Journal of Pharmacological Sciences xxx (2016) 1–7

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

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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ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form
19 September 2016
Accepted 4 October 2016
Available online xxx

Keywords:
SGLT2 inhibitor
DPP4 inhibitor
Canagliflozin
Combination treatment
Glucagon-like peptide-1

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-(β-D-Glucopyranosyl)-4-chloro-3-[5-(6-fluoro-2-pyridyl)-2-thienylmethyl]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.

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

Abbreviations: aGLP-1, active GLP-1; ANOVA, Analysis of variance; AUC, Area under the curve; CMA, (2*S*)-2-Cyano-1-[trans-4-(morpholinocarbonyl)cyclohexylamino]acetylpyrrolidine; DPP4, dipeptidyl peptidase-4; ELISA, Enzyme-linked immunosorbent assay; GLP-1, glucagon-like peptide-1; GTB, 1-(β-D-Glucopyranosyl)-4-chloro-3-[5-(6-fluoro-2-pyridyl)-2-thienylmethyl]benzene; OGTT, Oral glucose tolerance test; SD, Sprague–Dawley; SEM, Standard error of the mean; SGLT, sodium–glucose cotransporter; ZDF, Zucker diabetic fatty.

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Peer review under responsibility of Japanese Pharmacological Society.

http://dx.doi.org/10.1016/j.jphs.2016.10.006

Please cite this article in press as: Oguma T, et al., 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, Journal of Pharmacological Sciences (2016), http://dx.doi.org/10.1016/j.jphs.2016.10.006

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, enhanced GLP-1 secretion is likely to result from elevated glucose contents in the gastrointestinal tract after treatment with SGLT inhibitors. However, it is unclear whether other SGLT inhibitors that have different chemical structures also enhance GLP-1 secretion and 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*-glucosides; 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, (2*S*)-2-Cyano-1-[trans-4-(morpholinocarbonyl)cyclohexylamino]acetylpyrrolidine (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 4×10^5 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-methyl-D-glucopyranoside (AMG; Sigma–Aldrich, St. Louis, MO, USA) in the presence of 16.7 μCi/mL [^{14}C]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 $MgCl_2$, 1-mM $CaCl_2$, and 137-mM NaCl at pH 7.4. We confirmed the linear range of [^{14}C]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/DuCrlCrlj and Sprague–Dawley (SD) rats were purchased from Charles River Japan (Kanagawa, Japan). Male C57BL/KsJ-db/db Jcl (*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 μElution 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 taken as the plasma aGLP-1 concentrations at 0 h in the vehicle group.

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. IC_{50} values of GTB against hSGLT1 and hSGLT2 were 966 ± 223 nM ($n = 3$) and 1.5 ± 0.1 nM ($n = 6$), respectively. IC_{50} 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

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