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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.

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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.

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