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Novel GPR119 agonist AS1535907 contributes to first-phase insulin secretion in rat perfused pancreas and diabetic db/db mice

Shigeru Yoshida *, Takahide Ohishi, Tetsuo Matsui, Hirotsugu Tanaka, Hiroyuki Oshima, Yasuhiro Yonetoku, Masayuki Shibasaki

Drug Discovery Research, Astellas Pharma Inc., Tsukuba, Ibaraki, Japan

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ABSTRACT

G protein-coupled receptor (GPR) 119 is highly expressed in pancreatic β-cells and enhances the effect of glucose-stimulated insulin secretion (GSIS) on activation. The development of an oral GPR119 agonist that specifically targets the first phase of GSIS represents a promising strategy for the treatment of type 2 diabetes. In the present study, we evaluated the therapeutic potential of a novel small molecule GPR119 agonist, AS1535907, which was modified from the previously identified 2,4,6-tri-substituted pyrimidine core agonist AS1269574. AS1535907 displayed an EC_{50} value of 4.8 μM in HEK293 cells stably expressing human GPR119 and stimulated insulin secretion in rat islets only under high-glucose (16.8 mM) conditions. In isolated perfused pancreata from normal rats, AS1535907 enhanced the first phase of insulin secretion at 16.8 mM glucose, but had no effect at 2.8 mM glucose. In contrast, the sulfonylurea glibenclamide predominantly induced insulin release in the second phase at 16.8 mM glucose and also markedly stimulated insulin secretion at 2.8 mM glucose. In *in vivo* studies, a single 10 μM administration of AS1535907 to diabetic db/db mice reduced blood glucose levels due to the rapid secretion of insulin secretion following oral glucose loading. These results demonstrate that GPR119 agonist AS1535907 has the ability to stimulate the first phase of GSIS, which is important for preventing the development of postprandial hypoglycemia. In conclusion, the GPR119 agonist AS1535907 induces a more rapid and physiological pattern of insulin release than glibenclamide, and represents a novel strategy for the treatment of type 2 diabetes.

1. Introduction

Depletion of glucose-stimulated insulin secretion (GSIS), particularly loss of the first phase, is a characteristic feature in the pathology of type 2 diabetes mellitus (T2DM) and results in postprandial hyperglycemia [1,2]. In the clinical management of T2DM patients, sulfonylurea derivatives (SUs) are the most widely used hypoglycemic agents [3]; however, SUs can cause severe and prolonged hypoglycemia because of their long duration and glucose-independent mode of action [4]. Recent strategies for promoting normoglycemia have focused on enhancing GSIS through the targeting of G protein-coupled receptors (GPCRs), such as the glucagon-like peptide 1 (GLP-1) receptor. Although GLP-1 analogs, such as exendin-4, have been shown to effectively stimulate GSIS and preserve pancreatic β-cells, these analogs can cause gastrointestinal side effects, pancreatitis, and require parenteral administration [5–8]. The limitations of SUs and GLP-1 analags underly the need for the development of oral insulin secretagogues that are capable of inducing normoglycemia and preserving the first phase of GSIS in T2DM patients.

GPR119 represents a promising anti-diabetic therapeutic target as it is predominantly expressed in pancreatic β-cells and intestinal L-cells and promotes GSIS and indirectly increases GLP-1 level. Although the downstream pathways are unclear, activation of GPR119 by endogenous ligands, such as lysophosphatidylcholine (LPC) and oleoylethanol amide (OEA), or small-molecule agonists, leads to the accumulation of intracellular cAMP and subsequent insulin and GLP-1 release [9–14]. We previously identified a novel structural class of small-molecule GPR119 agonists, consisting of 2,4,6-tri-substituted pyrimidine cores, which were orally active and displayed higher activity than existing T2DM therapeutics, such as extendin-4 [5,8]. The first identified compound, AS1269574, was capable of inducing GSIS *in vitro* and *in vivo*, and improved glucose tolerance in normal mice [15]. In an effort to identify an agonist in the identical structural class with a lower effective dosage and the ability to specifically induce the first phase of GSIS, compound

Abbreviations: GPR119, G protein-coupled receptor 119; GSIS, glucose-stimulated insulin secretion; T2DM, type 2 diabetes mellitus; SU, sulfonylurea; GPCR, G protein-coupled receptor; GLP-1, glucagon-like peptide-1; HEK, human embryonic kidney; LPC, lysophosphatidylcholine; OEA, oleoylethanol amide.

* Corresponding author. Address: Drug Discovery Research, Astellas Pharma Inc., 21 Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan. Fax: +81 29 852 2955.
E-mail address: shigeru.yoshida@jp.astellas.com (S. Yoshida).

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AS1535907 (2-(4-bromophenyl)-6-methyl-*N*-[2-(1-oxidopyridin-3-yl) ethyl] pyrimidin-4-amine), which was modified from AS1269574, was selected as a promising candidate.

Here, we report the *in vitro* and *in vivo* characterization of the novel small-molecule GPR119 agonist AS1535907 in rat islets, isolated pancreata, and diabetic db/db mice. We specifically compared the effects of AS1535907 with the anti-diabetic SU glibenclamide on glucose tolerance, glucose levels, and insulin secretion in these animal systems.

2. Materials and methods

2.1. Animals and materials

Male diabetic db/db mice and male Sprague–Dawley (SD) rats were obtained from CLEA Japan, Inc. (Kanagawa, Japan). All surgical procedures performed were approved by the Animal Ethical Committee of Astellas Pharma Inc. The compounds AS1535907 (2-(4-bromophenyl)-6-methyl-N-[2-(1-oxidopyridin-3-yl) ethyl] pyrimidin-4-amine) was synthesized in-house at Astellas Pharma Inc. (Ibaraki, Japan), and was modified from the 2,4,6-tri-substituted pyrimidine core agonist AS1269574 [15] Glibenclamide was obtained from Sigma (St. Louis, MO, USA). Prior to use in assays, AS1535907 and glibenclamide were dissolved in dimethyl sulfoxide (DMSO) to yield a stock solution of 10 mM.

2.2. Plasmid construction and generation of HEK293-hGPR119 cells

The human GPR119 ORF (GenBank Accession No. BD169091) was amplified by PCR from human pancreas cDNA (Clontech, Tokyo, Japan) with the sense, 5′-AAAATCTAGAATGGAATCATCTTTCTC ATTTG-3′, and antisense, 5′-CGGCTCTAGATTAGCCATCAAACTCTG AGCTGG-3′, primers (*Xba*I restriction sites are underlined). The amplified product was subcloned into the mammalian expression vector plasmid pcDNA3.1 (Invitrogen, Tokyo, Japan). The resulting pcDNA-GPR119 plasmid and pCRE (cAMP responsive element)-Luc plasmid (Clontech) were cotransfected into human embryonic kidney (HEK) 293 cells using standard procedures. Candidate clones stably expressing human GPR119 and pCRE-Luc were selected by resistance to 400 μg/ml geneticin, and were confirmed by the activity of LPC, an endogenous ligand of GPR119.

2.3. Insulin secretion by islet cells

Islets of Langerhans were isolated from male SD rats by collagenase digestion as described previously [16] and subsequently handpicked under a stereomicroscope. The isolated islets were then cultured overnight at 37 °C in RPMI-1640 medium (Invitrogen) supplemented with 11.1 mM glucose and 10% fetal bovine serum. After 2 days of culture, the ilets were washed with HEPES-balanced Krebs–Ringer bicarbonate buffer (KRBB) containing 2.8 mM glucose and 0.1% BSA, and preincubated for 60 min at 37 °C in the same medium. After preincuation, cells were stimulated with test compound in HEPES-balanced KRBB (pH 7.4) containing 16.8 mM glucose at 37 °C for 60 min. Insulin secreted into the supernatant was collected and measured using an Insulin Radioimmunoassay Kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

2.4. Perfusion of isolated rat pancreata

The pancreata of male SD rats (300–400 g) were isolated as described previously [9], with the introduction of a few modifications. Briefly, the animals were anaesthetized using intrapertioneal sodium pentobarbital (50 mg/kg) followed by dissection of the pancreas and associated spleen, stomach and duodenum. The isolated pancreata were then perfused (flow rate, 2 ml/min) through the celiac artery with HEPES-balanced KRBB containing 2.8 or 16.8 mM glucose and 0.1% fatty acid-free BSA. The preparation was then placed in an acrylic chamber containing KRBB (37 °C) and the effluent perfusate from the portal vein cannula was collected at 1–2 min intervals in fraction tubes containing aprotinin (500 units/tube). The samples were stored at −20 °C until insulin concentrations were measured using an Insulin Radioimmunoassay Kit (Amersham Pharmacia Biotech).

2.5. Oral glucose torelance test (OGTT) in diabetic db/db mice

Six-week-old male db/db mice were fasted overnight and then orally administered 0.5% methyl-cellulose (vehicle, $n = 7$), or 10 mg/kg AS1535907 ($n = 7$), and 10 mg/kg glibenclamide ($n = 7$). After 30 min, glucose was given orally at a dose of 2 g/kg/10 ml, and blood samples were collected from tail veins after 0, 5, 10, 15, 30, 60, 120, and 180 min. Blood glucose levels was determined using the Glucose CII test (Wako, Osaka, Japan), while plasma insulin level was measured by radioimmunoassay (Amersham Biosciences), following the manufacturers' instructions.

2.6. Statistical analysis

For the statistical analysis in the OGTT experiment, the area under the curve (AUC) was calculated. Significant differences were determined using Dunnett's multiple comparison test. A value of $P < 0.05$ was considered to represent statistical significance. All data are expressed as the mean ± SE. All statistical analyses were performed using the SAS 8.2 software package (SAS Institute Japan Ltd., Tokyo, Japan).

3. Results

3.1. Human GPR119 agonist activity of AS1535907 and its effect on insulin secretion by rat islets

To evaluate the human GPR119 agonist activity of AS1535907, this compound was used to treat HEK293 cells stably expressing human GPR119 and pCRE-Luc. AS1535907 significantly evoked intracellular cAMP accumulation in a dose-dependent manner and displayed an EC_{50} value of 4.8 μM for human GPR119 (Fig. 1A). Significantly, AS1535907 had no effect on HEK293 cells expressing control vector and was inactive towards several other GPCRs, including the β-adrenergic and GLP-1 receptors (data not shown). To confirm that AS1535907 has direct effects on insulin secretion, we next examined the ability of AS1535907 to induce GSIS in isolated rat islets. The exposure of rat islets to 10 μM AS1535907 resulted in an increase of insulin secretion at high glucose levels (16.8 mM) (Fig. 1B).

3.2. Insulin secretion in isolated perfused rat pancreata

We next investigated the effect of AS1535907 on first-phase insulin secretion *in vitro* using isolated perfused rat pancreata. Specifically, we examined and compared the effects of AS1535907 and the T2DM therapeutic agent glibenclamide on the kinetics of insulin release in pancreata in the presence of 2.8 or 16.8 mM glucose. At a basal glucose concentration (2.8 mM), treatment of pancreata with 10 μM AS1535907 had no observable effect (Fig. 2A and C). This contrasted with the effect of glibenclamide, which markedly stimulated insulin secretion within 5 min of treatment under basal glucose levels. At 16.8 mM glucose, 10 μM AS1535907 specifically enhanced first-phase insulin secretion by pancreata, as indicated by the time–response curve and the AUC between 30–40 and