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HD047703, a New Promising Anti-Diabetic Drug Candidate: *In Vivo* Preclinical Studies

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Abstract

G-protein coupled receptor 119 (GPR119) has emerged as a novel target for the treatment of type 2 diabetes mellitus. GPR119 is involved in glucose-stimulated insulin secretion (GSIS) from the pancreatic β-cells and intestinal cells. In this study, we identified a novel small-molecule GPR119 agonist, HD047703, which raises intracellular cAMP concentrations in pancreatic β-cells and can be expected to potentiate glucose-stimulated insulin secretion from human GPR119 receptor stably expressing cells (CHO cells). We evaluated the acute efficacy of HD047703 by the oral glucose tolerance test (OGTT) in normal C57BL/6J mice. Then, chronic administrations of HD047703 were performed to determine its efficacy in various diabetic rodent models. Single administration of HD047703 caused improved glycemic control during OGTT in a dose-dependent manner in normal mice, and the plasma GLP-1 level was also increased. With respect to chronic efficacy, we observed a decline in blood glucose levels in *db/db*, *ob/ob* and DIO mice. These results suggest that HD047703 may be a potentially promising anti-diabetic agent.

Key Words: GPR119 agonist, Type 2 diabetes, GLP-1

INTRODUCTION

An agonist to the G-protein coupled receptor 119 (GPR119) has recently emerged as a promising new anti-diabetic drug target. GPR119 agonist offers promise in modulating glucose homeostasis in an incretin-dependent and incretin-independent manner (Jones and Leonard, 2009). Impaired insulin sensitivity causes chronic hyperglycemia which presents as Type 2 diabetes mellitus (T2DM) (DeFronzo *et al*., 1992; Taylor *et al*., 1994). Depletion of glucose-stimulated insulin secretion (GSIS) is a representative feature and leads to postprandial hyperglycemia in T2DM patients, especially in the early phase (Meece, 2007). In a clinical study, hypoglycemic agents such as metformin, α-glycosidase inhibitors, thiazolidines (TZDs) and sulfonylurea derivatives are used. However, these compounds have side effects including hypoglycemia, weight-gain, and cardiovascular problems (Ho *et al*., 2011). The development of novel diabetes medications, especially oral medications that do not have the side effects of the earlier medications is an active area of research both in the industry and in academics in order to meet the needs of diabetic patients (Xia *et al*., 2011)

GPR119 agonist is currently receiving significant attention for its therapeutic potential as an anti-diabetic agent and an appropriate treatment modality for the safe amelioration of metabolic diseases. In principle, a molecule acting via GPR119 to raise intracellular cAMP concentrations in pancreatic β-cells would be expected to potentiate GSIS in a manner analogous to that of the incretin hormones glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide/glucose-dependent insulinotropic peptide, which also act via Gαs-coupled, β-cell receptors (Furman *et al*., 2004). GLP-1 analogs increase glucose-dependent insulin secretion through the gastrointestinal mechanism, which causes GLP-1 release from pancreatic β-cells and enteroendocrine L-cells due to activation of GPR 119. Activation of GPR119 increases GLP-1 levels (Ross and

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Ekoé, 2010). Mimics of these ligands induce GLP-1 secretion and glucose tolerance in normal and diabetic mice (Chu *et al*., 2007; Jones *et al*., 2009).

In the current research, the *in vivo* efficacy profile of the oral dose of HD047703, a novel small-molecule GPR119 agonist, was evaluated in normal and diabetic mice. HD047703 was synthesized at Hyundai Pharm. We specifically compared the effects of HD047703 with those of the dipeptidyl peptidase-4 (DPP-4) inhibitor, sitagliptin, which also increases the GLP-1 level in the blood (Barnett, 2006), on glycemic control and insulin secretion in several animal models.

MATERIALS AND METHODS

Chemicals and animals

HD047703 was synthesized in-house at Hyundai Pharm Inc. HD047703 is composed of pyridinone derivatives (Patent No.: WO2012011707). Prior to their use in assays, HD047703 and sitagliptin (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 0.5% carboxy-methylcellulose (CMC; Sigma-Aldrich, St. Louis, MO, USA).

In vitro experiments

Cell line: Human GPR119 receptor stable cell lines were purchased from ChanTest (Cleveland, Ohio, USA). These cell lines were cultured in Ham's F12 media containing 10% FBS, 1% Non Essential Amino Acids (NEAA, Gibco), 400 μg/ml G418 (Sigma-Aldrich, St. Louis, MO, USA).

cAMP measurement: Human GPR119 receptor CHO stable cell lines were seeded in 96-well plates at densities of 2×10^4 cells/0.1 ml/well and 5×10^4 cells/0.1 ml/well. After overnight incubation, the cells were incubated for 10 min with the test medium of Hanks buffered salt solution (HBSS; Gibco) containing 5 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; Gibco), 0.5 mM of 3-isobutyl-1-methylxanthine (IBMX; Sigma-Aldrich, St. Louis, MO, USA), and 0.1% of bovine serum albumin (BSA; Sigma-Aldrich). Then, cells were treated with 10, 2, 0.4, 0.08, 0.016, 0.0032 μM concentrations of the test drug for 90 min. Measurement of cytoplasmic cAMP levels after the drug treatment was performed with homogeneous time-resolved fluorescence (HTRF; CISBIO, USA). At 3 h after mixing cAMP-d2 and Anti-cAMP cryptate conjugate, the fluorescence (excitation 337 nm, emission 665 nm, 620 nm) was measured by Flexstation (Molecular Devices, USA). All of the experiments were performed in duplicate.

Animals

All procedures involving animals were approved by the Animal Ethics Committee of Hyundai Pharma Inc. All animals were housed singly under a 12 hr/12 hr light/dark cycle and free-feeding conditions, in temperature and humidity controlled rooms. Normal C57BL/6J male mice, *db/db*, and *ob/ob* mice were obtained from NARA BIO Inc. (Seoul, KOR) and Korea Research Institute of Bioscience and Biotechnology (KRIBB; Ochang, KOR). To generate diet-induced obese (DIO) mice, 4-week-old, normal C57BL/6J mice were fed high-fat chow (Kcal 60% fat) for 8 weeks. The body weight of mice that were fed a high-fat diet was checked, and mice with a body weight of more than 40 g were considered as the DIO model.

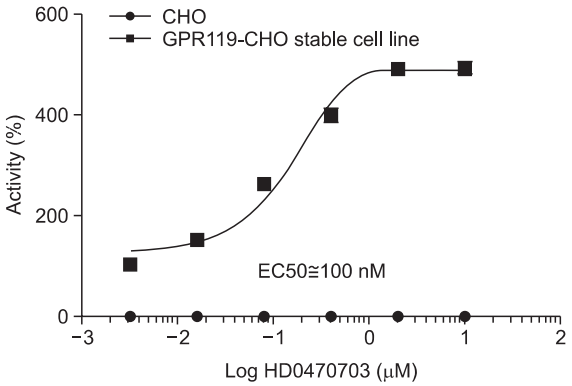

Fig. 1. HD047703 stimulated cAMP accumulation. (A) cAMP levels in GPR119-CHO stable cell line (■). CHO cells were used as control cells (●). The cells were treated with the test drug at 10, 2, 0.4, 0.08, 0.016, 0.0032 μM concentrations for 90 min. Measurement of cytoplasmic cAMP level after treatment was performed with homogeneous time-resolved fluorescence (HTRF). All data represent the means ± SEM from at least three independent experiments.

In vivo experiments

Oral glucose tolerance test (OGTT) and GLP-1 measurement: Acute pharmacological efficacy of the test drug was tested by the oral glucose tolerance test (OGTT). Eight-week-old male C57BL/6 mice were fasted overnight and then they were orally administered 0.5% CMC (vehicle) or 10, 50 mg/kg HD047703 through 10 ml/kg. After 30 min, glucose was given orally at a dose of 2 g/kg/10 ml, and blood samples were collected. Blood glucose levels were then monitored by tail snipping at -30, 0, 20, 40, 60, and 120 minutes after glucose loading. The plasma glucose level was immediately determined using Accu-Chek Active blood glucose meter (Roche, Switzerland).

To assess the improvement in active GLP-1 secretion abilities after administration of the test drug, separate experiments were performed in overnight-fasted mice. Thirty minutes following the administration of the test drug, a glucose bolus was administered, and then plasma samples were obtained after 20 min. The isolated plasma was analyzed with active GLP-1 Enzyme-linked immunosorbent assay (ELISA; Millipore, MO, USA).

Efficacy of repeated administration: The studies for assessing chronic efficacy were performed in *db/db*, *ob/ob*, and DIO mice. All of the animals were given the vehicle or the test drug bid and sitagliptin qd. To generate the DIO mice, 4-week-old, normal C57BL/6J mice were fed high-fat chow (Kcal 60% fat) for 8 weeks. The body weight of mice that were fed high-fat diet was checked, and mice with a body weight of more than 40 g were considered as the DIO model. The vehicle groups were administered 0.5% CMC (vehicle, n=7), and the test groups of *db/db* mice and *ob/ob* mice were administered 10, 20, 50 mg/kg HD047703 BID and sitagliptin 10 mg/kg as a positive agent (n=7) QD, through 10 ml/kg, and 10, 30 mg/kg HD047703 (n=7) BID through 10 ml/kg, respectively. In DIO model groups, the vehicle groups were administered 0.5% CMC (vehicle, n=7) BID, and the test groups were administered 10, 20, 50 mg/kg HD047703 (n=7) BID through 10 ml/kg. During the treatment period, body weight of each animal was checked. At 4 weeks after initiating the administration of, the

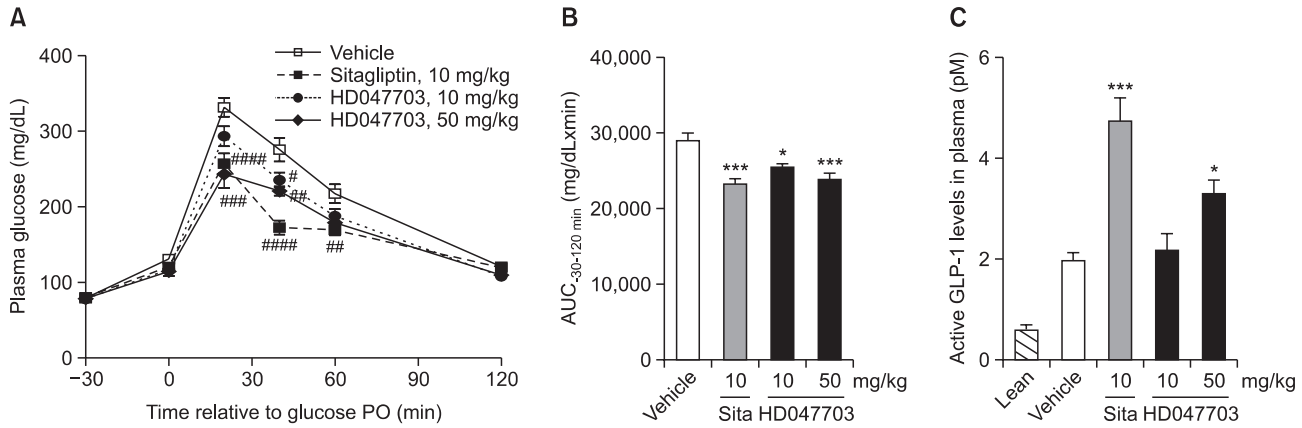

Fig. 2. Effect of single treatment with HD047703 on oral glucose tolerance under fasting conditions in normal mice. (A) The effect on plasma glucose levels in normal mice during an OGTT. (B) The area under the plasma glucose concentration-time curve for 2 h ($AUC_{0\text{-}2}$ h) in an OGTT. (C) The effect on plasma GLP-1 levels in normal mice during an OGTT. Sham controls are normal mice without glucose administration. Data are presented as the mean ± SE for each group (n=6). $^{\#\#\#\#}$<0.0001 vs. vehicle, $^{\#\#\#}$<0.001 vs. vehicle, $^{\#\#}$<0.01 vs. vehicle, $^{\#}$<0.05 vs. vehicle by two way ANOVA analysis, respectively; ***p<0.001 vs. vehicle, *p<0.05 vs. vehicle by one way ANOVA, respectively. The post-comparison of all results was performed by the Dunnett's test.

OGTT was performed in the administrated mice. To assess the glucose-lowering effect of GLP-l in DIO model groups, we measured the final body weight. The fat pads were immediately removed and their weights were measured.

Statistical analysis

Values are expressed as the mean standard error (SE). Group comparisons were carried out using one-way ANOVA with a Dunnett's comparison test and two-way ANOVA. Statistical analyses were conducted using 'GraphPad Prism 5' software (Graphpad Co., La Jolla, CA, USA), and a p-value of <0.05 was considered to be statistically significant.

RESULTS

Increase in the cAMP level by the GPR119 agonist

The GPR119 signaling is known to be triggered by elevation of the cAMP synthesis. Therefore, we tested whether HD047703 elevates the intracellular cAMP level in CHO cells stably expressing the human GPR119 receptor. HD047703 significantly stimulated intracellular cAMP accumulation in a dose-dependent manner and showed an EC_{50} value of 110 nM for human GPR119-CHO stable cell lines (Fig. 1).

Effect of single administration of HD047703 on glucose tolerance in normal mice

We evaluated the role of HD047703 in the acute regulation of insulin secretion *in vivo* by examining the blood glucose level and GLP-1 secretion during the OGTT in normal mice.

We also assessed the improvement in glycemic control through OGTT after single administration of drugs. Sitagliptin improved glycemic control in normal mice, and administration of 50 mg/kg HD047703 improved glycemic control, and the glucose levels were decreased at 20 min after glucose loading (Fig. 2A). After single administration of HD047703, the area under the curve (AUC) during the OGTT was significantly decreased in a dose-dependent manner, compared to that in the vehicle group (Fig. 2B).

We also tested whether elevation of the cAMP level by HD047703 increased the GLP-1 secretion. Overnight-fasted normal C57BL/6J mice were administered the 10, 50 mg/kg dose (Fig. 2C) of HD047703 before 30 min of glucose loading, then blood samples were collected after 20 min of glucose loading to analyze GLP-1 levels by ELISA. Sitagliptin, DPP-4 inhibitor, caused an increase in the plasma GLP-1 level (Fig. 2C, p<0.001 vs. vehicle group), and an increased GLP-1 level may lead to improved glucose-stimulated insulin secretion. After administration of 50 mg/kg of HD047703, the plasma GLP-1 level was significantly increased (Fig. 2C, p<0.05 vs. vehicle group).

Effect of chronic treatment with HD047703 in diabetic *db/db* and *ob/ob* mice

To evaluate the anti-diabetic efficacy of HD047703 in a diabetic model, 6-week-old diabetic *db/db* mice were treated with 10, 20, 50 mg/kg HD047703 or vehicle for 4 weeks. In the OGTT performed at 28 days after administration, the HD047703 treated groups showed lower blood glucose levels than the vehicle group, and AUC values during OGTT showed lower abundances in the vehicle group (Fig. 3A, B). In the 50 mg/kg HD047703 group, the glucose level was significantly decreased. To evaluate the efficacy of HD047703 in another type 2 diabetic model, 6-week-old diabetic *ob/ob* mice were treated with 10, 30 mg/kg HD047703 or vehicle for 4 weeks. In the OGTT performed at 28 days after administration, the HD047703 treated groups showed significantly lower blood glucose levels than the vehicle group in a dose-dependent manner, and AUC values during OGTT showed lower abundances in the vehicle group (Fig. 3C, D).

Effect of chronic treatment with HD047703 in diabetic DIO mice

To evaluate the effects of HD047703 on the combination of dietary and genetic influences, diabetic DIO mice were treated with 10, 20, 50 mg/kg HD047703 or vehicle for 4 weeks. In the OGTT performed at 28 days after administration, the HD047703 treated groups showed lower blood glucose levels

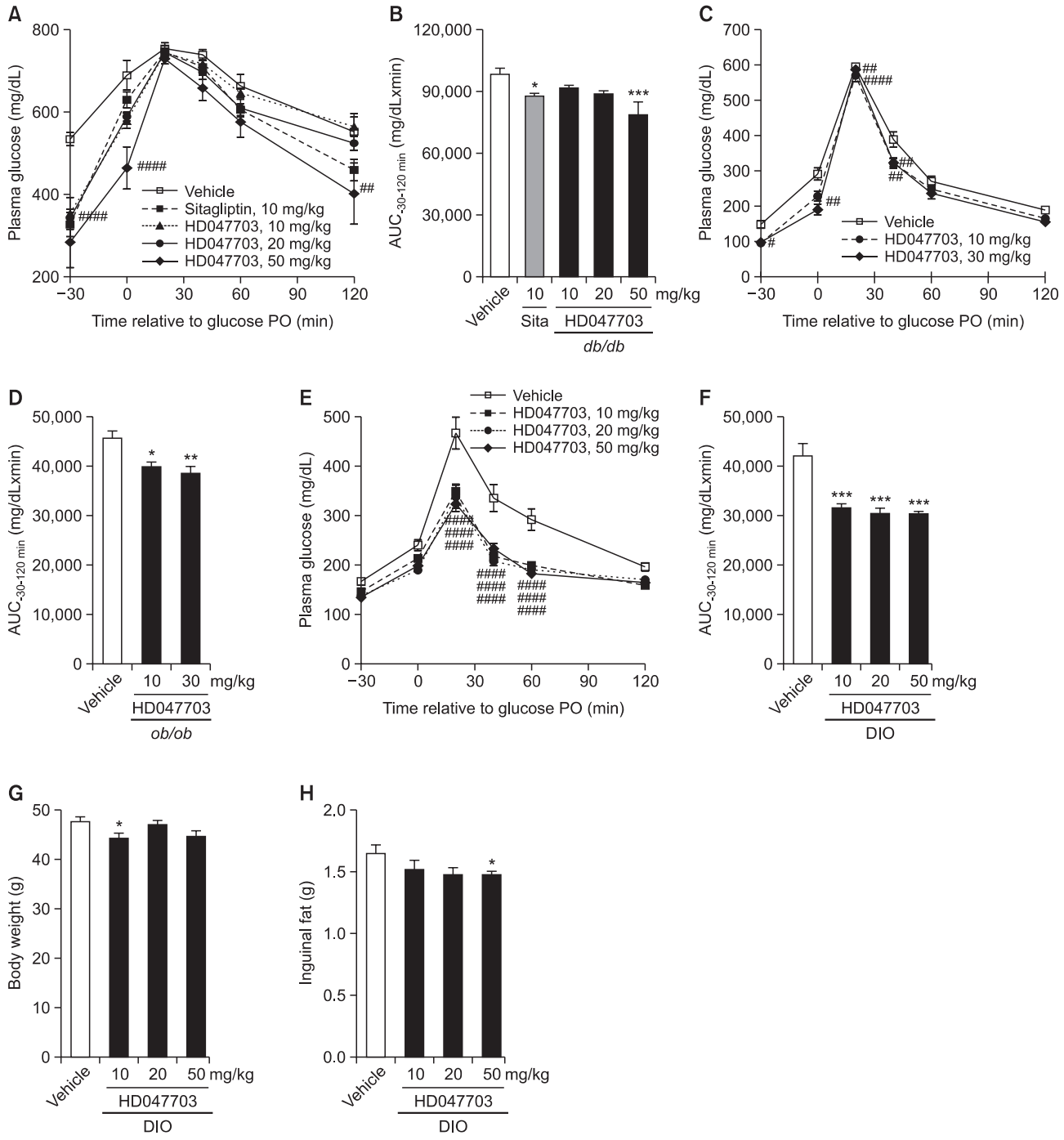

Fig. 3. Effect of chronic treatment in diabetic *db/db*, *ob/ob*, and DIO mice. A 10, 20, 50 mg/kg dose of HD047703 or vehicle was administered twice daily to diabetic *db/db* mice (n=7) for 4 weeks. (A) The effect on plasma glucose levels in *db/db* mice during an OGTT. (B) The area under the plasma glucose concentration-time curve for 2 h ($AUC_{0\text{-}2\ h}$) in an OGTT. (C) The effect on plasma glucose levels in *ob/ob* mice during an OGTT. (D) The area under the plasma glucose concentration-time curve for 2 h ($AUC_{0\text{-}2\ h}$) in an OGTT. (E) The effect on plasma glucose levels in DIO mice during an OGTT. (F) The area under the plasma glucose concentration-time curve for 2 h ($AUC_{0\text{-}2\ h}$) in an OGTT. (G) Final body weight. (H) Inguinal fat pad Weight. Data are presented as the mean ± SE. $^{\#\#\#\#}p<0.0001$ vs. vehicle, $^{\#\#}p<0.01$ vs. vehicle. vehicle by two way ANOVA analysis, respectively; $^{***}p<0.001$ vs. vehicle, $^{**}p<0.01$ vs. vehicle, $^{*}p<0.05$ vs. vehicle by one way ANOVA, respectively. The post-comparison of all results was performed by the Dunnett's test.

than the vehicle group, and AUC values during OGTT showed lower abundances in the vehicle group (Fig. 3E, F) and the body weight was decreased slightly but not significantly (Fig. 3G). Inguinal fat pad weight was decreased in a dose-dependent manner, but this decrease was not significant (Fig. 3H).

DISCUSSION

The incretin hormone GLP-1 is released in response to nutrient ingestion and it is required for maintenance of normal glucose homeostasis (Kreymann *et al*., 1987). Several orally effective GPR119 agonists have been discovered and reported to have anti-diabetic actions in animals (Yoshida *et al*., 2010) and in humans (Katz *et al*., 2012). Compared to other medications for T2DM, the advantage of incretin hormone-based therapy is not only improved glycemic control, but also loss of body weight (Amiel *et al*., 2008). Chronic treatment with exendin-4, a long acting GLP-1 analogue, in a rodent obesity model resulted in improved insulin sensitivity and loss of body weight (Iltz *et al*, 2006). Unfortunately, all incretin hormone-based drugs do not cause loss of body weight. Although selective DPP-4 inhibitors have been reported to have strong effects on improving insulin sensitivity and glycemic control, some reports have stated that sitagliptin does not decrease body weight in obese subjects (Barnett *et al*., 2007; Raun *et al*., 2007). Moreover, selective DPP-4 inhibitors have adverse effects on the immune system, thus causing rheumatoid arthritis and pancreatitis (Bekur *et al*., 2010; Iyer *et al*., 2012; Yokota and Igaki, 2012).

In the present study, we identified a novel small-molecule GPR119 agonist, HD047703, which not only improved the blood glucose level but also did not cause body weight gain.

The major sites of GPR119 expression are pancreatic beta cells and intestinal L cells. Its action begins by increasing intracellular cAMP levels, and this pathway leads to insulin or GLP-1 secretion by beta or L cells (Drucker *et al*., 1994; Chu *et al*., 2007). Treatment of the CHO stable cell line with HD047703 resulted in elevated cAMP levels. Therefore, the in vitro studies indicate that the GPR119 agonist increases intracellular cAMP levels in a dose-dependent manner leading to GLP-1 secretion.

Acute administration of HD047703 also increased the GLP-1 secretion in normal C57BL/6J mice, which is related to improved glycemic control in T2DM animal models (Bekur *et al*., 2010). These data suggest that HD047703 may activate GPR119, which regulates GLP-1 secretion in normal mice.

Chronic administration of HD047703 in the *db/db* and *ob/ob* mouse models of T2DM resulted in improved glycemic control as long as the treatment was continued, suggesting that HD047703 may improve glycemic control.

The DIO model resembles the human condition with respect to the combination of dietary as well as genetic influences on obesity and diabetes (Giorgino *et al*., 2011), and it also showed improved glycemic control. In addition, mice treated with HD047703 showed slight loss of body weight, although several studies have not shown this effect DPP-4 inhibitors on body weight (Barnett *et al*., 2007; Raun *et al*., 2007). In the OGTT performed at the 28th day, the HD047703 treated groups showed improved glucose tolerance compared to the vehicle-treated group. These data suggest that HD047703 improves glycemic control in the *db/db*, *ob/ob*, and DIO mice. GLP-1 agonists cause weight loss; conversely, the DPP-4 inhibitors appear to have a weight-neutral effect (Winzell and Ahrén, 2004). Also, body weight gain and inguinal fat pad weight were reduced in the HD047703 treated groups.

In conclusion, we evaluated a novel small-molecule GPR119 agonist, HD047703, which effectively reduced the glucose levels in normal and diabetic mice. A significant decrease was observed in the inguinal fat pad weight after *in vivo* chronic treatment. Although further studies on this compound are required, including the measurement of incretin hormone levels, and functional activity of β-cells and its proliferative activity, this study demonstrated that HD047703 can be a potential oral anti-diabetic agent that can improve glycemic control in the treatment of T2DM patients.

CONFLICT OF INTEREST

DH Kim and JK Phee are employees of Hyundai Pharm. Corp.

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