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Potentiation of insulin secretion and improvement of glucose intolerance by combining a novel G protein-coupled receptor 40 agonist DS-1558 with glucagon-like peptide-1 receptor agonists

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ABSTRACT

G protein-coupled receptor 40 (GPR40) is a Gq-coupled receptor for free fatty acids predominantly expressed in pancreatic β-cells. In recent years, GPR40 agonists have been investigated for use as novel therapeutic agents in the treatment of type 2 diabetes. We discovered a novel small molecule GPR40 agonist, (3*S*)-3-ethoxy-3-(4-{[(1*R*)-4-(trifluoromethyl)-2,3-dihydro-1*H*-inden-1-yl]oxy}phenyl)propanoic acid (DS-1558). The GPR40-mediated effects of DS-1558 on glucose-stimulated insulin secretion were evaluated in isolated islets from GPR40 knock-out and wild-type (littermate) mice. The GPR40-mediated effects on glucose tolerance and insulin secretion were also confirmed by an oral glucose tolerance test in these mice. Furthermore, oral administration of DS-1558 (0.03, 0.1 and 0.3 mg/kg) significantly and dose-dependently improved hyperglycemia and increased insulin secretion during the oral glucose tolerance test in Zucker fatty rats, the model of insulin resistance and glucose intolerance. Next, we examined the combination effects of DS-1558 with glucagon like peptide-1 (GLP-1). DS-1558 not only increased the glucose-stimulated insulin secretion by GLP-1 but also potentiated the maximum insulinogenic effects of GLP-1 after an intravenous glucose injection in normal Sprague Dawley rats. Furthermore, the glucose lowering effects of exendin-4, a GLP-1 receptor agonist, were markedly potentiated by the DS-1558 (3 mg/kg) add-on in diabetic *db/db* mice during an intraperitoneal glucose tolerance test. In conclusion, our results indicate that add-on GPR40 agonists to GLP-1 related agents might be a potential treatment compared to single administration of these compounds. Therefore the combinations of these agents are a novel therapeutic option for type 2 diabetes.

1. Introduction

G protein-coupled receptor 40 (GPR40) agonist has been investigated for use as a novel therapeutic agent in the treatment of type 2 diabetes with effects on glucose-stimulated insulin secretion (GSIS) in recent years. GPR40 is a Gq-coupled G protein-coupled receptor (GPCR) for saturated and unsaturated medium and long chain free fatty acids predominantly expressed in pancreatic β-cells (Itoh et al., 2003; Tomita et al., 2006). Activation of the Gq-coupled receptor results in the accumulation of second messengers, such as inositol trisphosphate (IP_3) and diacylglycerol, followed by a Ca^{2+} influx from endoplasmic reticulum (ER) and the stimulation of GSIS. Several small molecule GPR40 agonists have been reported, recently (Briscoe et al., 2006; Christiansen et al., 2012; Doshi et al., 2009; Lin et al., 2011; Luo et al., 2012; Negoro et al., 2012;; Schmidt et al., 2011; Tan et al., 2008). It is also reported that [(3S)-6-({2′,

Abbreviations: ATP, adenosine triphosphate; AUC, area under the curve; BSA, bovine serum albumin; cAMP, cyclic adenosine monophosphate; CHO, Chinese hamster ovary; DHA, docosahexaenoic acid; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; GLP-1, glucagon like peptide-1; GPCR, G protein-coupled receptor; GPR40, G protein-coupled receptor 40; GSIS, glucose-stimulated insulin secretion; IP_3, inositol trisphosphate; ipGTT, intraperitoneal glucose tolerance test; KO, knock-out; oGTT, oral glucose tolerance test; PKA, protein kinase A; PKC, protein kinase C; SD, Sprague Dawley; S.E.M., standard error

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6′-dimethyl-4′-[3-(methylsulfonyl)propoxy]biphenyl-3-yl}methoxy)-2,3-dihydro-1-benzofuran-3-yl]acetic acid hemi-hydrate (TAK-875), one of the GPR40 agonists, improved glycemic control in type 2 diabetes patients with minimum risk of hypoglycemia (Araki et al., 2012; Burant et al., 2012; Kaku et al., 2013; Negoro et al., 2012).

Glucagon like peptide-1 (GLP-1) is one of the insulinogenic hormones secreted from intestinal L-cells. GLP-1 increases the intracellular cyclic adenosine monophosphate (cAMP) level and enhances insulin secretion in a glucose-dependent manner via binding to its Gs-coupled GPCR, which is highly expressed in pancreatic β-cells (Holst, 2004; Holz et al., 1993). Hence, GLP-1 related agents including GLP-1 analogs, exendin-4, and dipeptidyl peptidase-4 inhibitors, which prevent the proteolytic degradation of GLP-1 (Drucker and Nauck, 2006), attracted attention as a new generation of insulin secretagogues without hypoglycemia risk (Derosa and Maffioli, 2012).

Combination therapy with anti-diabetic drugs is often used for treatment of type 2 diabetes (Bailey and Day, 2009). According to a United Kingdom prospective diabetes study, 50% of patients initially treated with a single agent required an additional second drug after three years. By their 9th year, 75% of patients needed multiple therapies (Turner et al., 1999). Among the combination therapies, it is reported that the combination of insulin secretagogues such as liraglutide, which is one of the GLP-1 analogs, plus sulfonylureas, such as glibenclamide, gliclazide and glimepiride, improved glycemic control (Kaku et al., 2010). On the other hand, there is no information about the combination effects of GPR40 and GLP-1 receptor agonists. Accordingly, research on the combination of these agents is important in order to know whether these agents are cooperative or not and whether they suggest a novel clinical option.

In the present report, we characterized a novel small molecule GPR40 agonist, (3*S*)-3-ethoxy-3-(4-{[(1*R*)-4-(trifluoromethyl)-2,3-dihydro-1*H*-inden-1-yl]oxy}phenyl)propanoic acid (DS-1558), by using human GPR40-expressing Chinese hamster ovary (CHO) cells and GPR40 knock-out (GPR40 KO) mice. The dose-dependent effects on glucose tolerance were evaluated by an oral glucose intolerance test (oGTT) in Zucker fatty rats. We also examined the combination effects of DS-1558 and GLP-1 receptor agonists on insulin secretion in normal Sprague Dawley (SD) rats. Furthermore, to explore the possibility of a novel therapeutic option for type 2 diabetes, the combination effects on glycemic control in diabetic *db*/*db* mice were examined by an intraperitoneal glucose tolerance test (ipGTT).

2. Materials and methods

2.1. Animals

Seven to nine-week-old male SD rats, 9-week-old male Zucker fatty rats (Crlj:ZUC-*Lepr*^{fa}) and 5-week-old male *db*/*db* mice (BSK. Cg-*Dock7*^{m}+/+*Lepr*^{db}/J) were purchased from Charles River Japan (Tokyo, Japan). GPR40 KO mice were purchased from DeltaGen (Palo Alto, CA, USA) and were custom-generated and genotyped by Charles River Japan. Six-week-old male GPR40 KO and littermate wild-type C57BL/6N mice were obtained. All animals were provided standard chow (FR-2, Funabashi Farm Co., Ltd.) and tap water *ad libitum* with controlled temperature (23 ± 2 °C), humidity (55 ± 10%) and a 12-hour light/dark cycle (lights on from 7:00 AM to 7:00 PM). All experimental procedures were performed in accordance with the in-house guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

2.2. Reagents

DS-1558 and TAK-875 were synthesized at the R&D Division in Daiichi Sankyo Co., Ltd. The chemical structure of DS-1558 is indicated in Fig. 1. Human GLP-1 (7-36)amide was purchased from Peptide Institute, Inc. (Osaka, Japan). Exendin-4 was purchased from AnaSpec, Inc. (Fremont, CA, USA).

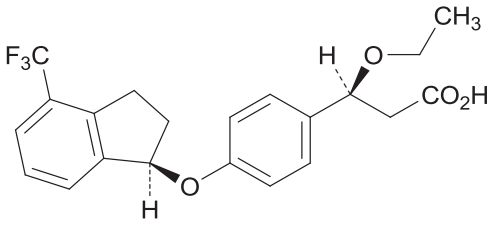

Fig. 1. Chemical structure of DS-1558 [(3*S*)-3-Ethoxy-3-(4-{[(1*R*)-4-(trifluoromethyl)-2,3-dihydro-1*H*-inden-1-yl]oxy}phenyl)propanoic acid].

2.3. Aequorin assay

Human GPR40-expressing CHO cells were purchased from PerkinElmer Life & Analytical Science (Waltham, MA, USA). Cells were diluted to the concentration of 1.5×10^6 cells/ml by D-MEM/Ham's F12/0.1% bovine serum albumin (BSA) containing HEPES and then coelenterazine h was added to the final concentration of 5 μM. Cells were allowed to incubate in coelenterazine h containing medium for 4 h and then were diluted to 1/3 with the medium prior to assay. DS-1558 and docosahexaenoic acid (DHA) (Cayman Chemical, Ann Arbor, MI, USA), which is one of the natural ligands of GPR40 (Itoh et al., 2003) and was used for a positive control. These compounds were dissolved in dimethyl sulfoxide (DMSO), the final concentration was 0.1%, and diluted to the desired concentration with assay buffer. The medium containing each compound was added to a 96-well plate then cell suspension was added to the well. The luminescence intensity was measured for 30 s. Assays were carried out with triplicate samples. Mean luminescence intensity of DHA at the maximum concentration was set as 100% and the response ratio of each intensity to that of DHA at the maximum concentration was calculated. The EC_{50} values were estimated by nonlinear regression using the logistic model. Analyses were performed by Microsoft Excel 2003 (Microsoft, Redmond, WA, USA) and EXSAS ver. 7.10 (CAC, Tokyo, Japan).

2.4. Glucose-stimulated insulin secretion in isolated islets.

Islets were isolated from GPR40 KO mice and wild-type mice by collagenase digestion and purified by Ficoll gradient separation. The islets were cultured overnight in RPMI1640 medium/10% fetal bovine serum with 11 mM glucose and then the islets (5 islets/well and 5 wells for each treatment) were incubated for 1 h at 37 °C with Krebs–Ringer bicarbonate buffer/0.2% BSA containing 2.8 mM glucose in a 24-well plate prior to assay. The buffer was then changed to Krebs–Ringer bicarbonate buffer/0.2% BSA containing glucose (2.8 and 16.7 mM) with or without DS-1558 (10 μM) or GLP-1 (10 nM) and incubated for 1 h at 37 °C. After incubation, supernatants were obtained from each well. Then, the insulin concentration was measured by enzyme-linked immunosorbent assay (ELISA) (Morinaga Institute of Biological Science, Inc., Tokyo, Japan). To evaluate the combination effect of DS-1558 and GLP-1, islets were also isolated from SD rats and GSIS was evaluated by a similar method to the above mentioned. Islets were treated with or without DS-1558 (0.1 μM), GLP-1 (1 nM) or combination of them.

2.5. Oral glucose tolerance test.

GPR40 KO and wild-type mice (n=5–6) were fasted overnight and then orally administered vehicle (0.5% methylcellulose) or DS-1558 (10 mg/kg) 30 min before the oral glucose (2 g/kg) load. Blood samples were obtained via the tail vein. Plasma glucose and