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G protein-coupled receptor 119 agonist DS-8500a effects on pancreatic β-cells in Japanese type 2 diabetes mellitus patients

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ABSTRACT

Aims/Introduction: Pancreatic β-cell dysfunction contributes to type 2 diabetes mellitus progression. Drugs that improve insulin secretion might be a valuable treatment approach. The present study aimed to evaluate the effect of the G protein-coupled receptor 119 agonist DS-8500a on insulin secretory capacity in Japanese type 2 diabetes mellitus patients.

Materials and Methods: This single-center, 4-week, randomized, double-blind, crossover study enrolled 21 Japanese drug-naïve type 2 diabetes mellitus patients aged ≥20 years with glycated hemoglobin ≥7.0 and <9.0% (NCT02669732, JapicCTI 163126). Patients received 75 mg of DS-8500a or a placebo orally daily for 4 weeks in a random order. A combined euglycemic-hyperinsulinemic and hyperglycemic clamp test was carried out to assess insulin secretion and insulin sensitivity before and after each 4-week treatment period. Primary end-points were first-phase insulin secretion (insulin area under the curve $[AUC]_{180–190\ min}$ and C-peptide $AUC_{180–190\ min}$ during the clamp test) and second-phase insulin secretion (insulin $AUC_{190–300\ min}$ and C-peptide $AUC_{190–300\ min}$). Insulin sensitivity (*M* and *M/I* values), disposition index and changes in lipid profile were also assessed.

Results: DS-8500a significantly increased first- and second-phase insulin AUC ($P = 0.0011$, $P = 0.0112$) and C-peptide AUC ($P = 0.0012$, $P < 0.0001$) compared with the placebo. At day 28, *M* and *M/I* values were comparable with those of the placebo, whereas the disposition index for insulin and C-peptide was significantly increased ($P = 0.0108$, $P = 0.0002$). Total cholesterol, low-density lipoprotein cholesterol and triglyceride concentrations were significantly reduced, and high-density lipoprotein cholesterol concentrations were significantly increased compared with the placebo. No significant treatment-emergent adverse events occurred.

Conclusion: DS-8500a enhanced insulin secretory capacity, but not insulin sensitivity.

INTRODUCTION

The global incidence of type 2 diabetes mellitus is increasing, and this trend is notable in Japan, where there has been an increase in Westernized dietary habits and lifestyle changes. The global age-standardized diabetes prevalence increased from 4.3% (95% credible interval 2.4–7.0%) in 1980 to 9.0% (7.2–11.1%) in 2014 in men, and from 5.0% (2.9–7.9%) to 7.9% (6.4–9.7%) in women. Furthermore, the number of adults with diabetes worldwide increased from 108 million in 1980 to 422 million in 2014 (28.5% due to the rise in prevalence, 39.7% due to population growth and aging, and 31.8% due to interactions between these two factors)[1]. Additionally, East Asians (including Japanese) were shown to have a lower innate insulin secretion ability than Caucasians and Africans[2]. In 2012, the

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number of adults with diabetes in Japan was estimated to be 9.5 million[3].

Diabetes is associated with many complications, including nephropathy, retinopathy and neuropathy, as well as increased risks of vascular events, such as ischemic heart disease, stroke and peripheral arterial disease, which have a high social and economic burden. Therefore, it is essential to develop treatments that provide good glycemic control[4]. Currently, a number of antidiabetic drugs are available, including sulfonylureas and short-acting insulin secretagogues that compensate for impaired insulin secretion, glucagon-like peptide-1 (GLP-1) receptor agonists that mimic the action of GLP-1 and stimulate the release of insulin, dipeptidyl peptidase (DPP)-IV inhibitors that block DPP-IV-mediated inactivation of incretin hormones, thiazolidines that reverse resistance to insulin, biguanides that suppress liver gluconeogenesis, sodium-glucose transporter 2 inhibitors that enhance glucose excretion in the urine and α-glucosidase inhibitors that suppress glucose absorption. In cases where diabetes patients show poor glycemic control with monotherapy, combinations of drugs with different modes of action are administered.

Because pancreatic β-cell dysfunction caused by impaired islet insulin responses and a reduction in β-cell mass contributes to the progression of type 2 diabetes mellitus, drugs that improve insulin secretion might be a valuable treatment approach. A characteristic feature of type 2 diabetes mellitus is a dramatic reduction in first-phase insulin secretion[5–8], which is thought to have a marked effect on postprandial plasma glucose excursions. Furthermore, the loss of early-phase insulin release is a common defect involved in the pathogenesis of post-meal hyperglycemia[9]. Therefore, specific therapeutic interventions that help insulin secretion from β-cells might result in improved glycemic control[10].

G protein-coupled receptor 119 (GPR119) is a Gαs protein-coupled receptor expressed in small intestinal L cells in the human gastrointestinal tract and in pancreatic β-cells[11,12]. GPR119 agonists mediate their effects through the dose-dependent increase in cyclic adenosine monophosphate concentration in cells expressing human GPR119[13]. Previous studies in mouse models have shown that GPR119 agonists promote GLP-1 secretion, have glucose-dependent insulinotropic effects and improve glucose tolerance[12,13]. They were also shown to upregulate insulin secretion and genes essential for controlling pancreatic β-cells in a mouse type 2 diabetes mellitus model[14]. Therefore, it has been hypothesized that long-term treatment with GPR119 agonists will promote insulin secretion to improve pancreatic β-cell function and lower blood glucose levels.

DS-8500a is a novel GPR119 agonist recently developed by Daiichi Sankyo Co., Ltd. Our previous non-clinical pharmacology study found that DS-8500a had agonistic activity in human GPR119-expressing Chinese hamster ovary-K1 cells, and that treatment of Zucker fatty rats improved glucose intolerance[15]. We also showed that DS-8500a preserved pancreatic β-cell function and islet morphology, and attenuated glycated hemoglobin increase in mouse models of type 2 diabetes mellitus[16]. A previous phase 1, randomized, double-blind, placebo-controlled study showed that the administration of up to 100-mg DS-8500a for 7 days was well tolerated by healthy Japanese men, and that plasma concentrations of DS-8500a reached steady-state levels by day 7[17]. Furthermore, a phase 2 study in Japanese type 2 diabetes mellitus patients showed that 75-mg DS-8500a once daily for 28 days was well tolerated with no hypoglycemic events. It also significantly improved glycemic and lipid variables compared with a placebo, with no loss of efficacy over time[18].

Because of the scarcity of GPR119 studies in type 2 diabetes mellitus patients, especially in the Japanese population, and based on these previous findings, the present study aimed to evaluate changes in insulin secretory capacity, as determined by the clamp technique, and safety in Japanese patients with type 2 diabetes mellitus after the 28-day oral administration of 75-mg DS-8500a.

METHODS

Trial design

This was a single-center, randomized, placebo-controlled, double-blind, cross-over study carried out in the SOUSEIKAI Hakata Clinic from 13 February 2016 to 11 August 2016.

Participants

We enrolled Japanese type 2 diabetes mellitus patients aged ≥20 years at the time of informed consent. Patients were included if they were treatment-naïve for antidiabetic drugs, had glycated hemoglobin ≥7.0 and <9.0% at the start of the run-in period and were following exercise therapy and diet therapy at the time of informed consent. The patients continued the exercise and diet therapy until the end of the study.

Patients were excluded if they had: type 1 diabetes; a history of diabetic coma, precoma or ketoacidosis; a history of clinically significant diabetic retinopathy, diabetic nephropathy or diabetic neuropathy; poorly-controlled blood pressure; clinically significant liver disease or renal disease; a body mass index of <18.5 kg/m^2 or >35.0 kg/m^2; or a fasting plasma glucose ≥240 mg/dL.

Interventions

Figure S1 shows the timeline for the study. Drug-naïve patients were enrolled, observed for 2 weeks and then randomly assigned into two groups that received an oral placebo or 75-mg DS-8500a for 4 weeks. This was followed by a 4-week washout period (based on a DS-8500a $t_{1/2}$ of 12.9 h in a multiple ascending dose study)[17], and then another 4-week administration of a placebo or 75-mg DS-8500a, whichever had not been administered in the first period. At the beginning and end of the two treatment periods, combined euglycemic-hyperinsulinemic and hyperglycemic clamp tests were carried out. The follow-up period was 14 days. The study drug was administered

as three 25-mg tablets (or three tablets of placebo) administered orally once daily for 28 days.

Clamp technique

Figure S1 illustrates the clamp procedure, which was carried out on day −1 and day 28 of each 28-day study period using an artificial pancreas (STG-22; Nikkiso, Tokyo, Japan). Figure S2 shows the euglycemic-hyperinsulinemic clamp and hyperglycemic clamp procedure.

The clamp procedure was carried out under fasting conditions. A tube to monitor blood glucose was inserted into either a dorsal hand vein or a vein around the wrist, and an electronic heat blanket was placed over the insertion site to warm it up and arterialize venous blood.

For the euglycemic-hyperinsulinemic clamp, insulin (Humulin®; Eli Lilly USA, Indianapolis, IN, USA) was injected to achieve a blood insulin level that inhibited glucose release from the liver, which corresponded to an insulin infusion rate of 1.25 mU/kg/min. Glucose was also injected to maintain a target glucose level of 90 mg/dL from 0 to 180 min. The *M* value was then calculated as the mean glucose infusion rate over 30 min from 90 to 120 min after the start of insulin infusion. When ending the euglycemic-hyperinsulinemic clamp, insulin infusion was stopped and the target glucose level of 90 mg/dL was maintained to minimize the effect of exogenous insulin.

For the hyperglycemic clamp, 180 min after the start of insulin infusion, a 50% glucose solution (0.3 g/kg) was injected by bolus for approximately 30 s followed by a 20% glucose solution to maintain a target glucose level of 270 mg/dL for 120 min.

The area under the plasma concentration–time curves $(AUC)_{180–190\ min}$ for insulin and C-peptide, and the $AUC_{190–300\ min}$ for insulin and C-peptide, were measured as first-phase and second-phase secretion, respectively.

End-points

The primary end-point of the present study was insulin secretory capacity measured as first-phase secretion and second-phase secretion.

Secondary end-points were insulin sensitivity from 90 to 120 min, the *M* value (mean glucose infusion rate from 90 to 120 min), which represents the cellular uptake of glucose, and the *M*/*I* value (*M* value/steady-state insulin [mean value of insulin at 90 and 120 min]) representing the insulin sensitivity index indicating the cellular uptake of glucose to the plasma concentration of insulin. The disposition index (product of first-phase secretion and *M* value) was another secondary end-point, representing insulin secretion/insulin resistance. We also calculated the metabolic clearance rate of insulin during the euglycemic-hyperinsulinemic clamp using the following equation[19]: metabolic clearance rate of insulin = $(IIR/[SS_I - \{B_I{*}SS_C/B_C\}])$, where IIR = insulin infusion rate, SS_I = steady-state insulin, B_I = baseline insulin (0 min), SS_C = steady-state C-peptide (mean value of C-peptide at 90 and 120 min), and B_C = baseline C-peptide (0 min). In addition, the lipid profile was assessed by measuring the percentage change in total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol and triglycerides from baseline to day 28.

Safety end-points of DS-8500a were adverse events (AEs), laboratory values, vital signs and electrocardiography. Treatment-emergent AEs (TEAEs) and drug-related TEAEs were assessed in relation to their seriousness, outcome, severity and whether the event was related to the study drug. Clinical and laboratory tests were also carried out to assess the status of the patients.

Sample size

The planned sample size was 20 participants (10 in each group). For first-phase insulin secretion, based on the results for exenatide[20], the difference between the DS-8500a treatment and placebo treatment of 250 mU min/L (approximately half of that for exenatide) and root mean square error of 250 mU min/L were assumed. At a two-sided significance level of 5% with a power of 80%, the necessary sample size was 18 participants (nine participants per group). Power would be sufficient if approximately two participants were excluded from the analyses.

Randomization and blinding

Randomization was carried out by an independent biostatistician using the permuted block method. The assignment of groups and treatments were blinded to all participants, investigators and the sponsor, excluding the independent biostatistician. Placebos were indistinguishable from the investigational drug in terms of appearance, packaging and other features.

Statistical methods

Efficacy was analyzed in the full analysis set (FAS) and sensitivity analysis in the per-protocol set. The FAS included randomized participants who did not meet the inclusion criteria, were not treated with even one dose of the study drug in the treatment period or for whom no data on 24-h weighted mean glucose on day −1 or after administration was obtained. The per-protocol set included participants who were included in the FAS and who did not meet any of the exclusion criteria; did not have a washout period of <28 days; did not have treatment compliance of <75% in the treatment period and did not have a serious protocol deviation. For each efficacy end-point, analyses were carried out using a linear model to compare DS-8500a with the placebo, and for each comparison the 95% confidence interval (CI) and *P*-value were calculated. The significance level was set at 5% (two-sided). For glucose, insulin and C-peptide, summary statistics were calculated for each treatment group at each time-point using the FAS, and figures showing their time-courses were prepared.

For safety, AEs were tabulated using the Medical Dictionary for Regulatory Activities (MedDRA®) version 19.0 using system organ class and preferred terms. MedDRA® trademark is registered by IFPMA on behalf of ICH (https://www.meddra.org/sites/default/files/page/documents/000198_statement_on_meddra_data_sharing.pdf). TEAEs were defined as AEs that occurred after the initiation of the treatment period, and their incidence was calculated per treatment group and then tabulated by event and severity. Adverse drug reactions were also tabulated in a similar manner. The incidence of significant AEs (anemia and severe/symptomatic/asymptomatic hypoglycemia, including values for hemoglobin levels and blood glucose levels) was calculated. Missing values were not supplemented with an estimate value or calculated value. Statistical analyses were carried out using SAS system release 9.2 (SAS Inc., Cary, NC, USA).

Ethical considerations

The study was carried out in compliance with the relevant standards of the Pharmaceutical Affairs Law, and by the "Ordinance Regarding Good Clinical Practice" and the ethical standards of the Declaration of Helsinki (as revised in Fortaleza, Brazil, October 2013). The study protocol received institutional review board approval and all participants provided written informed consent.

RESULTS

A total of 24 patients were enrolled in the study, 21 of whom were randomized into the two groups and three of whom were excluded because they did not meet the inclusion criteria. In treatment periods 1 and 2, 11 patients received 75-mg DS-8500a followed by the placebo, and 10 received the placebo followed by 75-mg DS-8500a. All 21 patients completed the study at follow up and their data were analyzed. The patients comprised 17 men and four women with a mean ± standard deviation (SD) age of 55.9 ± 7.7 years, and a mean ± SD body mass index of 24.2 ± 2.6 kg/m^2. The mean ± SD duration of diabetes was 3.6 ± 5.2 years, and the mean ± SD of glycated hemoglobin, fasting plasma glucose, fasting plasma insulin and fasting plasma C-peptide was 7.75 ± 0.51%, 149.7 ± 23.1 mg/dL, 3.98 ± 2.0 μU/mL and 1.52 ± 0.53 ng/mL, respectively (Table 1). All data were comparable between the groups.

The time-course of the change in plasma glucose during the clamp test indicated that there was no difference between DS-8500a and the placebo (Figure 1). Figure 2 shows that at 180–300 min there was no difference in insulin profile at baseline between DS-8500a and the placebo, but that insulin secretion was increased with DS-8500a compared with the placebo after the glucose bolus at day 28. Table 2 shows that for the C-peptide profile at 180–300 min, although there was no difference in C-peptide profile at baseline between DS-8500a and placebo, C-peptide secretion was increased with DS-8500a compared with the placebo at day 28.

The results of the primary efficacy end-points – first- and second-phase secretion of insulin and C-peptide – are shown in Figure 2 and Table 2. DS-8500a significantly increased first- ($AUC_{180–190\ min}$) and second-phase ($AUC_{190–300\ min}$) insulin secretion at day 28 compared with the placebo (least squares mean difference *vs* placebo of 11.63, 95% CI 5.32–17.93, $P = 0.0011$ for first-phase secretion; least squares mean difference *vs* placebo of 238.41, 95% CI 61.04–415.78, $P = 0.0112$ for second-phase secretion). Similarly, as shown in Table 2, DS-8500a significantly increased first- and second-phase C-peptide at day 28 compared with the placebo (least squares mean difference *vs* placebo of 2.07, 95% CI 0.94–3.19, $P = 0.0012$ for first-phase secretion; least squares mean difference *vs* placebo of 76.53, 95% CI 51.74–101.32, $P < 0.0001$ for second-phase secretion). In addition, DS-8500a significantly increased first-phase secretion of insulin and first- and second-phase secretion of C-peptide from baseline to day 28, whereas the placebo significantly decreased second-phase secretion of insulin from baseline to day 28 (Table S1).

There was no difference in *M* value or *M*/*I* value with DS-8500a compared with the placebo at day 28, indicating that DS-8500a did not affect insulin sensitivity (Table 3). The disposition index for insulin and C-peptide showed both were significantly increased compared with the placebo at day 28 ($P = 0.0108$ and $P = 0.0002$, respectively; Table 3). There was no difference in metabolic clearance rate of insulin with DS-8500a compared with the placebo at day 28 (Table 3).

Table 1 | Baseline characteristics

	DS-8500a followed by placebo	Placebo followed by DS-8500a	Total
n	11	10	21
Age (years)	54.6 ± 7.8	57.3 ± 7.7	55.9 ± 7.7
Male/female (*n*)	8/3	9/1	17/4
BMI (kg/m^2)	23.9 ± 2.1	24.6 ± 3.1	24.2 ± 2.6
Duration of diabetes (years)	4.8 ± 6.8	2.2 ± 2.4	3.6 ± 5.2
HbA1c (%)	7.82 ± 0.55	7.68 ± 0.48	7.75 ± 0.51
Fasting plasma glucose (mg/dL)	148.5 ± 26.2	151.0 ± 20.5	149.7 ± 23.1
Fasting plasma insulin (μU/mL)	3.72 ± 1.73	4.27 ± 2.33	3.98 ± 2.0
Fasting C-peptide (ng/mL)	1.42 ± 0.40	1.63 ± 0.66	1.52 ± 0.53

Results are presented as the mean ± standard deviation. BMI, body mass index; HbA1c, glycated hemoglobin.

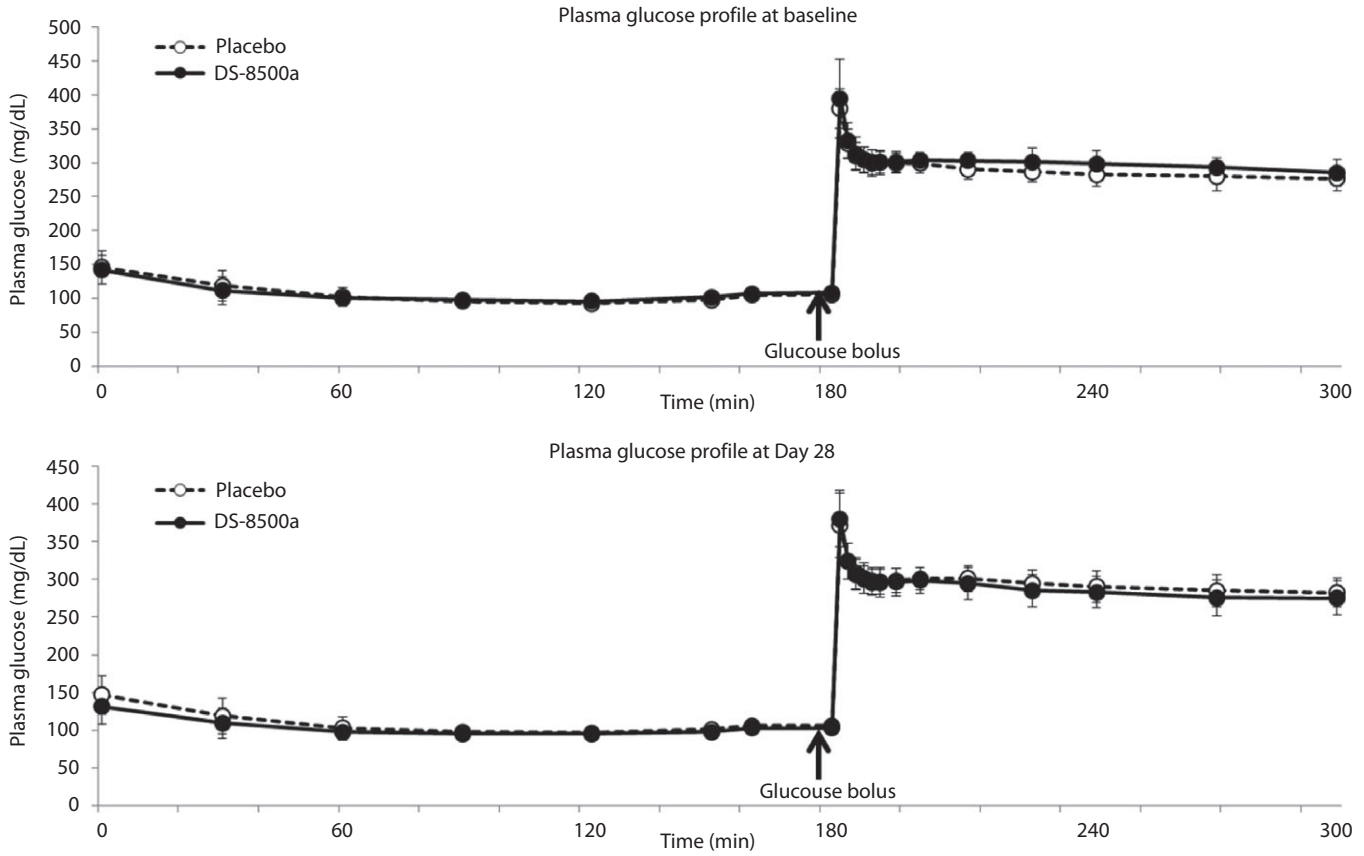

Figure 1 | Plasma glucose profile (0–300 min). Introduction of the glucose bolus at 180 min caused a spike in plasma glucose levels that was similar between the DS-8500a and placebo groups, and between baseline and day 28.

DS-8500a improved the lipid profile by significantly reducing total cholesterol, low-density lipoprotein cholesterol and triglycerides concentrations. Additionally, DS-8500a significantly increased high-density lipoprotein cholesterol concentrations at day 28 compared with the placebo (Table 3).

TEAEs were reported in five patients in the placebo group and in four in the DS-8500a group. None were considered serious or severe, and none caused study discontinuation. All AEs resolved. The five TEAEs reported in the placebo group were gingivitis, herpes zoster, nasopharyngitis, increased gamma-glutamyltransferase and laceration, which occurred in one patient each. The four TEAEs reported in the DS-8500a group were toothache, rash, arthritis and phlebitis at the infusion site, which also occurred in one patient each. There were no drug-related TEAEs or hypoglycemic symptoms or episodes of hypoglycemia. There were also no apparent safety concerns in terms of vital signs, laboratory tests, 12-lead electrocardiogram or physical examination.

DISCUSSION

This single-center, randomized, placebo-controlled, double-blind, cross-over study was designed to evaluate the effects of insulin secretory capacity of DS-8500a over 28 days in type 2 diabetes mellitus patients. GPR119 agonists have been confirmed to increase first-phase insulin secretion in non-clinical studies[21]. However, to date, no clinical studies have evaluated insulin secretion after GPR119 agonist administration. It has been reported that short-term treatment administration (e.g., 2 weeks) of other GPR119 agonists results in an attenuated or eliminated hypoglycemic effect[22,23]. Meanwhile, with DS-8500a treatment, the 24-h weighted mean glucose was significantly decreased compared with a placebo in a phase 2a study[18]; however, the underlying mechanism remains unknown. To our knowledge, the present study is the first to evaluate human insulin secretion ability after GPR119 agonist administration using the clamp technique.

Our main finding was that DS-8500a enhanced insulin secretory capacity, but did not affect insulin sensitivity. This improvement in insulin secretion capacity was assumed based on preclinical studies that observed the mechanism of action of the GPR119 agonist. Thus, these results support the proposed mechanism of action. In the hyperglycemic clamp, DS-8500a significantly increased first- and second-phase insulin secretion compared with the placebo. Three possible underlying

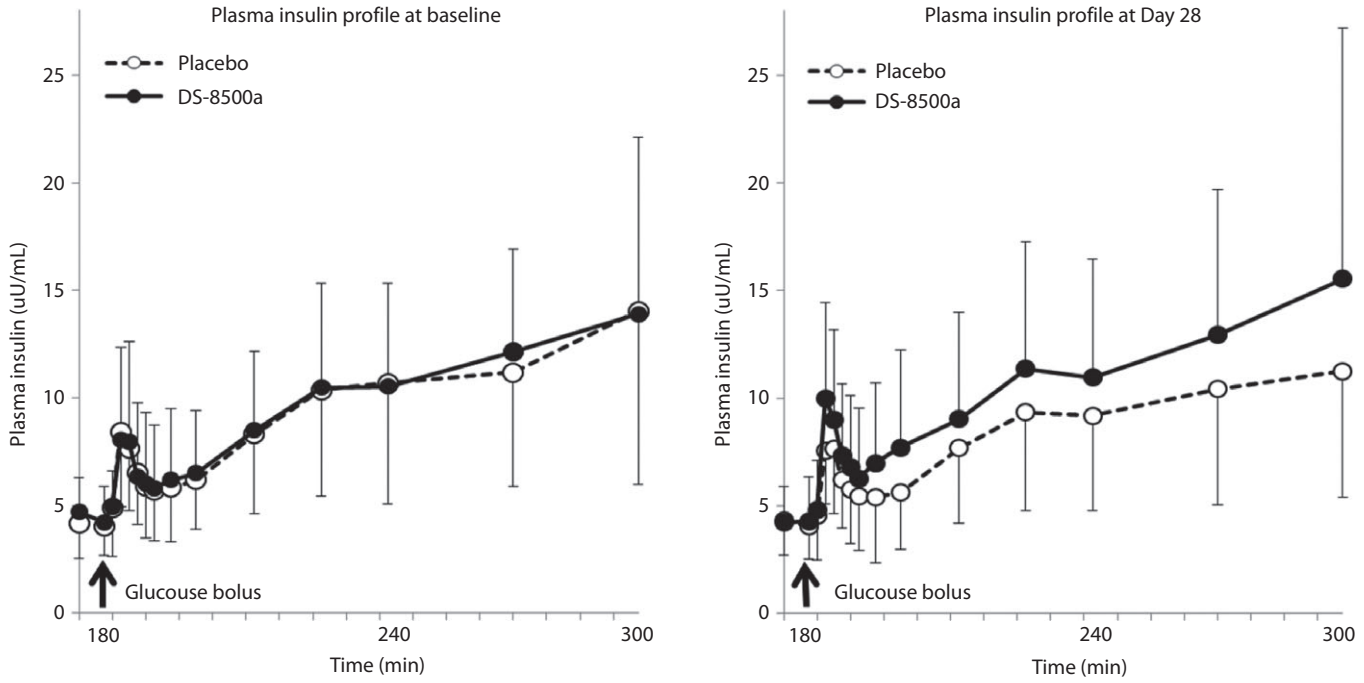

Figure 2 | Insulin profiles during the hyperglycemic clamp (180–300 min; full analysis set). At 180–300 min there was no difference in insulin profile at baseline between DS-8500a and the placebo.

mechanisms have been reported in studies of GPR119 agonists that increase insulin secretion as their mechanism of action: an increase in cyclic adenosine monophosphate concentration in pancreatic β-cells, followed by the recruitment of intracellular insulin secretory granules by cyclic adenosine monophosphate and increased glucose-dependent insulin secretion[13,24], the expression of pancreatic β-cell-related transcription factors (e.g., Nkx2.2, Bkx6.1, Neuro D), and an increased number of pancreatic β-cells[14] and the promotion of GLP-1 secretion by small intestinal L cells.[12] However, in the present study, we did not measure GLP-1; thus, the possible involvement of the third mechanism is unknown. In contrast, DS-8500a significantly decreased fasting plasma glucose (–14.5 mg/dL, $P < 0.001$) compared with the placebo at day 28; therefore, it is possible that insulin secretion was increased at least in part because of reduced glucotoxicity. In the present study, insulin sensitivity in the euglycemic-hyperinsulinemic clamp was unaffected. Additionally, improvement of the homeostatic model of assessment for insulin resistance was not observed in this study (data not shown). However, the exposure of DS-8500a in this study was short, and recruited patients had good insulin sensitivity (homeostatic model of assessment for insulin resistance 1.3–1.5), which might have precluded the appropriate evaluation of insulin sensitivity.

The present findings with DS-8500a are in accordance with studies of other antidiabetic agents that enhance the effect of GLP-1. Clinical trials of exenatide, a GLP-1 agonist, have shown a restoration of insulin secretion. The short-term administration of exenatide in 13 type 2 diabetes mellitus patients restored insulin secretion in the first (0–10 min) and second (10–180 min) phases after glucose challenge similar to healthy controls[20]. Other studies that administered exenatide to metformin-treated type 2 diabetes mellitus patients for 1 or 3 years reported improved β-cell function, as well as enhanced insulin secretion and glycemic control[25,26]. The administration of sitagliptin, a DPP-IV inhibitor, for 12 months in type 2 diabetes mellitus patients who had received metformin for 8 months improved glycemic control and β-cell function compared with metformin alone[27]. Furthermore, a study of vildagliptin, another DPP-IV inhibitor, administered for 52 weeks reported increased β-cell secretion ability in drug-naïve type 2 diabetes mellitus patients[28].

DS-8500a was well tolerated by the patients, causing no hypoglycemic events, and had no significant effects on bodyweight, blood pressure or pulse. In terms of safety, DS-8500a showed a similar profile to other GPR119 agonists. GSK263 in drug-naïve type 2 diabetes mellitus patients or type 2 diabetes mellitus patients taking metformin, as well as JNJ-38431055 and PSN821 in type 2 diabetes mellitus patients, were generally well tolerated[22,29,30]. The present study also showed an improved lipid profile after treatment with DS-8500a, similar to that reported for other GPR119 agonists GSK263 and PSN821[22,29]. The current study findings are also in accord with our previous non-clinical and clinical findings, further

Table 2 | Results of combined euglycemic-hyperinsulinemic and hyperglycemic clamp (full analysis set)

	Baseline		Day 28		
	Placebo	DS-8500a	Placebo	DS-8500a	LSM difference *vs* placebo (95% CI)
Insulin (min × μU/mL)					
First-phase secretion $AUC_{180–190\ min}$	65.28 ± 23.65	65.18 ± 31.13	61.84 ± 21.54	73.52 ± 32.01	11.63** (5.32, 17.93)
Second-phase secretion $AUC_{190–300\ min}$	1135.65 ± 515.63	1166.93 ± 478.02	1006.68 ± 469.86	1258.97 ± 668.41	238.41* (61.04, 415.78)
First-phase secretion $iAUC_{180–190\ min}$	21.85 ± 12.69	20.51 ± 18.90	19.65 ± 11.77	28.90 ± 15.66	9.84* (4.36, 15.33)
Second-phase secretion $iAUC_{190–300\ min}$	489.27 ± 370.48	502.21 ± 468.36	373.92 ± 371.59	510.44 ± 488.90	124.98 (–42.06, 292.02)
C-peptide (min × ng/mL)					
First-phase secretion $AUC_{180–190\ min}$	9.27 ± 2.41	9.61 ± 3.45	9.41 ± 2.83	11.55 ± 4.17	2.07** (0.94, 3.19)
Second-phase secretion $AUC_{190–300\ min}$	275.23 ± 93.60	286.55 ± 81.99	275.41 ± 99.07	345.45 ± 132.50	76.53*** (51.74, 101.32)
First-phase secretion $iAUC_{180–190\ min}$	2.89 ± 1.29	2.94 ± 1.98	2.75 ± 1.58	4.07 ± 1.97	1.35*** (0.92, 1.77)
Second-phase secretion $iAUC_{190–300\ min}$	165.76 ± 73.12	173.41 ± 76.34	163.84 ± 76.41	207.16 ± 100.69	45.06*** (25.63, 64.48)
M value	4.99 ± 2.03	5.03 ± 2.34	4.96 ± 2.35	4.92 ± 2.24	–0.04 (–0.54, 0.47)
M/*I* value	0.075 ± 0.038	0.074 ± 0.044	0.082 ± 0.042	0.080 ± 0.041	0.002 (–0.001, 0.009)
DI for insulin (iAUC180–190 min × *M* value)	95.45 ± 64.78	96.38 ± 95.21	97.27 ± 69.94	129.01 ± 91.27	33.3* (8.85, 57.80)
DI for C-peptide (iAUC180–190 min × *M* value)	13.51 ± 8.42	14.00 ± 10.48	13.52 ± 9.98	18.47 ± 10.63	5.2*** (3.16, 7.31)
MCRI	731.67 ± 354.88	651.61 ± 140.61	765.63 ± 194.96	747.04 ± 166.14	–2.05 (–69.51, 65.41)

Results are presented as the mean ± standard deviation. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs placebo. CI, confidence interval; DI, disposition index; iAUC, incremental area under the curve; LSM, least squares mean; MCRI, metabolic clearance rate of insulin.

Table 3 | Lipid profile (full analysis set)

	Placebo			DS-8500a			
	Baseline	Day 28	% change from baseline (95% CI)	Baseline	Day 28	% change from baseline (95% CI)	LSM difference *vs* placebo (95% CI)
Total cholesterol (mg/dL)	198.6 ± 32.2	196.6 ± 29.7	−0.493 (−4.56, 3.57)	198.1 ± 32.1	178.5 ± 24.5	−8.92*** (−13.50, −4.34)	−8.52††† (−12.59, −4.44)
HDL cholesterol (mg/dL)	50.2 ± 10.6	49.0 ± 9.4	−1.85 (−5.00, 1.31)	49.7 ± 10.8	51.5 ± 10.9	4.39 (−0.25, 9.03)	5.23† (0.17, 0.30)
LDL cholesterol (mg/dL)	129.9 ± 29.5	127.1 ± 26.6	−1.12 (−6.33, 4.09)	130.1 ± 28.9	112.0 ± 21.3	−12.29*** (−18.65, −5.93)	−10.96†† (−17.36, −4.56)
TG (mg/dL)	155.7 ± 108.5	157.3 ± 119.5	1.83 (−6.61, 10.27)	149.0 ± 96.7	106.5 ± 58.8	−23.26*** (−32.67, −13.85)	−27.34††† (−38.00, −16.68)

Results are presented as the mean ± standard deviation. ***$P < 0.001$ vs placebo; †$P < 0.05$, ††$P < 0.01$ and †††$P < 0.001$. CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LSM, least squares mean; TG, triglycerides.

demonstrating the potential of DS-8500a as a well-tolerated antidiabetic drug for glycemic control in type 2 diabetes mellitus patients.

The present study had some limitations. First, the sample size was small, which limited the power of statistical analysis. Additionally, the study was carried out using treatment-naïve patients who tended to have a short duration of type 2 diabetes mellitus, and no diabetic complications (e.g., retinopathy, nephropathy or neuropathy). Therefore, the generalizability to other type 2 diabetes mellitus populations is unclear. In addition, the administration period was short (28 days). Nevertheless, an improvement in β-cell function might be expected by long-term DS-8500a administration. β-Cell function in humans cannot be evaluated by biopsy. Thus, after long-term treatment administration, it is evaluated comprehensively using insulin secretion ability and other markers. As shown in the exenatide 3-year administration study[25], it is better to repeat a clamp test during the off-drug period after long-term treatment administration to evaluate β-cell function. Future studies should therefore enroll patients with advanced disease who are undergoing long-term treatment.

In conclusion, the present cross-over study evaluated the effects of DS-8500a on pancreatic β-cell function in Japanese type 2 diabetes mellitus patients. Overall, DS-8500a enhanced first- and second-phase insulin secretory capacity compared with the placebo, and it was well tolerated with no hypoglycemic events.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 | Study design, flow and procedures. This phase 2, randomized, placebo-controlled, double-blind, cross-over study evaluated changes in insulin secretory capacity and safety in patients with type 2 diabetes mellitus (T2DM) after a 28-day oral administration of 75-mg DS-8500a. Treatment-naïve patients were enrolled, observed for 2 weeks and randomly assigned into two groups to receive oral placebo or 75-mg DS-8500a for 4 weeks, followed by a 4-week washout period and then another 4-week administration of placebo or 75-mg DS-8500a. At the beginning and end of the two treatment periods, combined euglycemic-hyperinsulinemic and hyperglycemic clamp tests were carried out. The follow-up period was 14 days. HbA1c, glycated hemoglobin; wks, weeks

Figure S2 | Combined euglycemic-hyperinsulinemic and hyperglycemic clamp procedure. For the euglycemic-hyperinsulinemic clamp, insulin was injected at an infusion rate of 1.25 mU/kg/min. Glucose was injected to maintain a target glucose level of 90 mg/dL. The *M* value was calculated as the mean glucose infusion rate over 30 min from 90 to 120 min after the start of insulin infusion. For the hyperglycemic clamp, 180 min after the start of insulin infusion, a 50% glucose solution (0.3 g/kg) was injected by bolus for approximately 30 s followed by 20% glucose. The insulin secretory capacity was measured as first-phase secretion and second-phase secretion.

Table S1 | Change in insulin secretory capacities determined by insulin or C-peptide from baseline to day 28.