Full paper

Changes in glucose-induced plasma active glucagon-like peptide-1 levels by co-administration of sodium–glucose cotransporter inhibitors with dipeptidyl peptidase-4 inhibitors in rodents

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ABSTRACT

We investigated whether structurally different sodium–glucose cotransporter (SGLT) 2 inhibitors, when co-administered with dipeptidyl peptidase-4 (DPP4) inhibitors, could enhance glucagon-like peptide-1 (GLP-1) secretion during oral glucose tolerance tests (OGTTs) in rodents. Three different SGLT inhibitors—1-[(1-o-Glucopyranosyl)-4-chloro-3-[5-(6-fluoro-2-pyridyl)-2-thienyl]methyl]benzene (GTB), TA-1887, and canagliflozin—were examined to assess the effect of chemical structure. Oral treatment with GTB plus a DPP4 inhibitor enhanced glucose-induced plasma active GLP-1 (aGLP-1) elevation and suppressed glucose excursions in both normal and diabetic rodents. In DPP4-deficient rats, GTB enhanced glucose-induced aGLP-1 elevation without affecting the basal level, whereas metformin, previously reported to enhance GLP-1 secretion, increased both the basal level and glucose-induced elevation. Oral treatment with canagliflozin and TA-1887 also enhanced glucose-induced aGLP-1 elevation when co-administered with either teneligliptin or sitagliptin. These data suggest that structurally different SGLT2 inhibitors enhance plasma aGLP-1 elevation and suppress glucose excursions during OGTT when co-administered with DPP4 inhibitors, regardless of the difference in chemical structure. Combination treatment with DPP4 inhibitors and SGLT2 inhibitors having moderate SGLT1 inhibitory activity may be a promising therapeutic option for improving glycemic control in patients with type 2 diabetes mellitus. © 2016 The Authors. Production and hosting by Elsevier B.V. on behalf of Japanese Pharmacological Society. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Instead of the abbreviations: aGLP-1, active GLP-1; ANOVA, Analysis of variance; AUC, Area under the curve; CMA, (2S)-2-Cyano-1-[(trans-4-(morphpolinocarbonyl)cyclohexylamino)acetyl]pyrrolidine; DPP4, dipeptidyl peptidase-4; ELISA, Enzyme-linked immunosorbent assay; GLP-1, glucagon-like peptide-1; GTB, 1-[(1-o-Glucopyranosyl)-4-chloro-3-[5-(6-fluoro-2-pyridyl)-2-thienyl]methyl]benzene; GTT, Glucose tolerance test; SD, Sprague–Dawley; SEM, Standard error of the mean; SGLT, sodium–glucose cotransporter; ZDF, Zucker diabetic fatty.

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1. Introduction

Sodium–glucose cotransporters (SGLTs) are membrane proteins that actively transport glucose concomitantly with sodium ions across cell membranes. SGLT1 is highly expressed on the brush-border membrane in the proximal small intestine, and is responsible for dietary glucose absorption (1). By contrast, SGLT2 is mainly present in the proximal convoluted tubule of the kidney, and plays a critical role in renal glucose reabsorption (1). In patients with type 2 diabetes mellitus (T2DM), inhibition of SGLT2 increases urinary glucose excretion and reduces plasma glucose independently of insulin.

Canagliflozin is the first SGLT2 inhibitor approved in the United States for the treatment of T2DM (2) and has been reported to increase the concentrations of circulating glucagon-like peptide 1 (GLP-1) in healthy subjects (3). GLP-1 is an incretin hormone secreted from intestinal L-cells in response to dietary ingestion, and it has therapeutic potential in the treatment of T2DM because of its insulinotropic and glucagonostatic effects (4). Although the contribution of SGLTs to GLP-1 secretion is controversial, the dual

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SGLT1/2 inhibitor LX4211 increases GLP-1 concentration and suppresses glucose excursions after meal challenges in patients with T2DM (5). In addition, we have previously demonstrated that combined treatment with canagliflozin and a dipeptidyl peptidase-4 (DPP4) inhibitor enhanced plasma active GLP-1 (aGLP-1) levels during oral glucose tolerance tests (OGTTs) in normal and diabetic rats (6, 7). As glucose is a major stimulant for GLP-1 secretion, during oral glucose tolerance tests (OGTTs) in normal and diabetic effects of structurally diverse novel SGLT2 inhibitors, chemical structure. To address this hypothesis, we assessed the delay intestinal glucose absorption. We hypothesized that, when co-administered with DPP4 inhibitors, enhancement of plasma aGLP-1 elevation is the common pharmacological effect of all SGLT inhibitors, regardless of their chemical structure. To address this hypothesis, we assessed the effects of structurally diverse novel SGLT2 inhibitors, C-glucoses; 1-[(β-D-Glucopyranosyl)-4-chloro-3-[5-(6-fluoro-2-pyridyl)-2-thienylmethyl]-benzene (GTB) and canagliflozin, and an N-glucoside; TA-1887 on plasma aGLP-1 levels when each was co-administered with a DPP4 inhibitor or when administered to DPP4-deficient rats. In addition, we compared the effects of GTB and metformin, an antidiabetic drug that has been reported to enhance GLP-1 secretion in both humans and rodents (8), on plasma aGLP-1 levels with or without oral glucose-loading to determine the difference in the mode of action of these agents.

2. Materials and methods

2.1. Reagents and chemicals

GTB, (2S)-2-Cyano-1-[(morpholinocarbonyl)cyclohexylamino]acetylpyrrolidone (CMA), canagliflozin, TA-1887, teneligliptin, sitagliptin, and tofogliflozin were prepared by Mitsubishi Tanabe Pharma Corporation (Kanagawa, Japan). Metformin was purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of reagent grade or tissue culture grade.

2.2. Cell-based assays

Expression plasmids containing human SGLT1 (hSGLT1) and human SGLT2 (hSGLT2) were stably transfected into Chinese hamster ovary (CHO)–K1 cells, which were then seeded into 24-well plates at a density of 4 × 10⁵ cells/well in Ham’s F-12 medium containing 10% fetal bovine serum. To evaluate hSGLT1 and hSGLT2 transporter activities, the cells were incubated with 0.3 or 0.5 mM α-glucopyranoside (AMG; Sigma–Aldrich, St. Louis, MO, USA) in the presence of 16.7 μCi/ml [14C]AMG (PerkinElmer, Waltham, MA, USA) at 37°C for 2 h in an assay buffer containing 50-mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 20-mM Tris Base, 5-mM KCl, 1-mM MgCl₂, 1-mM CaCl₂, and 137-mM NaCl at pH 7.4. We confirmed the linear range of [14C]AMG uptake to 2.5 h, and selected 2 h to conduct the assay with a wide dynamic range. Radioactive counts in the cells were determined using a liquid scintillation counter (PerkinElmer). Protein concentrations were measured using the CoomassiePlus Protein Assay Kit (Pierce, Rockford, IL, USA).

2.3. In vivo studies

2.3.1. Animal procedures

All animal experimental procedures were approved by the Institutional Animal Care and Use Committees of Mitsubishi Tanabe Pharma Corporation and Ina Research (Nagano, Japan). Male F344/NSlc rats were purchased from Japan SLC (Shizuoka, Japan). Male F344/DuClCrj and Sprague–Dawley (SD) rats were purchased from Charles River Japan (Kanagawa, Japan). Male C57BL/KsJ-db/db mice were purchased from CLEA Japan (Tokyo, Japan). Experimental animals were housed in a temperature and humidity controlled room on a 12-h light/dark cycle, and were provided access to water and standard commercial diet, CRF-1 (Oriental Yeast Co., Tokyo, Japan) ad libitum. Test compounds for oral gavage were prepared in 0.5% carboxymethylcellulose containing 0.2% Tween 80, or 0.5% hydroxypropyl methylcellulose.

2.3.2. OGTT in F344 rats and db/db mice

Overnight-fasted 9-week-old F344 rats or 13-week-old db/db mice were orally administered test compounds and a glucose solution (2 g/kg) simultaneously. Blood was then collected from the tail vein into chilled tubes containing ethylenediaminetetraacetic acid dipotassium salt (EDTA-2K; final concentration: 2.5 mM) and a DPP4 inhibitor (Cat# DPP4-010; Millipore, Billerica, MA, USA; final concentration: 50 μM) at different sampling points. Plasma was separated by centrifugation and stored at –80°C until use for measurement.

2.3.3. Determination of metabolic parameters

Glucose concentrations in plasma and hydrolyzed samples as described above were determined using a Glucose CII-Test Wako Kit (Wako Pure Chemical Industries). Plasma insulin concentrations were measured using an enzyme-linked immunosorbent assay (ELISA) kit (Millipore). Plasma aGLP-1 concentrations were measured using ELISA kit (Millipore or Epitope Diagnostics, San Diego, CA) after solid-phase extraction with an Oasis HLB elution plate (Waters, Milford, MA, USA). Plasma aGLP-1 levels were expressed as relative values normalized to the baseline of plasma GLP-1 concentrations per figure because the absolute concentrations of GLP-1 measured using different kits considerably vary due to differences in standardization, whereas similar responses of GLP-1 were observed using either kit. The baseline values were considered statistically significant.

2.4. Statistical analysis

Data are presented per group as means ± standard error of the mean. The peak values were represented as the highest values of each plasma parameter during OGTT. The incremental change in the area under the curve (ΔAUC; defined as the AUC above the baseline value) was calculated by the trapezoidal rule (a technique for approximating AUC by dividing the AUC into several trapezoids and summing the area of these trapezoids). Statistical differences between vehicle and single treatment groups or between a DPP4 inhibitor and combination treatment groups were determined by one-way analysis of variance followed by a parametric Dunnett’s multiple comparison test or Student’s t-test, as appropriate. Statistical analyses were performed using either SAS (SAS Institute, Cary, NC, USA) or Prism software (GraphPad, San Diego, CA, USA). Probabilities less than 5% (P < 0.05) were considered statistically significant.

3. Results

3.1. SGLT and DPP4 inhibition

The chemical structures of GTB, TA-1887, and canagliflozin are shown in Supplementary Fig. 1. IC₅₀ values of GTB against hSGLT1 and hSGLT2 were 966 ± 225 nM (n = 3) and 1.5 ± 0.1 nM (n = 6), respectively. IC₅₀ values of the other SGLT inhibitors are shown in Supplementary Table 1. Among these SGLT inhibitors, GTB and TA-1887 were the potent SGLT2 inhibitor, and TA-1887 had the most
potent inhibitory activity against SGLT1. Supplementary Table 2 shows the IC50 values of CMA, teneligliptin, and sitagliptin against human DPP4 activity. CMA exhibited more potent inhibitory activity against hDPP4 than sitagliptin.

3.2. Effects of GTB plus a DPP4 inhibitor on plasma parameters in normal and diabetic rodents

To determine whether oral treatment with GTB affects GLP-1 secretion, we measured plasma parameters during OGTT in normoglycemic F344 rats (F344/NSlc) with and without the co-administration of CMA, a novel DPP4 inhibitor, which prevents aGLP-1 degradation. In rats treated with CMA (3 mg/kg), plasma glucose level slightly decreased and plasma insulin level markedly increased (Fig. 1). Plasma aGLP-1 level was elevated immediately after oral glucose-loading, but ΔAUC0–5h of plasma aGLP-1 was unchanged. By contrast, in rats treated with CMA (3 mg/kg) and GTB (10 and 30 mg/kg), plasma glucose elevation was further reduced compared with that in rats treated with CMA alone, and plasma aGLP-1 elevation was enhanced in a manner dependent on the dose of GTB.

We previously reported that combined treatment with canagliflozin and sitagliptin increased plasma GLP-1 levels in normal mice (7). To explore the effect of GTB on plasma aGLP-1 levels in diabetic animals, we examined combined treatment with GTB and CMA during OGTT in db/db mice. This strain develops significant obesity, fasting hyperglycemia, and hyperinsulinemia within 6 weeks of age and is used as a murine model of spontaneous T2DM (9). Inhibition of DPP4 by CMA (3 mg/kg) did not significantly change plasma glucose and insulin levels after glucose-loading, but additional GTB (10 mg/kg) treatment further reduced plasma glucose excursions (Fig. 2). Treatment with either CMA (3 mg/kg) or GTB (10 mg/kg) caused elevated plasma aGLP-1 levels, and treatment with both CMA (3 mg/kg) and GTB (10 mg/kg) augmented that plasma aGLP-1 elevation extensively. These data demonstrate that combined treatment with GTB and CMA increases plasma aGLP-1 levels in both normal and diabetic rodents.

3.3. Effect of GTB on plasma aGLP-1 levels with or without glucose-loading in DPP4-deficient rats

Subsequently, we examined the effects of GTB on plasma aGLP-1 levels with or without glucose-loading to analyze the involvement of SGLT1. To eliminate potential off-targeting effects of CMA, we used DPP4-deficient F344 rats (F344/DuCrlCrlj), which are a sub-strain of F344 rats lacking endogenous DPP4 enzymatic activity (10). In glucose-loaded DPP4-deficient rats, metformin (300 mg/kg) slightly reduced plasma glucose excursion and modestly elevated plasma aGLP-1 levels, consistent with data in previous reports (11) (Fig. 3). Similar to combined treatment with CMA, the use of GTB alone (at 10 and 30 mg/kg) suppressed plasma glucose elevations and robustly increased plasma aGLP-1 levels in a dose-dependent manner. However, in DPP4-deficient rats without glucose-loading, GTB did not change ΔAUC0–5h of plasma aGLP-1 at the same doses (Fig. 4). By contrast, metformin (300 mg/kg) significantly increased plasma aGLP-1 levels without glucose-loading in the DPP4-deficient rats. These data suggest that
glucose-loading is required for the elevation of plasma aGLP-1 induced by GTB, but not for that by metformin.

3.4. Effect of canagliflozin and TA-1887 in combination with DPP4 inhibitors on plasma aGLP-1 levels

To compare the combined effects of structurally diverse SGLT inhibitors with DPP4 inhibitors on plasma aGLP-1 levels, we examined canagliflozin and TA-1887 in combination with teneligliptin and sitagliptin in DPP4-positive F344 rats (F344/NSlc) during OGTT. Although inhibition of DPP4 by teneligliptin (5 mg/kg) or sitagliptin (10 mg/kg) alone did not elevate plasma aGLP-1 levels at 2 h after glucose-loading, additional treatment with canagliflozin (30 mg/kg) or TA-1887 (30 mg/kg) in combination with these agents significantly elevated plasma aGLP-1 levels (Fig. 5). These data suggest that dual treatment with structurally different SGLT2 inhibitors plus DPP4 inhibitors results in plasma aGLP-1 elevation, regardless of the difference in chemical structure. In addition, we assessed the effect of 3 mg/kg canagliflozin on plasma aGLP-1 levels and intestinal glucose contents. In glucose-loaded DPP4-deficient rats, 3 mg/kg canagliflozin prolonged plasma aGLP-1 elevation and significantly increased plasma aGLP-1 AUC0–4h (Supplementary Fig. 2). Compared with 1 mg/kg tofogliflozin, a highly selective SGLT2 inhibitor, 3 mg/kg canagliflozin significantly reduced plasma glucose excursions and increased the glucose content in the small intestine 1 h after sucrose-loading (Supplementary Fig. 3). These findings suggest that 3 mg/kg canagliflozin increases plasma aGLP-1 levels in glucose-loaded DPP4-deficient rats as well as GTB, and this effect may be caused through SGLT1 inhibition. The Cmax of the clinical dose of canagliflozin (100 mg/kg/day) and tofogliflozin (20 mg/day) in human plasma was similar to that in the plasma of SD rats after receiving 3 mg/kg canagliflozin and 1 mg/kg tofogliflozin, respectively. Supplementary Table 3 shows the Cmax values of canagliflozin (3 mg/kg) and tofogliflozin (1 mg/kg) in the plasma of SD rats. The Cmax values of unbound canagliflozin and tofogliflozin were estimated as 7.1–12.8 ng/mL (16.0–18.1 nM) and 58.1–61.7 ng/mL (150.3–170.0 nM), respectively, based on the plasma protein binding ratios in rats of 98.2%–99.0% for canagliflozin and 83%–84% for tofogliflozin (12, 13). Thus, plasma exposure to these compounds was higher than the respective hSGLT2 IC50 values.

4. Discussion

SGLT2 inhibitors reduce postprandial glucose levels in patients with T2DM by increasing excretion of glucose in urine. Although the plasma concentrations of most SGLT2 inhibitors are insufficiently high to inhibit SGLT1, some do show weak or moderate SGLT1 inhibitory activity in vitro. We hypothesized that oral administration of these SGLT2 inhibitors would inhibit small intestinal SGLT1 to increase luminal glucose concentrations, which would in turn stimulate the release of GLP-1 from intestinal L-cells. In the present study, we demonstrated that oral administration of structurally different SGLT2 inhibitors, when combined with DPP4 inhibitors, effectively elevated plasma aGLP-1 levels in both normal and diabetic rodents. Unlike with metformin, this aGLP-1 elevation was also dependent on oral glucose-loading, suggesting a possible involvement of intestinal SGLT1 inhibition in the enhanced GLP-1 secretion.
We have previously shown that phloridzin and canagliflozin, when combined with sitagliptin, increase plasma aGLP-1 levels after glucose-loading in SD rats (7). In this study, GTB and TA-1887 in combination with a DPP4 inhibitor similarly enhanced glucose-induced aGLP-1 elevation in F344 rats. The glucoside structures of these SGLT2 inhibitors are different: phloridzin is an \( \text{O} \)-glucoside, canagliflozin and GTB are \( \text{C} \)-glucosides, and TA-1887 is an \( \text{N} \)-glucoside. Canagliflozin has moderate hSGLT2/hSGLT1 selectivity (IC\(_{50}\) ratio = 158), favorable pharmacokinetic profile, and glucose-lowering effect. Compared with canagliflozin, GTB has higher selectivity (IC\(_{50}\) ratio = 644) and TA-1887 has more potent inhibitory activity against hSGLT1 (TA-1887 = 230 nM; canagliflozin = 663 nM). In addition, TA-1887 has similar pharmacokinetic properties, such as \( C_{\text{max}} \), half-life, and bioavailability, similar to those of canagliflozin, and increased urinary glucose excretion (UGE) in normal rats and exhibited antihyperglycemic effects in diabetic mice (14, 15). Although the pharmacokinetic parameters of GTB have not been measured to date, it also promoted UGE in normal rodents in a dose-dependent manner (unpublished data). This study demonstrated that structural variation in the glucosides did not affect the elevating effect of GLP-1. In addition, TA-1887 has similar pharmacokinetic properties, such as \( C_{\text{max}} \), half-life, and bioavailability, similar to those of canagliflozin, and increased urinary glucose excretion (UGE) in normal rats and exhibited antihyperglycemic effects in diabetic mice (14, 15). Although the pharmacokinetic parameters of GTB have not been measured to date, it also promoted UGE in normal rodents in a dose-dependent manner (unpublished data). This study demonstrated that structural variation in the glucosides did not affect the elevating effect of GLP-1. In addition, GTB did not affect plasma aGLP-1 levels in control conditions, but it did increase them under DPP4 inhibition and in DPP4-deficient animals. Equally, canagliflozin and TA-1887 also resulted in postprandial plasma GLP-1 elevation when combined with any of the DPP4 inhibitors, indicating that structurally different SGLT2 inhibitors enhance plasma GLP-1 levels by promoting GLP-1 secretion, provided there is sufficient DPP4 inhibition. Thus, we conclude that the increase of plasma aGLP-1 levels is the common pharmacological effect of combination treatment with SGLT/DPP4 inhibitors.

In the present study, combination treatment with GTB and CMA significantly enhanced plasma aGLP-1 elevations in both normoglycemic rats and diabetic db/db mice relative to that with CMA treatment alone. db/db mice develop hyperphagic obesity and nonketotic diabetes and are used as a murine model of spontaneous T2DM (9). This result is consistent with previous data obtained in ZDF rats treated with canagliflozin and sitagliptin (7). These findings suggested that combined treatment with SGLT/DPP4 inhibitors induces plasma aGLP-1 elevation in both normal and diabetic rodents.

Metformin, an antidiabetic drug that reduces glycated hemoglobin (HbA1c) by suppressing hepatic gluconeogenesis and improving glucose uptake, has been shown to increase plasma aGLP-1 levels in clinical and nonclinical studies (8). However, the mechanism underlying metformin-induced GLP-1 elevation is controversial. In the present study, we showed that metformin increased plasma aGLP-1 levels independent of glucose-loading in DPP4-deficient rats, suggesting that metformin directly stimulated GLP-1 secretion in L-cells via DPP4-independent mechanism. By contrast, GTB did not affect basal GLP-1 levels, but it did enhance glucose-induced GLP-1 elevation. This indicates that the metformin and GTB caused GLP-1 to increase through different mechanisms. Canagliflozin, at a dose of 3 mg/kg, and while under DPP4 inhibition, prolonged the elevation of plasma aGLP-1 during OGTT and significantly increased glucose content within the small intestine after sucrose-loading. These data may support the idea that SGLT2 inhibitors, GTB, and canagliflozin, could increase plasma GLP-1...
Fig. 4. Effects of GTB and metformin on plasma glucose, insulin, and aGLP-1 levels without glucose-loading in DPP4-deficient F344 rats. (A) Time course of plasma glucose levels. (B) Time course of plasma insulin levels. (C) Time course (upper panel) and ΔAUC<sub>0-5h</sub> (lower panel) of relative plasma aGLP-1 levels. GTB (10 and 30 mg/kg), metformin (300 mg/kg), and water instead of glucose were administered simultaneously to 9-week-old F344/DuCrI Crlj rats at time 0 by oral gavage. Data are presented as the mean ± SEM. (N = 6).
**P < 0.01 vs vehicle group by t-test; #P < 0.05 vs vehicle group by one-way ANOVA with Dunnett’s post hoc test.

Fig. 5. Effects of canagliflozin and TA-1887 combined with a DPP4 inhibitor on plasma aGLP-1 levels during OGTT in normal F344 rats. (A) Relative plasma aGLP-1 levels 2 h after glucose-loading when administered with canagliflozin and TA-1887 combined with teneligliptin. (B) Relative plasma aGLP-1 levels 2 h after glucose-loading when administered with canagliflozin and TA-1887 combined with sitagliptin. Canagliflozin (30 mg/kg), TA-1887 (30 mg/kg), teneligliptin (5 mg/kg), sitagliptin (10 mg/kg), and glucose solution (2 g/kg) were simultaneously administered to 9-week-old F344/NSlc rats at time 0 by oral gavage. Data are presented as the mean ± SEM. (N = 5–6). *P < 0.05, **P < 0.01 vs teneligliptin group; ##P < 0.01 vs sitagliptin group by one-way ANOVA followed by t-test.
levels through intestinal SGLT1 inhibition. Although GTB, canagliflozin, and TA-1887 are potent SGLT2 inhibitors, they also share mild or moderate inhibitory effects on hSGLT1. They are likely to show similar inhibitory activity against rodent SGLT1 because other SGLT2 inhibitors structurally close to GTB have shown no appreciable inter-species differences between humans and rodents (16, 17). Moreover, after a glucose-containing meal challenge, an increase in GLP-1 concentration was observed in SGLT1-deficient mice, but not in SGLT2-deficient mice (18). These data suggested that inhibition of SGLT2 does not contribute to plasma aGLP-1 elevation. These data support the idea that the GLP-1-elevating effect of SGLT2 inhibitors is related to their SGLT1 inhibition.

As an index for glycemic control to prevent macro- and microvascular complications, the roles of 2 h postprandial glucose, HbA1c, and fasting glucose levels are well documented. Elevation of the postprandial glucose level is a risk factor for the onset or progression of macroangiopathy. The present study demonstrated that combined treatment with structurally different SGLT2 inhibitors and DPP4 inhibitors act to reduce postprandial glucose excursions compared with the use of DPP4 inhibitors alone. SGLT2 and DPP4 inhibitors have different mechanisms of action for reducing plasma glucose levels and, because these actions are glucose-dependent, they are associated with a low risk of inducing hypoglycemia. Furthermore, moderate SGLT1 inhibition would partly contribute to the reduction of glucose excursions by delaying intestinal glucose absorption. In patients with T2DM, blood glucose levels rise gradually with time, making long-term maintenance of glycemic control difficult with a single oral antidiabetic agent; however, combination therapy with several agents can increase the risk of hypoglycemia. Combination therapy with a DPP4 inhibitor and an SGLT2 inhibitor might therefore be an effective and relatively safe therapeutic alternative for better glycemic control in patients with T2DM.

Chronic hyperglycemia impairs beta-cell function through glucotoxic mechanisms and the induction of structural disorganization in pancreatic islets. We showed that repeated administration of canagliflozin increased insulin levels in beta-cells and minimized the deterioration in islet structure in ZDF rats by ameliorating the progression of hyperglycemia (16). GLP-1 exhibits similar antidiabetic effects, including the protection of pancreatic function, so elevated levels after co-administration of canagliflozin with a DPP4 inhibitor might potentiate these protective effects. To explore whether plasma aGLP-1 elevation prevents pancreatic dysfunction in diabetic animals, the effects of long-term treatment with SGLT2/DPP4 inhibitors should be examined.

In summary, we showed that enhancement of glucose-induced plasma aGLP-1 elevation is the common pharmacological effect of structurally different SGLT2 inhibitors when co-administered with DPP4 inhibitors, regardless of their chemical structure. Based on this and the results of our previous studies, we propose the following hypothetical mechanism. First, SGLT2 inhibitors with moderate SGLT1 inhibitory activity transiently delay glucose absorption in the small intestine, and the non-absorbed glucose then stimulates GLP-1 secretion from L-cells in the lower gastrointestinal tract. Subsequently, DPP4 inhibitors likely prevent aGLP-1 degradation and prolong its half-life in the systemic circulation. Finally, mediated by SGLT1 inhibition in the small intestine, aGLP-1 enhances glucose-induced insulin secretion, protects against pancreatic beta-cell failure, and exerts multifunctional antidiabetic effects. It appears that canagliflozin exerts these effects at a dose that gives plasma drug concentrations equivalent to those in clinical practice. Thus, combination therapy with a DPP4 inhibitor and an SGLT2 inhibitor could provide better glycemic control for patients with T2DM.