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Full paper

The effect of combined treatment with canagliflozin and teneligliptin on glucose intolerance in Zucker diabetic fatty rats

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ABSTRACT

To assess the impact of concomitant inhibition of sodium-glucose cotransporter (SGLT) 2 and dipeptidyl peptidase IV (DPP4) for the treatment of type 2 diabetes mellitus (T2DM), the effect of combined treatment with canagliflozin, a novel SGLT2 inhibitor, and teneligliptin, a DPP4 inhibitor, on glucose intolerance was investigated in Zucker diabetic fatty (ZDF) rats. Canagliflozin potently inhibited human and rat SGLT2 and moderately inhibited human and rat SGLT1 activities but did not affect DPP4 activity. In contrast, teneligliptin inhibited human and rat DPP4 activities but not SGLT activities. A single oral treatment of canagliflozin and teneligliptin suppressed plasma glucose elevation in an oral glucose tolerance test in 13 week-old ZDF rats. This combination of agents elevated plasma active GLP-1 levels in a synergistic manner, probably mediated by intestinal SGLT1 inhibition, and further improved glucose intolerance. In the combination-treated animals, there was no pharmacokinetic interaction of the drugs and no further inhibition of plasma DPP4 activity compared with that in the teneligliptin-treated animals. These results suggest that the inhibition of SGLT2 and DPP4 improves glucose intolerance and that combined treatment with canagliflozin and teneligliptin is a novel therapeutic option for glycemic control in T2DM.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from decreased insulin secretion and increased insulin resistance. Although a number of oral antihyperglycemic agents are available and glucose-lowering therapy is effective to prevent and manage diabetes and its complications (1), it is still difficult to maintain good glycemic control with monotherapy over a long-term period because of their limited efficacy (2). Patients with inadequate glycemic control often require additional combination therapy with other oral agents or insulin to achieve the desired glycemic target levels (3).

Glucagon-like peptide-1 (GLP-1), an incretin hormone released from L cells, exerts multiple antidiabetic effects such as stimulating insulin secretion, inhibiting gastric emptying and glucagon secretion, and suppressing appetite (4). Dipeptidyl peptidase IV (DPP4) inhibitors prevent inactivation of incretins including GLP-1, facilitate incretin-induced insulin secretion, and control postprandial blood glucose levels. Sodium glucose co-transporter (SGLT) 2 plays a critical role in renal glucose reabsorption (5,6). SGLT2 inhibitors, which enhance renal glucose excretion and reduce blood glucose levels independent of insulin action, have been identified as a new class of antihyperglycemic agents (7–10). Their use is associated with a slight increase in the incidence of adverse events such as urogenital infection and osmotic diuresis, which are considered to be drug class effects (6). However, preclinical and clinical studies of SGLT2 inhibitors have demonstrated the additional beneficial effects of reducing body weight and blood pressure with low risk of hypoglycemia (11–15).

A recent study has shown that canagliflozin, an SGLT2 inhibitor, increases plasma GLP-1 concentrations in a mixed-meal tolerance test in healthy subjects (16). Plasma concentrations of active and total GLP-1 were further augmented in the combination treatment with canagliflozin and a long-acting DPP4 inhibitor, teneligliptin, in normoglycemic subjects (17). However, it is not known whether the combination treatment controls hyperglycemia better than each individual drug treatment in type 2 diabetes.

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In the present study, to explore the antidiabetic potential of concomitant inhibition of SGLT2 and DPP4, the combined effect of teneligliptin and canagliflozin on glucose intolerance in an oral glucose tolerance test (OGTT) was investigated in 13-week-old Zucker diabetic fatty (ZDF) rats.

2. Materials and methods

2.1. Reagents and chemicals

Teneligliptin (98.8% purity) and canagliflozin (>99.95% purity) were synthesized at Mitsubishi Tanabe Pharma Corporation (Todashi, Saitama, Japan).

2.2. Cell-based assays

2.2.1. SGLTs inhibition assay

Expression plasmids containing human SGLT1 (hSGLT1), human SGLT2 (hSGLT2), rat SGLT1 (rSGLT1), and rat SGLT2 (rSGLT2) were stably transfected into Chinese hamster ovary (CHO)–K1 cells. Cells were seeded into 24-well plates at a density of 4×10^5 cells/well in Ham's F-12 medium containing 10% fetal bovine serum and were incubated at 37 °C in an assay buffer containing 50 mM HEPES, 20 mM Tris Base, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, and 137 mM NaCl at pH 7.4. SGLT1 and SGLT2 transporter activities were assayed after 2 h of reaction time with 0.3 or 0.5 mM α-methyl-D-glucopyranoside (AMG; Sigma–Aldrich, St. Louis, MO) in the presence of [^{14}C]AMG (PerkinElmer, Waltham, MA). Radioactive counts in the cells were determined using a liquid scintillation counter (PerkinElmer). Protein concentration was measured using the CoomassiePlus Protein Assay Kit (Pierce, Rockford, IL).

2.2.2. DPP4 inhibition assay

Inhibitory activities against human DPP4 were measured using a fluorogenic DPP4 Assay Kit (BPS Bioscience, San Diego, CA). Test compound solution, DPP substrate, and human recombinant DPP4 were mixed to initiate the enzyme reaction. After a 10 min reaction at room temperature, the fluorescence intensity was measured using a microplate reader (Molecular Devices, Sunnyvale, CA).

Inhibitory activities against rat DPP4 were measured using serum collected from 7-week-old male Sprague–Dawley (SD) rats (Charles River Japan, Yokohama, Japan). Rat serum and Glycyl-L-proline 4-methylcoumaryl-7-amide (MCA) were mixed with PBS containing 0.003% Brij-35 to initiate the enzyme reaction, as previously described (18). The fluorescence intensity of MCA was measured using a microplate reader after a 1-h incubation at 37 °C.

2.3. In vivo studies

2.3.1. Animals and test compound administration

All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Mitsubishi Tanabe Pharma Corporation or LSI Medience Corporation (Tokyo, Japan) and met the Japanese Experimental Animal Research Association standards, as defined in the Guidelines for Animal Experiments (1987). Male ZDF-*Lepr*^{fa}/CrlCrlj rats were purchased from Charles River Japan. During an acclimatization period of 6 weeks, the animals were housed with a 12-h light/dark cycle and controlled temperature and humidity. Rats were provided water *ad libitum* and a standard commercial diet. Test compounds for oral gavage were prepared in 0.5% hydroxypropyl methylcellulose.

2.3.2. OGTT in ZDF rats

Because female ZDF rats rarely exhibit hyperglycemia, despite obesity and insulin resistance comparable to males (20,23), we performed the experiments only in male rats. We used 13-week-old animals because previous studies have demonstrated normal creatinine clearance in the age range of 16–20 weeks in ZDF rats (20–24). After overnight fasting, test drugs were orally administered to 13-week-old ZDF rats in a volume of 5 mL/kg. The administered doses were 0.3 mg/kg for teneligliptin, 3 and 10 mg/kg for canagliflozin, and a combination of 0.3 mg of teneligliptin and 3 or 10 mg/kg of canagliflozin. Teneligliptin at 0.1 mg/kg and 1 mg/kg inhibits >50% of plasma DPP4 activity for 30 min and significantly reduces postprandial hyperglycemia in Zucker fatty rats (18), and canagliflozin at 3 mg/kg and 30 mg/kg inhibits >70% of renal glucose reabsorption in ZDF rats (19). Glucose solution was orally administered at 2 g/kg body weight 15 min after the administration of the test compounds, and blood was collected from the tail vein into chilled tubes containing EDTA and a DPP4 inhibitor 15 min before (−15) and 0, 10, 30, 60, and 120 min after the oral glucose administration. Plasma was separated by centrifugation and stored at −80 °C until the measurement of plasma glucose, insulin, and aGLP-1 concentrations.

2.3.3. Determination of metabolic parameters

Plasma glucose concentrations were determined using a Glucose CII-Test WAKO Kit (Wako Pure Chemical Industries, Osaka, Japan). Plasma active GLP-1 (aGLP-1) concentrations were measured using an ELISA kit (Epitope Diagnostics, Inc., San Diego, CA) after solid phase extraction. Plasma insulin concentrations were measured with an enzyme-linked immunosorbent assay kit (Morinaga Institute of Biologic Science, Yokohama, Japan). Plasma DPP4 activities were measured as described above.

2.3.4. Pharmacokinetic study

Plasma concentrations of canagliflozin and teneligliptin were determined in satellite groups of 13 week-old ZDF rats by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after solid-phase extraction from plasma. In brief, test compounds and glucose were orally administered to ZDF rats as mentioned above. The same volume of drug or vehicle was administered in both single and combination treatment groups. Plasma samples were collected at 0.25, 0.42, 0.75, 1.25, 2.25, 4, 8, 10, 24, and 32 h after test compound administration. Each sample was loaded onto an OASIS HLB μElution 96-well plate (30 μm, Waters Corporation, Milford, MA) and eluted with acetonitrile. The eluates were injected into an API4000 mass spectrometer (AB SCIEX, Framingham, USA) equipped with an Atlantis C18 column (5 μm, 2.1 mm I.D. × 50 mm; Waters Corporation) for teneligliptin or a Cadenza CD C-18 column (2.0 mm I.D. × 50 mm, 3 μm, Imtakt Corporation, Kyoto, Japan) for canagliflozin. The pharmacokinetic parameters were determined using the pharmacokinetic analysis software Phoenix WinNonlin 6.3 (Pharsight Corporation, RealMountain View, USA).

2.4. Statistical analysis

Data were presented as the mean ± S.E.M. for each group. Statistical analyses were performed using an SAS-based system (SAS Institute, Cary, NC, USA) or Prism software (GraphPad, San Diego, CA, USA), and significant differences were identified using a parametric Dunnett's multiple comparison test, *t*-test, or two way analysis of variance, as appropriate. Probabilities less than 5% ($P < 0.05$) were considered to be statistically significant. Integrated plasma glucose, aGLP-1, and insulin levels during OGTT were expressed as the incremental area under the curve (ΔAUC_{0-2h}), calculated by the trapezoidal rule. The peak value of plasma glucose, aGLP-1, and insulin above baseline levels measured at 0 min was calculated up to 120 min after glucose loading.

Table 1
IC_{50} values for SGLT1, SGLT2, and DPP4 activities of canagliflozin and teneligliptin.

IC_{50} (nmol/L)	Human			Rat		
	SGLT1	SGLT2	DPP4	SGLT1	SGLT2	DPP4
Canagliflozin	663 ± 180[a]	4.2 ± 1.5[a]	>10,000	555 ± 31[a]	3.7 ± 0.2[a]	>10,000
Teneligliptin	>10,000	>10,000	3.66 (1.29–10.45)	>10,000	>10,000	0.76 (0.33–1.77)

Values were expressed as the geometric mean of three sets of experiments with 95% confidence intervals.
[a] Values were expressed as mean ± SEM of 3–4 experiments. Data from Kuriyama et al. (12).

3. Results

3.1. SGLT and DPP4 inhibition

Table 1 shows the IC_{50} values of canagliflozin and teneligliptin against human and rat SGLT1, SGLT2, and DPP4 activities. As previously reported, canagliflozin inhibited SGLT2 potently and moderately inhibited both human and rat SGLT1 activities (19). The IC_{50} ratio for SGLT1 to SGLT2 was approximately 150–160. Teneligliptin did not inhibit SGLT1 and SGLT2 activities (IC_{50} > 10 μmol/L). Human and rat DPP4 activities were inhibited by teneligliptin but not by canagliflozin.

3.2. Effect of the combination of canagliflozin and teneligliptin on plasma levels of glucose, insulin, and aGLP-1 during OGTT in ZDF rats

The fasting plasma glucose level was 423.2 mg/dL, and the plasma glucose level was elevated to 692.0 mg/dL in the vehicle-treated ZDF rats, which showed development of marked fasting hyperglycemia and glucose intolerance. Both teneligliptin (0.3 mg/kg) and canagliflozin (3 and 10 mg/kg) significantly suppressed the elevation of plasma glucose levels after glucose loading (Fig. 1A and Table 2). Treatment with a combination of teneligliptin and canagliflozin further inhibited the excursion of plasma glucose levels compared to each of the single treatments.

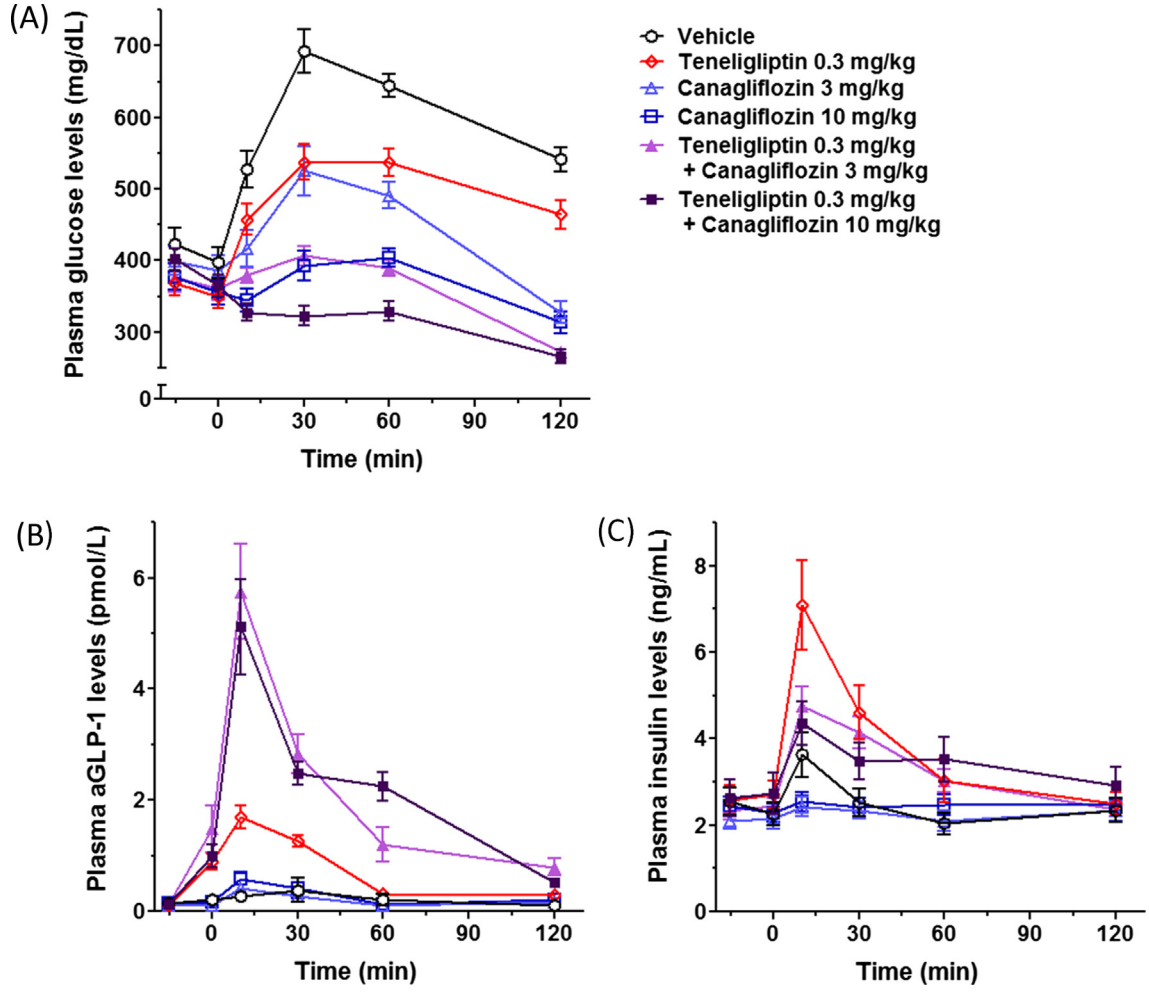

Fig. 1. Effects of treatment with a combination of canagliflozin and teneligliptin during OGTT in ZDF rats. Thirteen-week-old ZDF rats were administered canagliflozin, teneligliptin, or both canagliflozin and teneligliptin by oral gavage. After 15 min, glucose solutions at 2 g/kg were orally administered to the rats, and blood was collected from the tail vein at time points of −15, 0, 10, 30, 60, and 120 min after oral glucose administration. (A) Time course of plasma glucose levels (mg/dL). (B) Time course of plasma aGLP-1 levels (pmol/L). (C) Time course of plasma insulin levels (ng/mL). Data are presented as the mean ± S.E.M. (N = 8).

The basal plasma aGLP-1 level was 0.20 pmol/L in vehicle treated ZDF rats and increased to 0.90 pmol/L 15 min after teneligliptin treatment, confirming systemic DPP4 inhibition following teneligliptin treatment. Glucose administration transiently increased the plasma aGLP-1 level. The peak level of plasma aGLP-1 was elevated by teneligliptin alone; however, the ΔAUC_{0-2h} of aGLP-1 levels was not significantly different (Fig. 1B and Table 2). Canagliflozin did not affect the ΔAUC_{0-2h} and peak plasma aGLP-1 levels in the OGTT at either doses studied. The difference in the ΔAUC_{0-2h} and peak plasma aGLP-1 levels from basal levels was enhanced in the combined treatment group compared with in the teneligliptin treatment group (Fig. 1B and Table 2).

ZDF rats exhibited fasting hyperinsulinemia and markedly diminished glucose-induced insulin secretion compared with lean rats (20,21). The plasma insulin levels in the OGTT were increased with teneligliptin treatment (Fig. 1C and Table 2) but were not affected with canagliflozin treatment. Combined drug treatment significantly increased glucose-induced insulin secretion compared with that in canagliflozin treatment group. The peak of insulin level during the OGTT in the combination treatment group was lower than that in the teneligliptin treatment group, although the ΔAUC_{0-2h} did not differ between the two treatment groups.

Plasma DPP4 activity was inhibited by approximately 75% with teneligliptin treatment and combined canagliflozin and teneligliptin treatment in ZDF rats (Fig. 2).

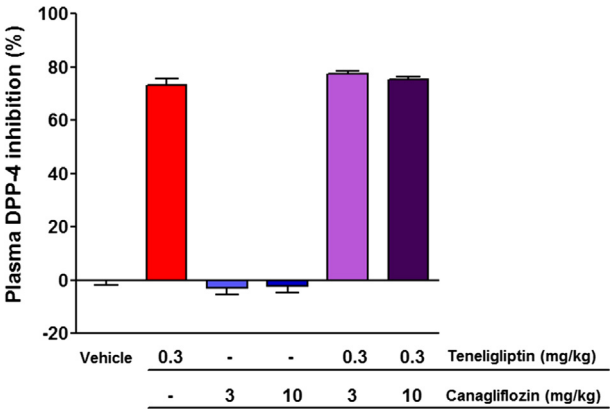

Fig. 2. The effect of combined treatment with canagliflozin and teneligliptin on plasma DPP4 activity in ZDF rats. Canagliflozin, teneligliptin, or both canagliflozin and teneligliptin were administered to 13-week-old ZDF rats by oral gavage, and blood was collected from the tail vein 15 min after the treatment. Inhibition rate was expressed as [1 − DPP4 activity after drug/DPP4 activity after vehicle] × 100 (%). Data are presented as the mean ± S.E.M. (N = 8).

3.3. Pharmacokinetic study in ZDF rats

The plasma concentrations of unchanged canagliflozin (3 and 10 mg/kg) increased and reached the peak levels within 4 h after administration, and then gradually decreased with $t_{1/2}$ values of approximately 6 h (Fig. 3A). The time course of plasma concentration and $t_{1/2}$ was almost the same with the concomitant treatment of teneligliptin (0.3 mg/kg). The peak plasma teneligliptin level was approximately 30 min after treatment and the plasma teneligliptin level gradually decreased with $t_{1/2}$ values of approximately 10 h (Fig. 3B). Time course changes and $t_{1/2}$ in plasma levels of teneligliptin were not affected by the concomitant administration of canagliflozin at 3 and 10 mg/kg.

4. Discussion

In the present study, combined treatment with canagliflozin and teneligliptin improved glucose intolerance and synergistically increased plasma aGLP-1 levels in ZDF rats, a widely used genetic model of obese T2DM (20–24). Canagliflozin did not affect DPP4 activity, and teneligliptin did not inhibit SGLTs activity *in vitro*. In addition, pharmacokinetic analysis revealed that there was no drug–drug interaction between canagliflozin and teneligliptin. Plasma DPP4 activity in ZDF rats was similarly inhibited by the treatment with teneligliptin alone and with the combination of these two drugs. Thus, the increase of glucose-induced plasma aGLP-1 levels is likely to be due to enhanced secretion of GLP-1 by canagliflozin and not potentiation of each mechanism.

SGLT1 plays a critical role in dietary glucose absorption in the gastrointestinal tract, and acts as a glucose sensor for GLP-1 secretion from L cells (25,26). In contrast, SGLT1 inhibitors increase plasma GLP-1 levels in diabetic rodents (27,28). Thus, the role of SGLT1 in secreting GLP-1 is still controversial. We have previously demonstrated the elevation of plasma aGLP-1 levels by SGLT1 inhibition during OGTT in normal rodents (29). Clinical studies in healthy subjects have also revealed delayed glucose absorption and elevated plasma GLP-1 levels in a mixed-meal test with canagliflozin, which inhibits SGLT1 activity at a concentration approximately 150–160 times higher than that for SGLT2 (16). The intraluminal concentration of canagliflozin is likely to be high enough to inhibit SGLT1 in the small intestines after oral administration, whereas the plasma concentration of

Table 2
Descriptive statistics of the peak levels and ΔAUC_{0-2h} of plasma glucose, aGLP-1, and insulin during OGTT in ZDF rats.

	Glucose		aGLP-1		Insulin	
	Peak (mg/dL)	ΔAUC_{0-2h} (h·mg/dL)	Peak (pmol/L)	ΔAUC_{0-2h} (h·pmol/L)	Peak (ng/L)	ΔAUC_{0-2h} (h·ng/L)
Vehicle	302.11 ± 13.11	24846.0 ± 1100.7	0.258 ± 0.136	3.1 ± 3.1	1.383 ± 0.293	20.4 ± 8.8
Teneligliptin (0.3 mg/kg)	199.86 ± 7.03$^{\#\#}$	18207.4 ± 554.4$^{\#\#}$	0.820 ± 0.179$^{\#}$	−24.1 ± 18.4	4.375 ± 0.775$^{\#\#}$	120.1 ± 27.2$^{\#\#}$
Canagliflozin (3 mg/kg)	146.20 ± 15.51**	6935.3 ± 1178.7**	0.460 ± 0.096	10.5 ± 2.7	0.438 ± 0.087**	11.3 ± 6.2
Canagliflozin (10 mg/kg)	53.00 ± 10.35**	1622.4 ± 847.8**	0.506 ± 0.124	12.5 ± 4.6	0.401 ± 0.080**	20.4 ± 8.1
Teneligliptin (0.3 mg/kg) + Canagliflozin (3 mg/kg)	45.81 ± 9.48$^{\$\$,\dagger\dagger}$	21.3 ± 963.2$^{\$\$,\dagger\dagger}$	4.324 ± 0.974$^{\$\$,\dagger\dagger}$	64.4 ± 34.9$^{\$}$	2.405 ± 0.324$^{\$,\dagger\dagger}$	102.3 ± 21.6††
Teneligliptin (0.3 mg/kg) + Canagliflozin (10 mg/kg)	0.91 ± 0.91$^{\$\$,\ddagger\ddagger}$	−6215.8 ± 1129.4$^{\$\$,\ddagger\ddagger}$	4.265 ± 0.735$^{\$\$,\ddagger\ddagger}$	141.1 ± 21.1$^{\$\$,\ddagger\ddagger}$	1.636 ± 0.136$^{\$\$,\ddagger\ddagger}$	84.5 ± 8.6‡‡

All values are presented as mean ± SEM (N = 8).
$^{\#}P < 0.05$.
$^{\#\#}P < 0.01$ vs. vehicle-treated group (*t*-test).
$^{**}P < 0.01$ vs. vehicle-treated group (Dunnett's test).
$^{\$}P < 0.05$.
$^{\$\$}P < 0.01$ vs. teneligliptin-treated group (Dunnett's test).
$^{\dagger\dagger}P < 0.01$ vs. canagliflozin (3 mg/kg)-treated group (*t*-test).
$^{\ddagger\ddagger}P < 0.01$ vs. canagliflozin (10 mg/kg)-treated group (*t*-test).

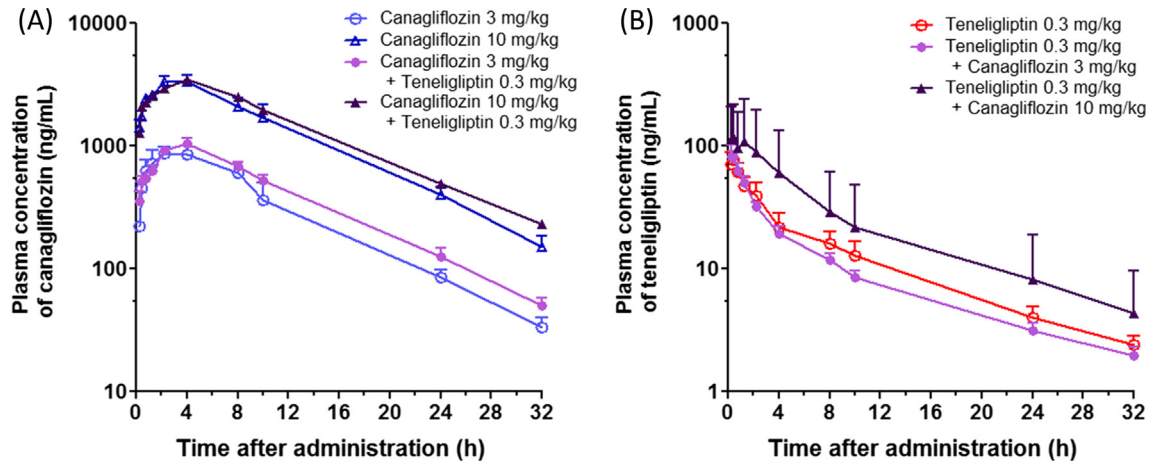

Fig. 3. Time course of plasma levels of unchanged canagliflozin and teneligliptin after single or concomitant administration in ZDF rats. Thirteen-week-old ZDF rats were administered canagliflozin, teneligliptin, or both canagliflozin and teneligliptin by oral gavage. After 15 min, glucose solution at 2 g/kg was orally administered to the rats, and blood was collected from the subclavian vein at time points of 0.25, 0.42, 0.75, 1.25, 2.25, 4, 8, 10, 24, and 32 h after test compound administration. Plasma concentrations of canagliflozin (A) and teneligliptin (B) were determined using LC-MS/MS as described in the methods. Data are presented as the mean + S.D. (N = 4).

canagliflozin was sufficient to inhibit SGLT2 but not SGLT1 systemically (19). A previous study has shown that suppressed glucose excursion is at least partly mediated by enhanced GLP-1 secretion during OGTT in SGLT1$^{-/-}$ mice (30). Similar to this SGLT1 knockout model, the combination treatment, compared with the teneligliptin alone treatment, markedly augmented plasma aGLP-1 concentrations along with a much improved glucose tolerance in ZDF rats in the present study. Thus, it is likely that intestinal SGLT1 inhibition contributes to improve glucose tolerance by canagliflozin when combined with teneligliptin. Systemic SGLT2 inhibition does not affect GLP-1 secretion because there are no changes in plasma GLP-1 levels in SGLT2-deficient mice after glucose-loading (28). Thus, although canagliflozin is an SGLT2 inhibitor, SGLT2 inhibition would not contribute to the elevation in plasma aGLP-1 levels in ZDF rats. It is likely that orally administered canagliflozin delays the absorption of glucose through inhibition of SGLT1 in the upper part of the small intestine and that GLP-1 is secreted because of increased glucose levels in the distal part of the small intestine.

While the glucose excursion in the combination group was greatly suppressed compared with that in the teneligliptin group in the OGTT, the increase of insulin levels was similar between these groups. Canagliflozin decreases blood glucose levels by enhancing excretion of glucose from the kidney independent of insulin action in hyperglycemic conditions. The similar insulin release with a small increment of glucose level shows that the glucose-induced insulin release mechanism was greatly enhanced with the addition of canagliflozin in the teneligliptin-treated hyperglycemic rats. In contrast to the insulin level, the plasma aGLP-1 level was greatly elevated in the rats with combination treatment group compared with those in the teneligliptin treatment group. Because aGLP-1 enhances glucose-induced insulin release, it is suggested that the plasma aGLP-1 elevation by combined treatment facilitated glucose-induced insulin release in the OGTT.

Glucose excursion in the OGTT was greatly reduced with combination treatment compared with the canagliflozin treatment alone. GLP-1 is an incretin hormone that enhances postprandial insulin secretion. Both aGLP-1 and insulin concentrations were greatly enhanced by the combination treatment compared with the canagliflozin treatment. It is reasonable to assume that increased plasma insulin levels further reduces glucose excursion during OGTT with combined treatment. Therefore, elevation of aGLP-1 levels in combination with teneligliptin may add therapeutic value in the control of glucose intolerance.

A number of SGLT2 inhibitors have been reported in non-clinical and clinical studies. Among them, canagliflozin is an SGLT2 inhibitor with moderate inhibitory activity for hSGLT1, which is unlike other highly selective SGLT2 inhibitors (31). Thus, it is not certain whether the synergistic effect on GLP-1 secretion with combined treatment of a DPP4 inhibitor is shared with other highly selective SGLT2 inhibitors. In addition to established treatments with SGLT2 inhibition and DPP4 inhibition in T2DM patients, treatment with a combination of canagliflozin and a DPP4 inhibitor would be associated with multiple antidiabetic effects of GLP-1 in the treatment of T2DM.

Taken together, the combined treatment of canagliflozin and teneligliptin improved glucose intolerance with similar insulin release when compared to teneligliptin treatment alone. Combined treatment also stimulated insulin release when compared to the canagliflozin treatment alone. Although canagliflozin is an SGLT2 inhibitor, the combination effect is likely to be partly mediated by its inhibitory action on intestinal SGLT1. Given the low incidence of side effects of hypoglycemia, weight gain, and abdominal symptoms with SGLT2 and DPP4 inhibitors (32,33), combined treatment with canagliflozin and teneligliptin would be a good option for treatment of T2DM.

Conflicts of interest

All the authors are employees of Mitsubishi Tanabe Pharma Corporation.

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