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ORIGINAL ARTICLE

Effects of saxagliptin on β-cell stimulation and insulin secretion in patients with type 2 diabetes

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Aim: To study the effect of dipeptidyl peptidase-4 (DPP-4) inhibition with saxagliptin on β-cell function as reflected by the stimulated insulin secretion rate after an enteral glucose load in patients with type 2 diabetes.

Methods: Patients in this randomized, parallel-group, double-blind, placebo-controlled study were drug-naïve, aged 43–69 years, with baseline haemoglobin A1c (HbA1c) 5.9–8.1%. Twenty patients received saxagliptin 5 mg once daily; 16 received placebo. Patients were assessed at baseline and week 12 by intravenous hyperglycaemic clamp (0–180 min, fasting state), and intravenous-oral hyperglycaemic clamp (180–480 min, postprandial state) following oral ingestion of 75 g glucose. Primary and secondary endpoints were percent changes from baseline in insulin secretion during postprandial and fasting states, respectively. Insulin secretion was calculated by C-peptide deconvolution.

Results: After 12 weeks, saxagliptin significantly increased insulin secretion percent change from baseline during the postprandial state by an 18.5% adjusted difference versus placebo ($p = 0.04$), an improvement associated with increased peak plasma concentrations of intact glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide. In the fasting state, saxagliptin significantly increased insulin secretion by a 27.9% adjusted difference versus placebo ($p = 0.02$). Saxagliptin also improved glucagon area under the curve in the postprandial state (adjusted difference −21.8% vs. placebo, $p = 0.03$).

Conclusions: DPP-4 inhibition with saxagliptin improves pancreatic β-cell function in postprandial and fasting states, and decreases postprandial glucagon concentration. Given the magnitude of enhancement of the insulin response in the fasting state, further study into the effect of DPP-4 inhibition on the β-cell is warranted.

Keywords: β cell, DPP-4 inhibitor, GIP, GLP-1, insulin secretion, type 2 diabetes

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Introduction

The endogenous incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) regulate blood glucose via mechanisms that include stimulation of insulin secretion (GLP-1 and GIP) and inhibition of glucagon secretion (GLP-1) in a glucose-dependent manner. GLP-1 and GIP are secreted in response to enteral nutrient loads, but are rapidly cleaved by the ubiquitous enzyme dipeptidyl peptidase-4 (DPP-4). DPP-4 inhibitors lower postprandial blood sugar by preventing rapid degradation of incretins, thereby enhancing glucose-mediated insulin release and reducing postprandial glucagon secretion. Data from preclinical studies show that incretins can inhibit β-cell apoptosis and necrosis, as well as stimulate β-cell proliferation and increase β-cell mass [1–3]. Because the progression of type 2 diabetes results in an inexorable decline in β-cell mass and function over time, slowing, preventing or reversing β-cell deterioration could potentially alter disease progression.

Saxagliptin is a potent, selective DPP-4 inhibitor specifically designed for extended inhibition of the DPP-4 enzyme. Previous reports describe the efficacy and safety of saxagliptin as treatment for type 2 diabetes [4–9], and homeostatic model assessment-2 of beta cell function (HOMA-2β) assessments show that saxagliptin improves β-cell function [4–6,9]. Other studies with the DPP-4 inhibitors sitagliptin [10–14] and vildagliptin [15–19] have incorporated various homeostatic, mathematical and infusion clamp models to investigate the effects of DPP-4 inhibition on insulin secretion, incretin levels and glucagon concentration in patients with type 2 diabetes. For this trial (CV181-041) (Clinical trial reg. no. NCT00374907, clinicaltrials.gov), we used a sequential intravenous and intravenous-oral hyperglycaemic clamp to study the effects of saxagliptin in fasting and postprandial states in patients with type 2 diabetes. An advantage of the hyperglycaemic

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clamp method is the ability to maintain plasma glucose—the major stimulus of β-cell function—at a constant level. Thus, insulin secretory capacity in fasting and postprandial states can be measured directly, rather than through mathematical modelling that is dependent on dynamically changing glucose concentrations.

We hypothesized that DPP-4 inhibition with saxagliptin for 12 weeks would improve β-cell function as reflected by an increase in the stimulated insulin secretion rate after an enteral glucose load. Additionally, we assessed the postprandial responses of GLP-1, GIP and glucagon under clamped glucose conditions, and the acute insulin response to intravenous arginine as an indicator of total β-cell secretory capacity.

Methods

Patients

Men and women aged 18–70 years were eligible. Major inclusion criteria were haemoglobin A1c (HbA1c) 6–8% at screening, body mass index $\leq$40 kg/m^2, fasting C-peptide level $\geq$1 ng/ml, and no antihyperglycaemic therapy for more than 3 consecutive days or a total of 7 non-consecutive days during 8 weeks prior to screening. Major exclusion criteria were poorly controlled diabetes, history of diabetic ketoacidosis or hyperosmolar nonketotic coma, aspartate transaminase and/or alanine transaminase $\geq$2 times the upper limits of normal (ULN), creatine phosphokinase $\geq$3 times ULN, and an unstable condition or serious cardiovascular or renal disease.

Trial Design

This phase 3, randomized, parallel-group, double-blind, placebo-controlled study was conducted at three sites in the USA, in accordance with the Declaration of Helsinki. Institutional review boards for participating centres approved the protocol. All patients provided written informed consent.

After screening for patient eligibility and a 2-week single-blind placebo lead-in period with dietary and exercise instruction, patients were randomized (1 : 1) to saxagliptin 5 mg or placebo for 12 weeks of double-blind treatment. Baseline values were obtained at randomization, separate from the values obtained at screening. Treatment was administered orally, once daily, prior to the morning meal.

On days −1 and 84, relative to the day of randomization, patients underwent a sequential intravenous hyperglycaemic clamp, intravenous-oral hyperglycaemic clamp [20], and arginine stimulation test [21], described below. Samples for glucose, insulin, glucagon, GLP-1 and GIP levels were drawn at designated intervals prior to and throughout the infusion. During the intravenous clamp (0–180 min), patients received a primed continuous infusion of 20% glucose, adjusted by variable rate to maintain hyperglycaemia at 280 mg/dl—a level sufficiently elevated to stimulate β-cell activity. Additionally, this level of hyperglycaemia permitted the infusion to be titrated down, but not suspended, to keep the plasma glucose clamped at 280 mg/dl after an oral glucose challenge. Bedside plasma glucose was determined every 5–10 min using a Yellow Springs Instrument (Yellow Springs, OH, USA), and adjusted by infusion rate as previously described [22]. For testing on day 84, saxagliptin 5 mg or placebo was administered 30 min before the start of the glucose infusion. During the intravenous-oral clamp (180–480 min), patients received a 75-g oral glucose challenge at 180 min, and the glucose infusion rate was adjusted to maintain plasma levels at 280 mg/dl. At 480 min, the glucose infusion was abruptly increased to achieve and maintain a plasma glucose level of 450 mg/dl at which a robust and near-maximal acute insulin response could be observed following stimulation with intravenous arginine. At 505 min, arginine 5 g was administered intravenously over 30 s. The glucose infusion rate was adjusted to maintain plasma glucose at 450 mg/dl through the end of the test at 515 min.

Insulin secretion rate was measured by C-peptide deconvolution using the ISEC (insulin secretion) program [22–24], which uses a population model to derive parameters of C-peptide kinetics from gender, diabetes status, age, weight and height of patients. ISEC was programmed with a discontinuity at the start of the oral glucose challenge to avoid smoothing of postprandial calculated secretion rates into fasting time-points by the deconvolution algorithm. Intact GLP-1 [25] and GIP [26] were measured as previously described, after ethanol extraction of plasma samples. Free-standing oral glucose tolerance tests (OGTTs) (0–300 min) were conducted separately during the trial, that is 2 days prior to the clamp procedures. Patients completing 12 weeks of study were eligible for continuation into a long-term extension totalling 102 weeks. Results of the OGTTs performed outside the setting of the hyperglycaemic clamp and results of the long-term extension are not reported here.

Efficacy and Safety Criteria (Saxagliptin 5 mg vs. Placebo)

The primary objective was to determine percent change from baseline at week 12 in area under the curve (AUC) for insulin secretion rate during the intravenous-oral clamp (180–480 min) following the oral glucose challenge (180 min). The secondary objective was to determine percent change from baseline in AUC for insulin secretion rate during the intravenous clamp (120–180 min). Tertiary objectives included determination of changes from baseline in insulin secretion following intravenous arginine stimulation, measured as the acute insulin response in the first 5 min to arginine (AIR_{arg}); intact GLP-1 and GIP concentrations during the intravenous-oral clamp; glucagon concentration during the intravenous-oral and intravenous clamp; and glycaemic control. Safety analyses included adverse events summarized by preferred term [Medical Dictionary for Regulatory Activities (MedDRA version 10.1)], laboratory results, electrocardiograms and vital signs.

Statistical Analysis

Efficacy analyses were conducted utilizing last observation carried forward (LOCF) methodology. In cases of early discontinuation, the week 12 clamp procedure was not allowed to be performed earlier than week 8. The primary endpoint was evaluated by analysis of covariance (ANCOVA) on logarithms of post- to pretreatment ratios with treatment group as an effect and the logarithm of baseline value as the covariate.

The secondary efficacy analysis utilized the same method and ANCOVA model to compare the geometric mean percent changes from baseline at week 12 between treatment groups. Interpretation of p-value was contingent upon significance of the primary endpoint; statistical testing of secondary endpoints proceeded sequentially to control overall type I error rate at the 0.05 level. Estimates and 95% confidence intervals (CIs) were calculated for adjusted geometric mean percent change from baseline within each treatment group, as well as for comparisons between groups.

AIR_{arg} was calculated as incremental (i.e., above pre-infusion of arginine values) differences in mean values [21]. The difference between AIR_{arg} values of saxaglitpin 5 mg and placebo was tested using the Kruskal–Wallis method, and reported as median and quartile values, to account for skewed distribution of data. Week 12 changes from baseline in glucagon AUC during intravenous-oral clamp, glucagon concentration during intravenous clamp, and glycaemic control were assessed using ANCOVA.

Results

Patients

Of 156 patients who were enrolled and screened, 46 met inclusion/exclusion criteria and entered the lead-in period. Following lead-in, 37 patients were randomized. Of the 36 patients who were treated, 32 completed the 12-week trial. The four discontinuations were for withdrawal of consent (saxagliptin group, 3; placebo group, 1).

Demographic and baseline characteristics were generally balanced between groups (Table 1). Baseline HbA1c, drawn at randomization, ranged from 5.9 to 8.1% for all patients. Mean body weight was 94 kg and mean body mass index (BMI) was 33 kg/m^2. Although mean weight and BMI were generally similar in both groups, the median body weight was 10 kg higher in the saxagliptin group than in the placebo group. Mean duration of exposure to double-blind treatment was shorter in the saxagliptin group (mean 76.2 days) than in the placebo group (85.2 days); the shorter exposure in the saxagliptin group was attributable to discontinuations for withdrawn consent.

Table 1. Patient characteristics.

	Saxagliptin 5 mg (n = 20)	Placebo (n = 16)
Age (years)	55.2 ± 8.6	56.2 ± 6.9
Female patients	12 (60.0)	10 (62.5)
Weight (kg)	95.0 ± 15.0	92.5 ± 13.5
BMI (kg/m^2)	33.5 ± 3.7	32.2 ± 3.9
Duration of type 2 diabetes (years)	2.7 ± 4.4	3.7 ± 4.0
HbA1c (%)	6.9 ± 0.5	6.6 ± 0.6
FPG (mmol/l) [mg/dl]	7.3 ± 1.2 [131.5 ± 22.0]	6.9 ± 1.2 [124.7 ± 21.5]
HOMA-2β (%)	102.2 ± 27.4	121.9 ± 62.3

BMI, body mass index; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c. Data are means ± standard deviation or n (%).

Thirty-one patients (saxagliptin group, 16; placebo group, 15) had baseline assessments and ≥1 post-randomization assessment of the primary endpoint, and were included in the primary efficacy analysis. At week 12, mean change from baseline in body weight was −1.3 kg (95% CI −2.6 to 0.0) for the saxagliptin group and −0.4 kg (95% CI −1.5 to 0.7) for the placebo group, with median changes of −0.7 and −0.6 kg, respectively.

Efficacy

Saxagliptin treatment for 12 weeks increased glucose-stimulated insulin secretion. Figure 1A shows the insulin secretion rates deconvoluted by ISEC, with the programmed discontinuity at 180 min reflecting the start time of the oral glucose challenge. The primary and secondary endpoints were derived from the AUCs of these curves during the respective 180–480 min and 120–180 min intervals. In spite of randomization, average β-cell function was better at baseline in both fasting and postprandial states for the placebo group compared with the saxagliptin group. These differences were no longer apparent at week 12 when insulin secretion had increased with saxagliptin but slightly decreased with placebo in both fasting and postprandial states.

Primary Endpoint. At week 12, there was a statistically significant mean increase in insulin secretion percent change from baseline with saxagliptin versus placebo during the intravenous-oral clamp (180–480 min). In the saxagliptin group, insulin secretion increased from a geometric mean of 2818 pmol/kg (baseline) to 3303 pmol/kg (week 12); in the placebo group, there was a decrease from 3687 pmol/kg (baseline) to 3564 pmol/kg (week 12). Figure 1B shows adjusted percent changes from baseline; the difference for saxagliptin versus placebo was an increase of 18.5% (95% CI 1.3 to 38.7, p = 0.04).

Secondary Endpoint. Saxagliptin treatment resulted in a statistically significant mean increase in insulin secretion percent change from baseline compared to placebo during the intravenous clamp (120–180 min). In the saxagliptin group, insulin secretion increased from a geometric mean of 446 pmol/kg (baseline) to 552 pmol/kg (week 12); in the placebo group, insulin secretion decreased from 594 pmol/kg (baseline) to 563 pmol/kg (week 12). Figure 1C shows adjusted percent changes from baseline; the difference for saxagliptin versus placebo was an increase of 27.9% (95% CI 4.2 to 57.1, p = 0.02).

Tertiary Endpoints. Improvement in glucose disposal was measured by the glucose infusion rate, adjusted during the hyperglycaemic clamp to maintain plasma glucose at ~280 mg/dl. Increased glucose disposal—a function of greater insulin secretion and decreased glucagon secretion—would require upward adjustment of the infusion rate. Plasma glucose levels were similar during the clamp procedures in both groups (figure 2A, B). At week 12, postprandial glucose disposal improved with saxagliptin treatment, as shown by a higher mean infusion rate required to maintain plasma glucose at 280 mg/dl (figure 2C, D). During the intravenous-oral clamp, the adjusted mean change from baseline at week

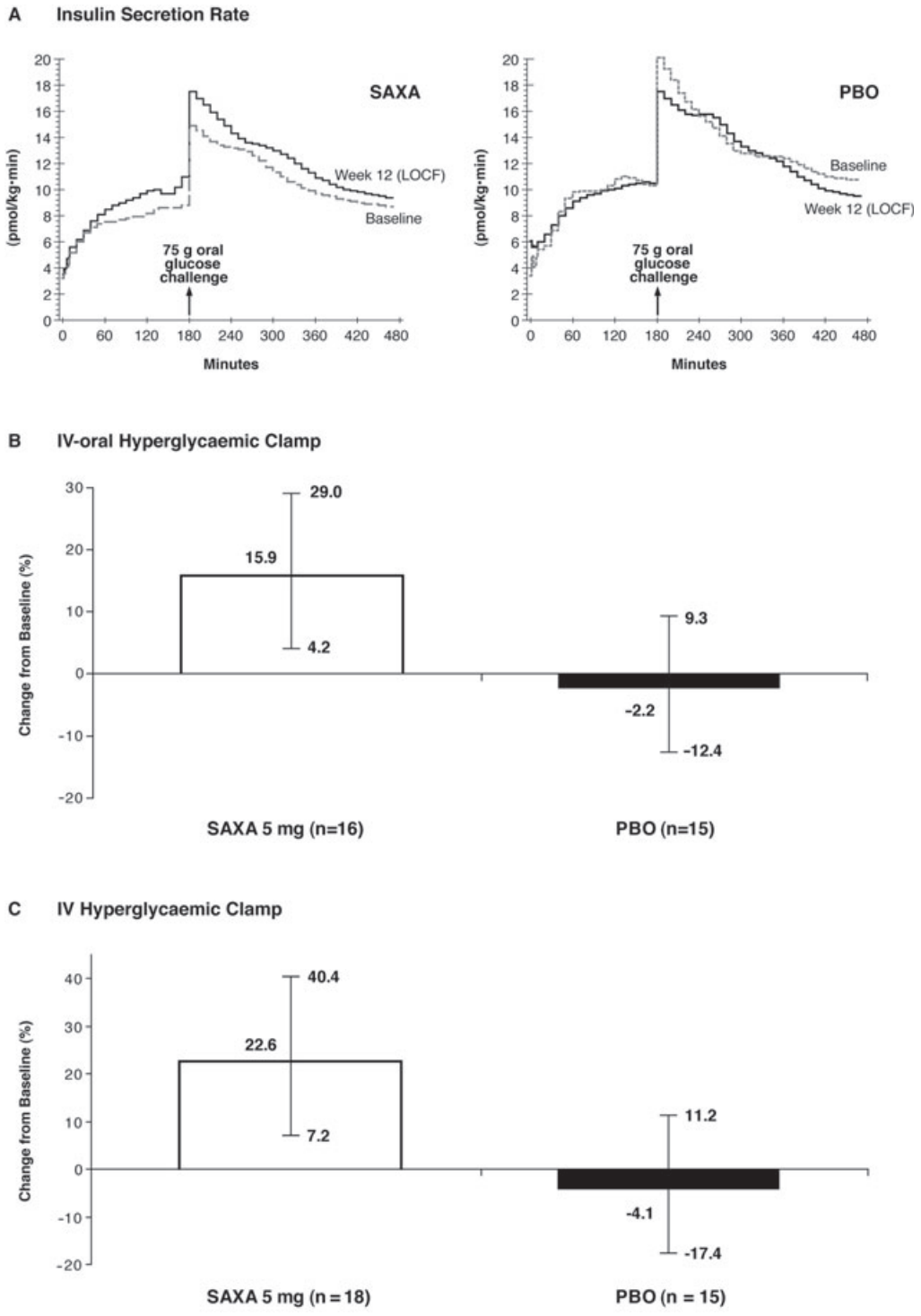

Figure 1. (A) Mean insulin secretion rate during hyperglycaemic clamp. (B and C) Insulin secretion change at week 12 during intravenous-oral hyperglycaemic clamp (IV-oral hyperglycaemic clamp) and intravenous hyperglycaemic clamp (IV hyperglycaemic clamp), shown as adjusted geometric mean percent change from baseline and 95% confidence interval. LOCF, last observation carried forward; PBO, placebo; SAXA, saxagliptin.

12 in glucose infusion rate with saxagliptin was 1.9 mg/kg min (s.e. 0.77) versus −0.7 mg/kg min (s.e. 0.85) with placebo, a significant difference of 2.6 mg/kg min (s.e. 1.18, $p = 0.03$). During the intravenous clamp, the adjusted mean change from baseline at week 12 with saxagliptin was 1.7 mg/kg min (s.e. 0.59), compared with 0.4 mg/kg min (s.e. 0.65) with placebo, a non-significant difference of 1.3 mg/kg min (s.e. 0.90, $p = 0.17$).

Initially, AIR_{arg} was greater in the placebo group, but decreased over the course of the trial from a median of

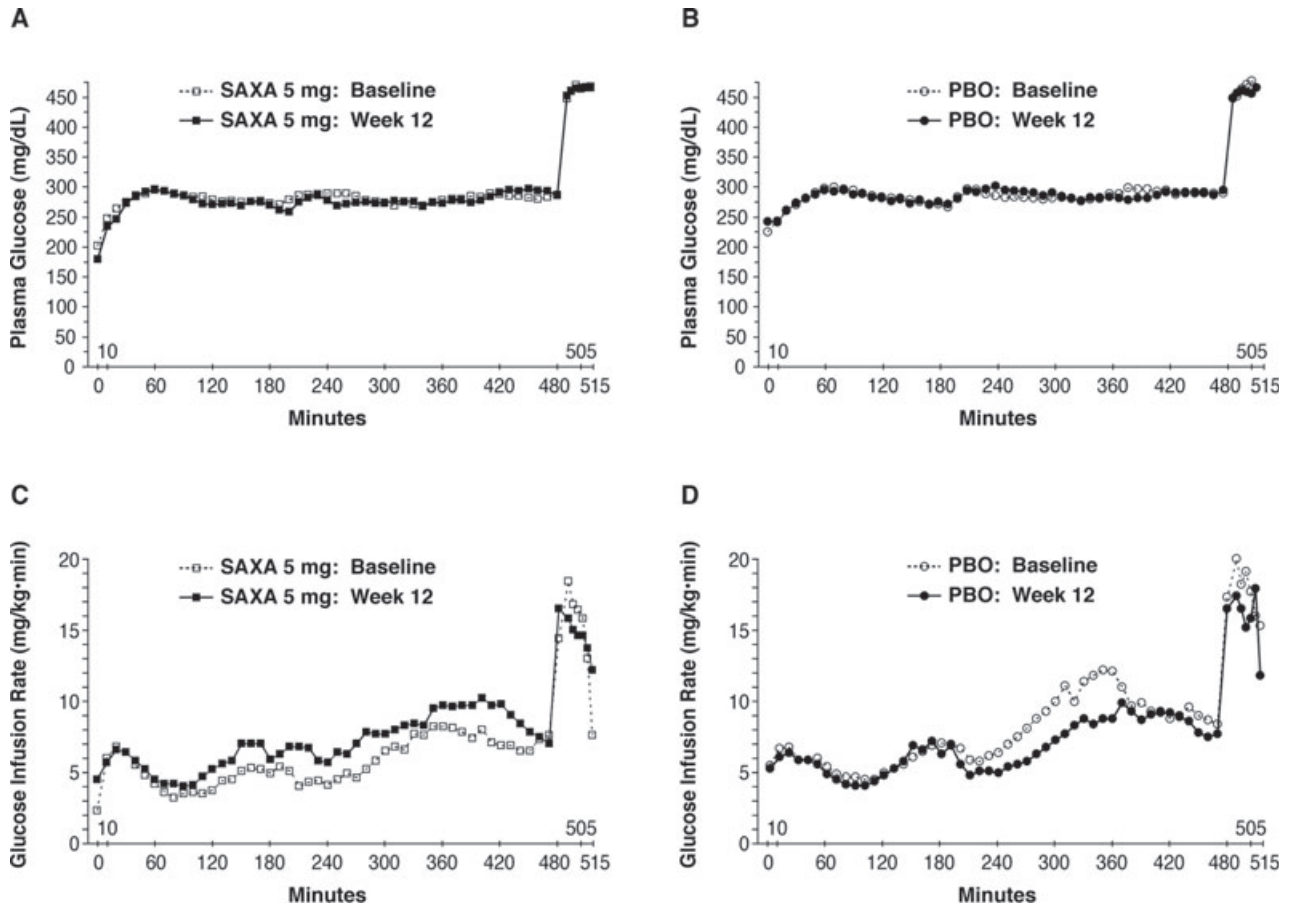

Figure 2. (A and B) Mean glucose concentration during hyperglycaemic clamp. (C and D) Mean glucose infusion rate during hyperglycaemic clamp. PBO, placebo; SAXA, saxagliptin.

204 µU/ml (Q1, Q3: 175, 268) at baseline to 185 µU/ml (Q1, Q3: 147, 208) at week 12. By contrast, median AIR_{arg} in the saxagliptin group increased from 164 µU/ml (Q1, Q3: 107, 203) at baseline to 172 µU/ml (Q1, Q3: 136, 228) at week 12, although the difference in change from baseline between these small-size groups was not statistically significant ($p = 0.074$) (figure 3).

Peak concentrations of intact GLP-1 and GIP increased at week 12 in the saxagliptin group following oral glucose challenge during the intravenous-oral clamp (figure 4A, B). There was little change, however, in the incretin effect during the hyperglycaemic clamp, calculated as the ratio of AUCs (normalized for time) of insulin or of C-peptide following oral glucose challenge versus AUCs prior to oral glucose challenge. The incretin effect on insulin was 57.8% (baseline) and 56.3% (week 12) for the saxagliptin group, compared with 78.5% (baseline) and 76.5% (week 12) for the placebo group. The incretin effect on C-peptide was 37.4% (baseline) and 31.9% (week 12) for the saxagliptin group, compared with 42.0% (baseline) and 36.1% (week 12) for the placebo group.

Glucagon levels were assessed by AUC from 180 to 480 min during intravenous-oral clamp. Mean glucagon AUC was lower at baseline in the placebo group (Table 2, figure 5). At week 12, mean glucagon AUC from 180 to 480 min decreased with saxagliptin and increased with placebo (significant difference of −3352 pg min/ml; $p = 0.03$). Percent changes between baseline and week 12 were −15.4% with saxagliptin versus 8.2% with placebo ($p = 0.03$). During the intravenous clamp,

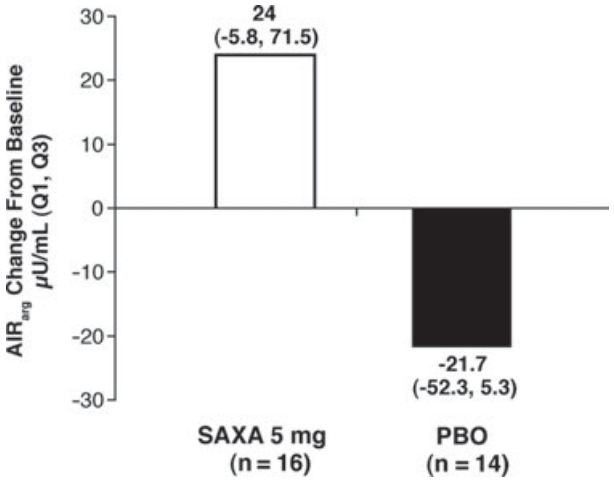

Figure 3. Acute insulin response to arginine (AIR_{arg}): median change (Q1, Q3) from baseline at week 12. PBO, placebo; SAXA, saxagliptin.

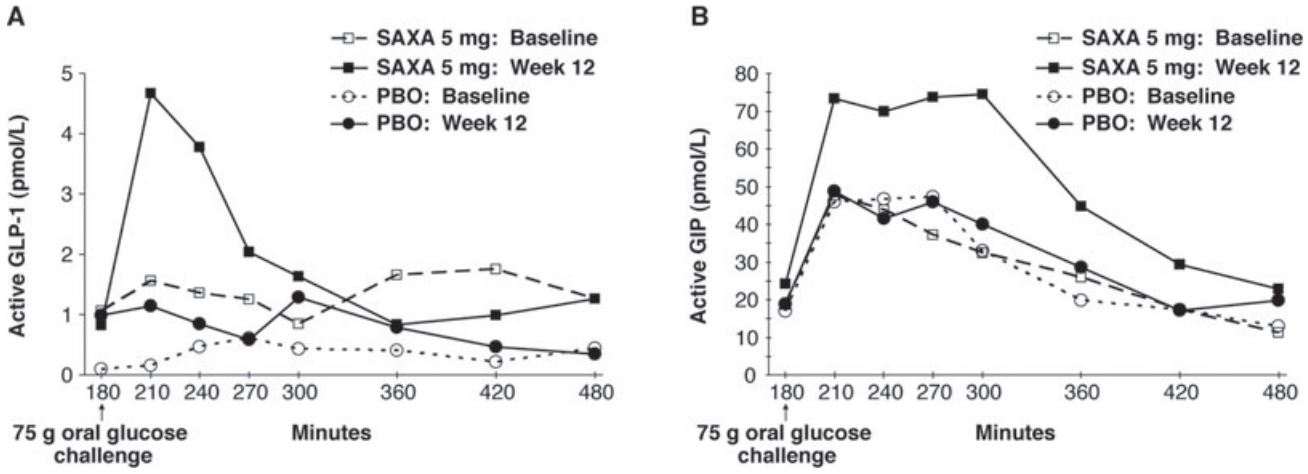

Figure 4. Mean active GLP-1 (A) and GIP (B) concentrations during intravenous-oral hyperglycaemic clamp at baseline and week 12. PBO, placebo; SAXA, saxagliptin.

Table 2. Glucagon secretion: changes from baseline at week 12.

	Saxagliptin 5 mg	Placebo
Glucagon AUC during intravenous-oral hyperglycaemic clamp (pg min/ml)	n = 17	n = 14
Baseline	14 279 ± 1228	11 177 ± 880
Week 12 (LOCF)	11 571 ± 1113	12 965 ± 1273
Week 12 adjusted change from baseline	−2191 (−4153 to −229)	1161 (−1014 to 3336)
Week 12 difference versus placebo	−3352* (−6371 to −333)	
Glucagon concentration during intravenous hyperglycaemic clamp (pg/ml)	n = 18	n = 15
Baseline	50.2 ± 3.54	36.8 ± 3.43
Week 12 (LOCF)	41.7 ± 3.75	45.5 ± 3.95
Week 12 adjusted change from baseline	−5.7 (−12.6 to 1.3)	5.5 (−2.2 to 13.2)

Data are means ± s.e. or means (95% CI). AUC, area under the curve; CI, confidence interval; LOCF, last observation carried forward; ANCOVA, analysis of covariance model.
*p value versus placebo = 0.03.

mean glucagon concentration also decreased at week 12 with saxagliptin and increased with placebo, although the difference was not statistically significant (Table 2).

Glycaemic control was not markedly different between the groups. Mean HbA1c values were 6.9% at baseline and 6.8% at week 12 in the saxagliptin group, and 6.6% at both baseline and week 12 in the placebo group.

Safety

Saxagliptin 5 mg daily was well tolerated. There were no deaths, serious adverse events, or discontinuations due to adverse events in either group. Seventeen (85.0%) saxagliptin-treated patients and 11 (68.8%) placebo-treated patients experienced at least one adverse event, including confirmed or unconfirmed events of hypoglycaemia, with no single preferred term explaining the difference in proportions. Adverse events reported by preferred term for more than one patient per treatment group included headache (saxagliptin, n = 3; placebo, n = 1), muscle spasms (saxagliptin, n = 3), sinusitis (saxagliptin, n = 2), paraesthesia (saxagliptin, n = 2), infusion site pain (saxagliptin, n = 2), fatigue (placebo, n = 2), cough (placebo, n = 2) and sinus congestion (placebo, n = 2). Two (10.0%) saxagliptin-treated patients had an adverse event of hypoglycaemia versus one (6.3%) placebo-treated patient; none was confirmed by a fingerstick glucose value ≤50 mg/dl. No cardiovascular adverse events were reported in either group.

Discussion

In this study we report that DPP-4 inhibition with saxagliptin for 12 weeks significantly improved insulin secretion in patients with type 2 diabetes during both postprandial and fasting states, as well as improved glucose disposal, and significantly lowered glucagon levels during the postprandial state. Patients were in the early course of diabetes and had β-cell function that was fairly well preserved. Baseline HOMA-2β [27] estimates of β-cell function were greater than 100% for both groups (Table 1). The mild diabetes of these patients minimized the concern that glucotoxicity would confound baseline β-cell function measurements, or that relief of glucotoxicity by treatment would confound interpretation of results. At week 12, the adjusted mean change from baseline in HbA1c was −0.14% for the saxagliptin group and 0.02% for the placebo group.

Although demographic and diabetes characteristics were generally well balanced, β-cell function at baseline was better in the placebo group which on average secreted more insulin during the clamp procedures. However, over 12 weeks, β-cell function slightly declined with placebo but improved with saxagliptin. Improvements in the insulin secretion rates

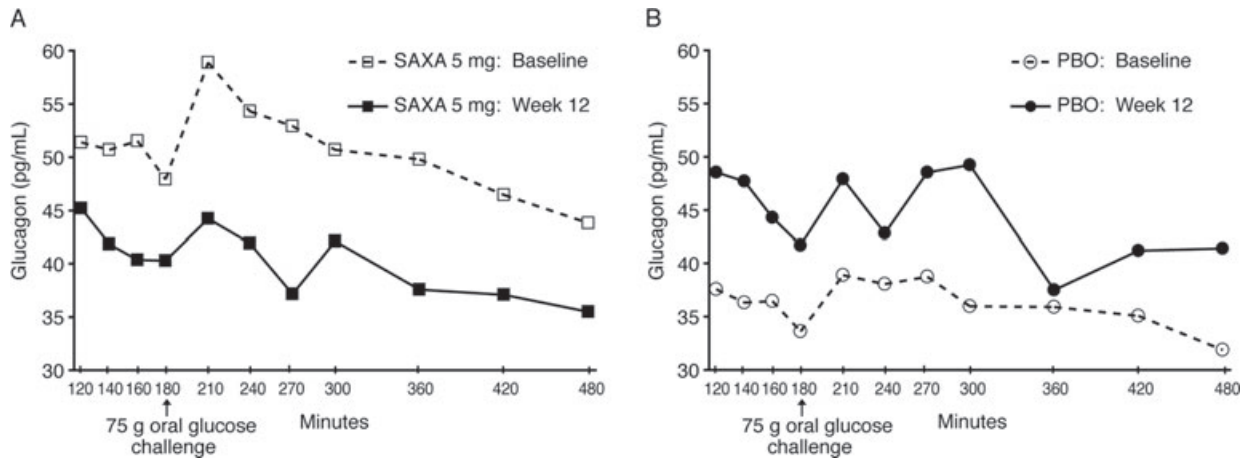

Figure 5. Mean baseline and week 12 glucagon concentrations from minutes 120 to 480 of hyperglycaemic clamp. PBO, placebo; SAXA, saxagliptin.

during both the postprandial and fasting states were statistically significant with saxagliptin versus placebo.

A number of sitagliptin [12–14] and vildagliptin [15,16, 28–31] trials have utilized mathematical or statistical methodologies to describe changes in insulin levels or secretory rates that accompany DPP-4 inhibition. Fewer studies have employed stepped clamp [17,19] or ramp [32] procedures to measure insulin response, such as trials with vildagliptin using glucose-insulin infusions. The present trial employed a different approach that utilized a hyperglycaemic clamp without, and with, enteral stimulation. An attribute of this technique is the ability to maintain a prespecified, absolute level of glycaemia while measuring the insulin response. Thus, β-cell responses can be measured against identical levels of plasma glucose [20]. During the intravenous-oral clamp, saxagliptin treatment produced an 18.5% improvement over placebo in the mean percent change from baseline in insulin secretion at week 12. This enhanced insulin secretion indicated significant improvement in β-cell responsiveness in the setting of an oral glucose stimulus to incretin secretion. Also significant, and somewhat unexpected in degree, was the increase in insulin secretion during the intravenous clamp which measured β-cell response in the fasting state. Even without a prandial stimulus to incretin secretion, patients receiving saxagliptin showed a 27.9% improvement over placebo in mean percent change from baseline in insulin secretion at week 12.

Surprisingly, our assessment of the incretin effect on insulin and C-peptide levels during the clamp procedure did not show a qualitative difference between saxagliptin and placebo groups. This finding reflects that saxagliptin was associated with augmentation of the insulin response not only to prandial glucose but also to intravenous glucose, so that no net change in incretin effect was evident. Salehi et al. reported that in patients with well-controlled type 2 diabetes, GLP-1 blockade with exendin-(9–39) reduced insulin secretion in response to intravenous glucose alone [33]. In contrast, our study was designed around incretin potentiation rather than incretin blockade. Recently, Vardarli et al. reported that DPP-4 inhibition with vildagliptin increased insulin secretion after both oral and isoglycaemic intravenous glucose, without a numerical change in the incretin effect [34]. Results from each of these studies suggest that endogenous GLP-1 plays a role in promoting insulin secretion regardless of the mode of glucose administration. However, the full mechanisms behind the balanced positive effect that we observed during both the fasting and postprandial states have yet to be explained. Conceptually, it is possible that DPP-4 inhibition preserves β-cell mass or improves global β-cell health. Islets isolated from mice that underwent long-term DPP-4 inhibition showed not only increased insulin responsiveness and glucose sensitivity, but also stabilization of islet size, suggesting that islet function *per se* had improved with DPP-4 inhibition [35]. Yet longer-term clinical investigations of 1 year with the incretin mimetic exenatide [36] and 2 years with vildagliptin [30] have not shown maintained improvement in β-cell function after cessation of therapy. Perhaps our result is more likely explained by an effect of saxagliptin to increase intact GLP-1 in the basal state, leading to improvements in β-cell responsiveness to glucose stimulation. Kjems et al. described how even a low-dose infusion of GLP-1 produced an increased rate of insulin secretion in response to infused glucose in patients with type 2 diabetes [37]. Whether these or other underlying mechanisms fully explain our result is presently unclear.

Despite the global increase in β-cell function with saxagliptin treatment that we observed irrespective of the prandial state, postprandial incretin concentrations did respond to saxagliptin (figure 4A, B), with peak levels of intact GLP-1 and GIP increasing several-fold after oral glucose stimulation. While these postprandial elevations in incretins are comparable to results from other studies with DPP-4 inhibitors [12,16,17], our results are particularly interesting when interpreted against the paradox of what appeared to be a lack of a net incretin effect. Indeed, while the strongest incretin response followed the oral glucose challenge (figure 4A, B), the effect was additive as indicated by the significant increase in insulin secretion during the preceding fasting intravenous clamp. To some extent these results qualify the conventional hypothesis of DPP-4 inhibition which focuses on prandial stimulation when describing incretin effects.

In type 2 diabetes, fasting glucagon is dysregulated and inappropriately elevated, which in turn contributes to increased

hepatic glucose production [38]. After 12 weeks of treatment, mean fasting glucagon concentrations decreased with saxagliptin but increased with placebo, although the difference between groups was not statistically significant. The effects of DPP-4 inhibition on glucagon levels were more pronounced in the postprandial state. Generally, patients with type 2 diabetes show a paradoxical increase in glucagon secretion after an enteral stimulus [38]. At week 12, the placebo group experienced an increase from baseline in mean postprandial glucagon AUC during the intravenous-oral clamp, whereas the saxagliptin group showed a significant decrease ($p = 0.03$). This result is consistent with other saxagliptin [5,7–9], vildagliptin [16–19] and sitagliptin studies [12] that show that DPP-4 inhibition lowers postprandial glucagon. Several mechanisms by which DPP-4 inhibition may decrease postprandial glucagon have been postulated. In diabetic mice with disproportionate elevations of glucagon-positive α-cells, chronic inhibition of DPP-4 with sitagliptin restored β-cell mass and normalized the β-cell-to-α-cell ratio [39,40]. These findings were accompanied by improvements in glycaemic control [39,40], an elevation in postprandial intact GLP-1, and a reduction in postprandial circulating glucagon [40]. In human subjects, the mechanisms of DPP-4 inhibition on islet morphology and α-cell regulation have yet to be fully characterized and await further investigation.

Arginine depolarizes the β-cell membrane leading to insulin secretion [41]. The AIR_{arg} in a hyperglycaemic state is therefore an indicator of total β-cell secretory capacity and has been used as a proxy measure of functional β-cell mass [42–44]. D'Alessio et al. studied patients treated with vildagliptin, and reported that potentiation of β-cell secretion by arginine bolus following a 60-min glucose ramp produced a modest non-significant effect of increasing maximal capacity for insulin release [32]. In this study, AIR_{arg} increased with saxagliptin treatment, although not to a statistically significant extent. Whether these results signal an improvement or stabilization of β-cell mass is unknown without longer-term data and further analyses.

In conclusion, this study showed that DPP-4 inhibition with saxagliptin significantly augments insulin secretion and decreases glucagon concentration in the postprandial state. Insulin secretion also improved significantly when the prandial stimulus to incretin secretion was absent. In light of the magnitude of enhanced insulin response that we observed in the fasting state, further investigation is needed to describe the scope of effects of DPP-4 inhibition on the β-cell.

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Conflict of Interest

R. R. H. and S. R. M. researched data, contributed to discussion, and reviewed/edited manuscript. S. R. S., S. L. S. and R. Y. D. researched data and reviewed/edited manuscript. C. F. D. and J. J. H. contributed to discussion and reviewed/edited manuscript. R. S. C. and J. F. L. researched data, contributed to discussion, wrote manuscript, and reviewed/edited manuscript.

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