β-cell dysfunction, associated with impaired insulin secretion and biosynthesis. There is considerable evidence linking hyperglycemia to nonenzymatic reactions of sugars with proteins and accelerated formation of the advanced glycation end products (AGEs) (13) which play a major pathogenic role in glucose toxicity. Hyperglycemia reportedly induces several other detrimental effects, including increased oxidative stress (14) and abnormal signal transduction, *i.e.,* enhanced protein kinase C (PKC) activation (15). As a result, prolonged hyperglycemia in diabetic patients ultimately results in declining insulin secretion from pancreatic β-cells and deterioration of the diabetic state. Similarly, the fatty diabetic *db/db* mouse shows hyperglycemia with hypoinsulinemia after prolonged hyperglycemia with hyperinsulinemia. The age-related decrease in plasma insulin and loss of pancreatic insulin content could be prevented by long-term T-1095 treatment (10, 16, 17). Thus, T-1095 may be able to prevent pancreatic β-cell exhaustion via alleviation of glucose toxicity.

SGLT inhibitor improves hyperglycemia-induced insulin resistance

Insulin resistance is essential to the occurrence and development of type 2 diabetes mellitus. Obesity (resulting from excessive caloric intake), insufficient exercise, excessive dietary fat, aging and other factors are regarded as causes of insulin resistance. Once the insulin needed to maintain normal glucose metabolism in the setting of insulin resistance exceeds the ability of pancreatic β-cells to secrete insulin, blood glucose rises. Hyperglycemia is considered to contribute to the worsening of peripheral insulin resistance associated with both type 1 and type 2 diabetes. It is also recognized that insulin resistance can be at least partially reversed by strict blood glucose control.

Using neonatally streptozotocin-treated (nSTZ) (11) and Zucker diabetic rats (12), we investigated whether normalizing hyperglycemia with T-1095 could reverse insulin resistance. Although apparent insulin resistance is seen in STZ-treated rats, they are regarded as a model of insulin-deficient diabetes. As shown in Table I, T-1095 treatment normalized the glucose infusion rate (GIR) and glucose utilization rate (GUR) in STZ-induced diabetic rats during a hyperinsulinemic euglycemic clamp study, while these parameters were unaffected in normal rats (11). These observations indicate that insulin resistance in STZ-treated rats is attributable to hyperglycemia. The improvement by T-1095 of impaired insulin sensitivity in STZ-treated rats was evidenced by increases in whole-body and skeletal muscle insulin-mediated glucose utilization, with a normalized muscle GLUT4 content and a decreased hepatic glucose production rate. Furthermore, insulin-induced translocation of GLUT4 from the low-density microsome (LDM) fraction to the plasma membrane (PM) was markedly suppressed in STZ-treated diabetic rats, and T-1095 treatment nearly normalized this impairment of translocation (Fig. 3).

Subsequently, we examined the altered insulin signaling which follows insulin injection via the portal vein. In STZ-treated rat muscle and liver, insulin-induced tyrosine phosphorylation of the insulin receptor substrates IRS-1 and IRS-2 and phosphatidylinositol 3-kinase (PI3-kinase) activation were all markedly enhanced in comparison to normal rats, despite insulin resistance, in the STZ-treated rats (IRS-1: Fig. 4A, B and C; IRS-2: data not shown) (18). Interestingly, however, the phosphorylation of Akt and resultant Akt kinase activation were both significantly impaired in STZ-treated rats (Fig. 4D, E). These abnormalities in the pathway from the insulin receptor to PI3-kinase activation and impaired Akt activation were reversed when blood glucose was normalized by administering T-1095 (18). The data strongly suggest that chronic hyperglycemia reduces the efficiency of the PI3-kinase to Akt kinase activation step and that this is the molecular mechanism underlying hyperglycemia-induced insulin resistance (19). Thiazolidinediones, recently used as insulin sensitizers, have been reported to enhance relatively upstream insulin signaling molecules such as IRS-1/2 and PI3-kinase. It is thus probable that these drugs would not be effective in treating hyperglycemia-induced insulin resistance. We therefore consider T-1095 to be the only agent which improves hyperglycemia-induced insulin resistance.

Possible favorable effects of SGLT inhibitors on diabetic complications

Both microvascular and macrovascular complications of diabetes constitute a major health burden for patients. Clinical and epidemiological data suggest that the magnitude and duration of hyperglycemia in diabetic patients are closely associated with the severity of these complications, although other factors, including age, sex, hypertension, genetic factors and cigarette smoking, are also involved in the pathogenesis of diabetic complications.

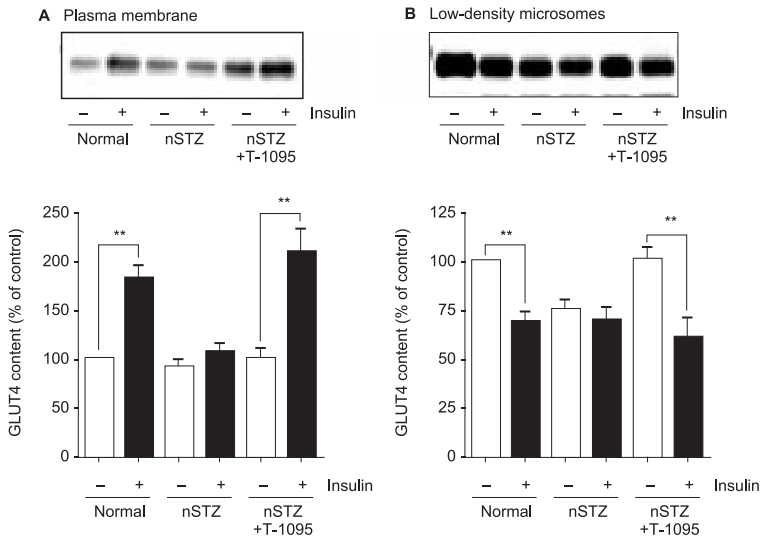

Fig. 3. Insulin-stimulated GLUT4 translocation in skeletal muscles of normal rats and rats neonatally treated with streptozotocin (nSTZ). The plasma membrane (PM) and low-density microsomes (LDM) isolated from skeletal muscles in the basal or insulin-stimulated state were subjected to SDS-PAGE followed by immunoblotting with anti-GLUT4. A representative autoradiograph (upper) and the quantified data (lower) on GLUT4 protein in the PM (A) and LDM (B). Results are expressed as means ± SE for 3 animals. $^{**}p < 0.01$.

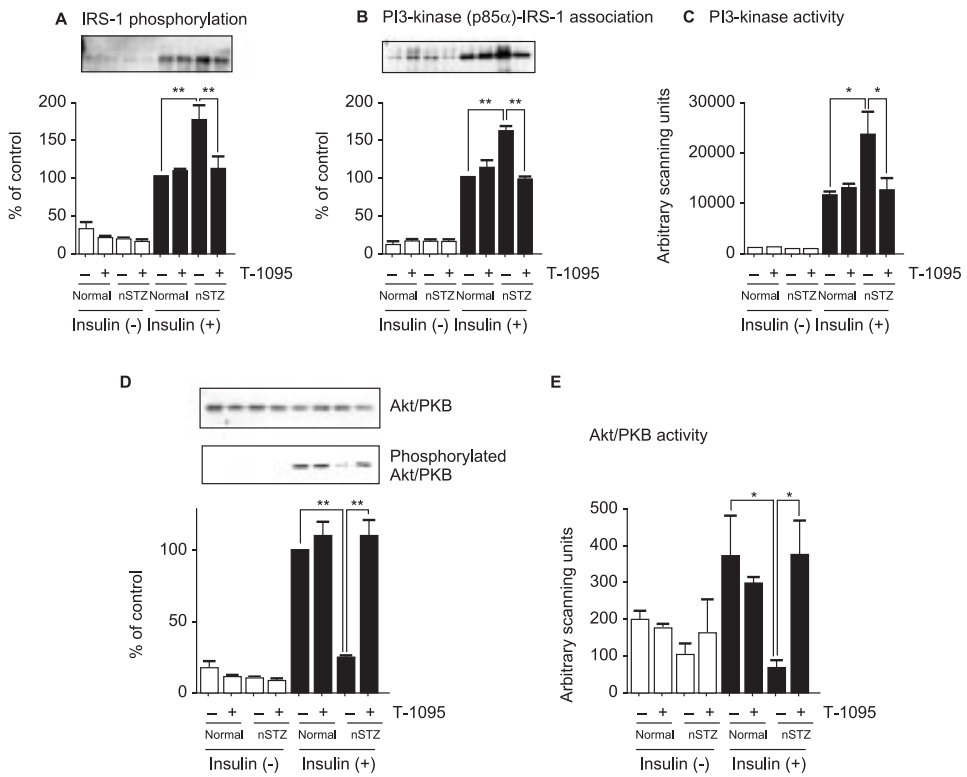

Fig. 4. Tyrosine phosphorylation (A) of IRS-1 and amount (p85α) (B) and activity (C) of PI3-kinase associated with IRS-1, and the protein expression, phosphorylation (Ser473) (D) and activity (E) of Akt/PKB in basal and insulin-stimulated skeletal muscles of normal and nSTZ rats with or without T-1095 treatment. Proteins immunoprecipitated with anti-IRS-1 were subjected to SDS-PAGE, then immunoblotting with anti-phosphorylated tyrosine (4G10) (A) or anti-p85α, or determination of PI3-kinase activity (C). A: A representative autoradiograph (upper) and the quantified data (lower) on tyrosine phosphorylation of IRS-1. B: A representative autoradiograph (upper) and the quantified data (lower) on p85α associated with IRS-1. C: PI3-kinase activity associated with IRS-1. The total lysate was subjected to SDS-PAGE followed by immunoblotting with anti-Akt/PKB or anti-phosphorylated (Ser473) Akt/PKB (D). Equal amounts of muscle protein were immunoprecipitated with anti-Akt/PKB followed by determination of Akt/PKB activity (E). D: Representative autoradiographs of Akt /PKB (upper) and phosphorylated Akt/PKB (middle), and the quantified data on Akt/PKB phosphorylation (lower). N.D.: not detected. E: Akt/PKB activity in skeletal muscle. Results are expressed as means ± SE for 3 animals. $^{*}p < 0.05$, $^{**}p < 0.01$.

Numerous clinical trials have demonstrated that normalization of hyperglycemia contributes to preventing the occurrence and worsening of diabetic complications. To date, it has been shown that normalizing hyperglycemia by administering T-1095 promotes urinary albumin excretion in diabetic *db/db* mice, which is an established parameter reflecting glomerular dysfunction in diabetes (10). In addition, expansion of the glumerular mesangial area in aged *db/db* mice was also apparently ameliorated. Thus, it is likely that the SGLT inhibitor itself is not only not harmful to the kidneys, but may even be useful for preventing diabetic nephropathy. Protective effects against other diabetic complications, *e.g.,* diabetic retinopathy, neuropathy and atherosclerosis, have not yet been demonstrated, but it is not unreasonable to speculate that SGLT inhibitors would be useful for preventing these complications as well. Although further studies are necessary, SGLT inhibitor treatment of diabetes may be more effective in preventing diabetic complications than therapy with other antidiabetic drugs.

Conclusions and future outlook

A recently developed, orally administered SGLT inhibitor has been shown to improve hyperglycemia by increasing urinary glucose secretion. This novel class of antidiabetic agents was demonstrated, in a series of experiments in diabetic animals, to not only lower elevated plasma glucose concentrations, but also to normalize insulin resistance and prevent exhaustion of pancreatic β-cells. In addition, SGLT inhibitors may effectively prevent diabetic complications involving AGEs. Whether additional beneficial or adverse effects other than the antidiabetic effects emerge during continuous suppression of SGLT activity by the inhibitor remains to be determined. Preliminary results using rodent models revealed that continuous T-1095 treatment induced neither compensatory overexpression of SGLT nor renal toxicity. However, these possibilities warrant detailed examination in future clinical trials.

References

1. Leahy, J., Bonner-Weir, S., Weir, G. *β-Cell dysfunction induced by chronic hyperglycemia: Current ideas on mechanism of impaired glucose induced insulin secretion.* Diabetes Care 1992, 15: 442-5.

2. Rossetti, L., Giaccari, A., DeFronzo, R. *Glucose toxicity.* Diabetes Care 1990, 13: 610-30.

3. Rossetti, L., Cusin, I., Tiengo, A. et al. *Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats.* J Clin Invest 1987, 79: 1510-5.

4. Blondel, O., Bailbe, D., Portha, B. *Insulin resistance in rats with non-insulin-dependent diabetes induced by neonatal (5 days) streptozotocin: Evidence for reversal following phlorizin treatment.* Metabolism 1990, 39: 787-97.

5. Oku, A., Ueta, K., Arakawa, K. et al. *T-1095, an inhibitor of renal Na^+-glucose cotransporters, may provide a novel approach to treating diabetes.* Diabetes 1999, 48: 1794-800.

6. Turk, E., Wright, E.M. *Membrane topology motifs in the SGLT cotransporter family.* J Membr Biol 1997, 159: 1-20.

7. Hediger, M.A., Turk, Y., Wright, E.M. *Homology of the human intestinal Na^+/glucose and Escherichia coli Na^+/proline cotransporters.* Proc Natl Acad Sci USA 1989, 86: 5748-52.

8. Kanai, Y., Lee, W., You, G. et al. *The human kidney low affinity Na^+/glucose cotransporter SGLT2. Delineation of the major renal reabsorptive mechanism for D-glucose.* J Clin Invest 1994, 93: 397-404.

9. Oku, A., Ueta, K., Arakawa, K. et al. *Antihyperglycemic effect of T-1095 via inhibition of renal Na^+-glucose cotransporters in streptozotocin-induced diabetic rats.* Biol Pharm Bull 2000, 23: 1434-7.

10. Arakawa, K., Ishihara, T., Oku, A. et al. *Improved diabetic syndrome in C57BL/KsJ-db/db mice by oral administration of the Na^+-glucose cotransporter inhibitor T-1095.* Brit J Pharmacol 2001, 132: 578-86.

11. Oku, A., Ueta, K., Nawano, M. et al. *Antidiabetic effect of T-1095, an inhibitor of Na^+-glucose cotransporter, in neonatally streptozotocin-treated rats.* Eur J Pharmacol 2000, 391: 183-92.

12. Nawano, M., Oku, A., Ueta, K. et al. *Hyperglycemia contributes to insulin resistance in hepatic and adipose tissue but not skeletal muscle of ZDF rats.* Am J Physiol Endcrinol Metab 2000, 278: E535-43.

13. Stern, D., Yan, S.D., Yan, S.F. et al. *Receptor for advanced glycation endproducts: A multiligand receptor magnifying cell stress in diverse pathologic settings.* Adv Drug Deliv Rev 2002, 54: 1615-25.

14. Pennathur, S., Heinecke, J.W. *Mechanisms of oxidative stress in diabetes: Implications for the pathogenesis of vascular disease and antioxidant therapy.* Front Biosci 2004, 9: 565-74.

15. Evans, J.L., Goldfine, I.D., Maddux, B.A. et al. *Oxidative stress and stress-activated signaling pathways: A unifying hypothesis of type 2 diabetes.* Endocrin Rev 2002, 23: 599-622.

16. Nunoi, K., Yasuda, K., Adachi, T. et al. *Beneficial effect of T-1095, a selective inhibitor of renal Na^+-glucose cotransporters, on metabolic index and insulin secretion in spontaneously diabetic GK rats.* Clin Exp Pharmacol Physiol 2002, 29: 386-90.

17. Yasuda, K., Okamoto, Y., Nunoi, K. et al. *Normalization of cytoplasmic calcium response in pancreatic β-cells of spontaneously diabetic GK rat by the treatment with T-1095, a specific inhibitor of renal Na+-glucose co-transporters.* Horm Metab Res 2002, 34: 217-21.

18. Oku, A., Nawano, M., Ueta, K. et al. *Inhibitory effect of hyperglycemia on insulin-induced Akt/protein kinase B activation in skeletal muscle.* Am J Physiol Endocrinol Metab 2001, 280: E816-24.

19. Nawano, M., Ueta, K., Oku, A. et al. *Hyperglycemia impairs the insulin signaling step between PI 3-kinase and Akt/PKB activations in ZDF rat liver*. Biochem Biophys Res Comm 1999, 266: 252-6.