

Short Report

Liraglutide, a once-daily human GLP-1 analogue, improves pancreatic B-cell function and arginine-stimulated insulin secretion during hyperglycaemia in patients with Type 2 diabetes mellitus

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Abstract

Aims To assess the effect of liraglutide, a once-daily human glucagon-like peptide-1 analogue on pancreatic B-cell function.

Methods Patients with Type 2 diabetes ($n = 39$) were randomized to treatment with 0.65, 1.25 or 1.9 mg/day liraglutide or placebo for 14 weeks. First- and second-phase insulin release were measured by means of the insulin-modified frequently sampled intravenous glucose tolerance test. Arginine-stimulated insulin secretion was measured during a hyperglycaemic clamp (20 mmol/l). Glucose effectiveness and insulin sensitivity were estimated by means of the insulin-modified frequently sampled intravenous glucose tolerance test.

Results The two highest doses of liraglutide (1.25 and 1.9 mg/day) significantly increased first-phase insulin secretion by 118 and 103%, respectively ($P < 0.05$). Second-phase insulin secretion was significantly increased only in the 1.25 mg/day group vs. placebo. Arginine-stimulated insulin secretion increased significantly at the two highest dose levels vs. placebo by 114 and 94%, respectively ($P < 0.05$). There was no significant treatment effect on glucose effectiveness or insulin sensitivity.

Conclusions Fourteen weeks of treatment with liraglutide showed improvements in first- and second-phase insulin secretion, together with improvements in arginine-stimulated insulin secretion during hyperglycaemia.

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Keywords B-cell function, clinical trial, glucagon-like peptide-1, insulin secretion, liraglutide

Abbreviations AUC, area under curve; CI, confidence interval; FSIGT, insulin-modified frequently sampled intra-venous glucose tolerance test; PG, plasma glucose; S_i , insulin sensitivity

Introduction

At the time of diagnosis of Type 2 diabetes, pancreatic B-cell function is already markedly impaired and the disease is characterized by a progressive failure of the B-cells [1]. Furthermore, first-phase insulin secretion is almost invariably absent [2]. Treatment with the once-daily human glucagon-like peptide-1 (GLP-1) analogue, liraglutide, demonstrates sustained improvement in glycaemic control, reduction of

body weight, low risk of hypoglycaemia and is well tolerated [3–7]. Furthermore, liraglutide has been shown to increase B-cell mass in rodent models of Type 2 diabetes [8–10], decrease B-cell apoptosis in isolated neo-natal rat cells [11] and increase B-cell differentiation in immature human pancreatic cells [12]. In patients with Type 2 diabetes, daily injection of liraglutide for 1 week improved B-cell function as measured by a significant improvement in both first- and second-phase glucose-induced insulin secretion [13]. Another study has shown that a single dose of liraglutide is capable of restoring B-cell responsiveness to glucose during a step-wise glucose clamp [14]. The objective of the current study was to assess the effect of 14 weeks of liraglutide treatment on B-cell function as compared with placebo.

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Table 1 Baseline characteristics for subjects completing the sub-study

Baseline characteristics	Patients with Type 2 diabetes				Control subjects
	Placebo	Liraglutide 0.65 mg/day	Liraglutide 1.25 mg/day	Liraglutide 1.9 mg/day	Untreated
Randomized (<i>n</i>)	10	8	10	11	12
Completers (<i>n</i>)	5	7	9	7	12
Gender [female : male (%)]	20:80	0:100	0:100	14:86	25:75
Age (years)	55.4 ± 6.7	61.1 ± 7.6	56.9 ± 10.1	58.6 ± 10.3	51.3 ± 10.5
Duration of diabetes (years)	1.8 ± 0.8	7.1 ± 2.5	7.9 ± 2.7	4.6 ± 3.2	NA
Previous treatment [diet : mono OGLA (%)]	40:60	29:71	33:67	14:86	NA
HbA _{1c} (%)	8.1 ± 0.3	8.7 ± 0.7	8.4 ± 0.6	8.2 ± 0.6	NA
BMI (kg/m ²)	30.3 ± 4.3	26.6 ± 2.8	29.1 ± 3.2	31.4 ± 3.3	29.2 ± 3.5

Mean ± standard deviation (SD).
BMI, body mass index; HbA_{1c}, glycated haemoglobin; NA, not applicable; OGLA, oral glucose-lowering agent.

Patients and methods

The study was part of a larger double-blind, placebo controlled, randomized trial conducted over 14 weeks comparing three doses of liraglutide (0.65, 1.25 or 1.9 mg/day) vs. placebo (subcutaneous injections in the evening) [7]. Subjects treated with oral glucose-lowering drugs entered a 4-week wash-out period before randomization. At randomization and after 14 weeks of treatment, an insulin-modified frequently sampled intravenous glucose tolerance test (FSIGT) and an arginine-stimulated insulin secretion during hyperglycaemia [clamped at 20 mmol/l + injection of 5 g L-arginine 45 min after elevation of plasma glucose (PG)] were conducted on two separate days. The first tests (baseline) were performed prior to initiation of trial medication and the last tests were performed while the subjects were still on trial medication (injected the evening before the experimental days). Thirty-nine patients were randomized and 28 patients completed the study (Table 1). In addition, an untreated group of healthy control subjects also underwent the two tests (*n* = 12).

During the FSIGT, 23 blood samples were drawn from -15 up to 240 min after elevation of the PG. First- and second-phase insulin responses were estimated as the incremental area under the curve from 0–10 min and 19–40 min using plasma insulin and C-peptide, respectively (C-peptide was used for estimation of the second-phase response as 0.05 IU Actrapid/kg was infused 20 min after the test began) (Actrapid, NovoNordisk, Bagsvaerd, Denmark). Insulin sensitivity (*S*_i) and glucose effectiveness (*G*_E) were estimated using the MINMOD model [15].

During the clamp, 21 blood samples were drawn from -15 to 90 min after glucose infusion. Arginine-stimulated insulin secretion during hyperglycaemia was estimated as the mean from 47–52 min [2], and as the incremental area under curve (AUC) over the first 10 min after the arginine stimulation (45–55 min).

Statistical analyses for comparisons of liraglutide groups compared with placebo were performed on the change from baseline of the end point using an ANOVA with treatment and previous glucose-lowering treatment (diet or oral glucose-lowering monotherapy) as fixed effects and baseline value as

covariate. Furthermore, an exploratory analysis of first-phase insulin was performed, adding the covariate baseline *S*_i to the above model. Comparisons of the week-14 value to healthy subjects were made using an ANOVA with treatment as fixed effect. The per cent changes were calculated directly, and not based on the ANOVA model.

Results

Effects on glycated haemoglobin (HbA_{1c}), fasting plasma glucose (FPG) and weight are presented in Table 2. There was a dose-dependent reduction in glycaemic control in the actively treated subjects, and deterioration in glycaemic control with associated weight loss in the placebo group. In Fig. 1, the glucose profiles during the hyperglycaemic clamp and FSIGT after 14 weeks of treatment for all groups are shown. Results from the B-cell functional tests are presented in Table 4, and plasma concentrations of liraglutide during the clamp in Table 3.

FSIGT

After 14 weeks of liraglutide treatment, first-phase insulin response (AUC_{insulin,0–10 min}) in comparison with placebo increased by 1.63 pmol/l×h [95% confidence interval (CI); -8.51; 11.78]; *P* = 0.74] corresponding to 34% in the 0.65 mg/day liraglutide group, by 10.62 pmol/l×h [95% CI (2.27; 18.96); *P* = 0.02] corresponding to 118% in the 1.25 mg/day liraglutide group, and by 9.09 pmol/l×h [95% CI (-0.14; 18.33); *P* = 0.05] corresponding to 103% in the 1.9 mg/day liraglutide group. Similar results were seen in the exploratory analysis where baseline *S*_i was added as a covariate to the model. The healthy control group had significantly greater first-phase insulin secretion compared with any of the liraglutide groups and the placebo group. Similar results were seen for acute insulin response to glucose as measured by incremental AUC_{insulin,0–10 min}. Second-phase insulin response (AUC_{C-peptide,19–40 min}) increased

Table 2 Effects on HbA_{1c}, fasting plasma glucose and body weight

	Patients with Type 2 diabetes			
	Placebo	Liraglutide 0.65 mg/day	Liraglutide 1.25 mg/day	Liraglutide 1.9 mg/day
HbA_{1c} (%)				
Randomization	8.1 ± 0.3	8.7 ± 0.7	8.4 ± 0.6	8.2 ± 0.6
Week 14	9.6 ± 1.8	7.7 ± 1.3	7.1 ± 0.6	6.6 ± 0.6
Change	1.5 ± 1.8	-1.0 ± (0.8)	-1.3 ± (0.7)	-1.5 ± (0.7)
FPG (mmol/l)				
Randomization	9.5 ± 1.5	13.2 ± 2.0	12.2 ± 2.3	10.9 ± 1.6
Week 14	12.5 ± 4.5	9.6 ± 2.5	8.8 ± 2.1	7.7 ± 1.5
Change	3.0 ± 3.1	-3.6 ± 2.1	-3.4 ± 2.2	-3.9 ± 1.5
Weight (kg)				
Randomization	84.5 ± 19.5	83.1 ± 13.1	94.3 ± 10.4	93.1 ± 19.7
Week 14	80.5 ± 15.7	81.8 ± 13.7	92.0 ± 11.2	90.3 ± 17.8
Change	-4.0 ± 4.8	-1.3 ± 2.0	-2.4 ± 2.4	-2.8 ± 2.7

Mean ± standard deviation (SD).
FPG, fasting plasma glucose; HbA_{1c}, glycated haemoglobin.

Table 3 Summary table of measurements of pancreatic B-cell function

	Patients with Type 2 diabetes				Control subjects
	Placebo	Liraglutide 0.65 mg/day	Liraglutide 1.25 mg/day	Liraglutide 1.9 mg/day	Untreated
First-phase insulin response (pmol/lxh)					
Estimated change from baseline	0.4 (-6.4; 7.2)	2.0 (-4.2; 8.3)	11.0 (6.6; 15.4)	9.5 (3.5; 15.5)	NA
Adjusted mean at week 14	18.4 (10.3; 26.6)	6.4 (-1.8; 14.5)	19.4 (13.3; 25.5)	18.1 (10.6; 25.5)	58.0 (40.4; 75.5)
First-phase insulin response with S₁ as covariate (pmol/lxh)					
Estimated change from baseline	-0.4 (-7.7; 6.8)	1.0 (-5.8; 7.8)	11.3 (6.1; 16.5)	9.0 (1.7; 16.3)	NA
Adjusted mean at week 14	7.9 (-2.6; 18.3)	2.3 (-5.9; 10.4)	14.9 (6.6; 23.1)	14.4 (6.0; 22.8)	70.7 (54.8; 86.5)
Acute insulin response to glucose (pmol/lxh)					
Estimated change from baseline	0.1 (-5.5; 5.6)	1.5 (-3.2; 6.2)	7.5 (3.9; 11.0)	6.8 (1.9; 11.6)	NA
Adjusted mean at week 14	4.7 (-0.5; 9.9)	1.6 (-3.7; 6.8)	7.4 (3.5; 11.3)	7.7 (3.0; 12.5)	39.4 (13.7; 65.1)
Second phase insulin response (pmol/lxh)					
Estimated change from baseline	-57 (-252; 139)	71 (-131; 273)	339 (184; 494)	139 (-80; 357)	NA
Adjusted mean at week 14	542 (313; 770)	378 (149; 606)	755 (574; 935)	724 (495; 952)	719 (520; 918)
Arginine stimulated insulin secretion (pmol/l)					
Estimated change from baseline	-141 (-501; 219)	64 (-203; 332)	376 (175; 578)	429.9 (199.4; 660.4)	NA
Adjusted mean at week 14	690 (302; 1077)	247 (-46; 540)	706 (448; 964)	856 (563; 1149)	1786 (1190; 2381)
Incremental AUC for arginine stimulated insulin secretion (pmol/l)					
Estimated change from baseline	-13 (-42; 16)	2.8 (-19; 24)	21 (4.6; 38)	15 (-3.5; 34)	NA
Adjusted mean at week 14	63 (29; 97)	20 (-5.8; 45)	50 (27; 72)	58 (32; 83)	166 (115; 218)

Mean (95% confidence interval).
AUC, area under curve.

in comparison with placebo by 127.4 pmol/lxh [95% CI (-174.1; 428.9); $P = 0.38$] corresponding to 53% in the 0.65 mg/day liraglutide group, by 395.8 pmol/lxh [95% CI (143.3; 648.4); $P = 0.005$] corresponding to 79% in the 1.25 mg/day liraglutide group, and by 195.3 pmol/lxh [95%

CI (-100.0; 481.5); $P = 0.17$] corresponding to 23% in the 1.9 mg/day liraglutide group after 14 weeks of treatment. There was no significant treatment effect on fractional glucose disposal rate (glucose effectiveness) or the ability of insulin to enhance glucose disposal (insulin sensitivity). The control

Table 4 Plasma concentrations of liraglutide during the FSIGT (nmol/l)

Time (min)	Liraglutide 0.65 mg/day	Liraglutide 1.25 mg/day	Liraglutide 1.9 mg/day
-15	6470 ± 1308	15 713 ± 1600	19 440 ± 58 556
60	5923 ± 1106	14 867 ± 1657	17 073 ± 5106
140	5986 ± 1309	13 484 ± 1801	17 324 ± 4200
240	5816 ± 1206	12 993 ± 1810	16 804 ± 4125

Mean ± standard deviation (sd).
FSIGT, insulin-modified frequently sampled intra-venous glucose tolerance test.

group had significantly greater insulin sensitivity and borderline significantly higher glucose effectiveness compared with the patients with Type 2 diabetes mellitus.

Arginine-stimulated insulin secretion during hyperglycaemia

Insulin secretion during hyperglycaemia with arginine stimulation increased in comparison with placebo by 206 pmol/l [95% CI (-287; 698); $P = 0.39$] corresponding to 32% at the 0.65 mg/day group, by 518 pmol/l [95% CI (92; 943); $P = 0.02$] corresponding to 114% at the 1.25 mg/day group, and by 571 pmol/l [95% CI (149; 993); $P = 0.01$] corresponding to 94% at the 1.9 mg/day liraglutide group. The analysis of incremental AUC (45–55 min) showed no significant increases at the two highest dose levels in comparison with placebo (1.25 mg/day: 34 pmol/l [95% CI (-0.85; 69); $P = 0.055$ and 1.90 mg/day: 28.3 pmol/l [95% CI (-5.7; 62); $P = 0.098$]. The healthy control group had significantly greater insulin secretion compared with any of the liraglutide groups and the placebo group.

Discussion

The present study demonstrated that treatment (14 weeks) in patients with Type 2 diabetes with the two higher doses of liraglutide (1.25 and 1.9 mg/day) substantially increased arginine-stimulated insulin secretion and first-phase insulin secretion. The increased insulin responses seen in this study following liraglutide treatment might be mediated through a combination of a direct acute potentiation of the glucose- or arginine-induced insulin secretion, together with stimulation of the steps in insulin biosynthesis, including insulin gene transcription. The latter provides a continued and augmented supply of insulin with an increased size of the rapid releasing pool of insulin granules, known to be of major importance in respect to determination of the magnitude of the first-phase insulin response [16,17]. Beyond the direct effects of liraglutide on B-cell function, the effect may also be mediated indirectly by factors known to affect B-cell function, such as glucose and lipid toxicity [18]. Whether the amplification of the insulin response seen during treatment with liraglutide was amplified

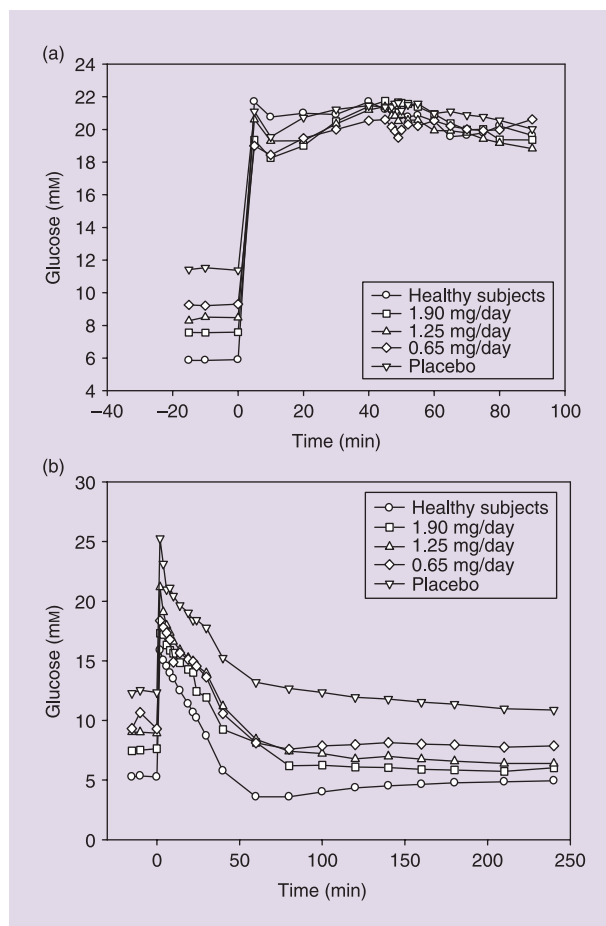


FIGURE 1 Mean glucose profiles during the clamp (a) and FSIGT (b) in mmol/l. Both plots represent values after 14 weeks of treatment for all groups except the healthy subjects that were not treated with either placebo or liraglutide.

by the improved glycaemic control seen after 14 weeks of treatment with liraglutide cannot be completely excluded. The fact that liraglutide partially restored the diminished first-phase insulin response to glucose in patients with diabetes may indicate that the loss of first-phase insulin secretion to glucose is not a permanent feature of the B-cell in diabetes and that defective B-cell function cannot be attributed to reduced B-cell mass alone. An early effect on B-cell function was previously demonstrated with both liraglutide and exenatide [13,14,19,20]. In the current study, no early test was performed and it is therefore not possible to differentiate early, immediate and chronic effects of liraglutide treatment. The current study demonstrates a significant and sustained effect of liraglutide on B-cell function in patients with Type 2 diabetes mellitus. We found no changes in insulin sensitivity in spite of the reduction in body weight, which may perhaps improve insulin sensitivity. However, the number of patients included in this trial is inadequate to exclude an effect on insulin sensitivity.

In conclusion, this present study showed a significant improvement in B-cell function following treatment with liraglutide.

Competing interests

The study was sponsored by Novo Nordisk. Parts of the results were presented as abstracts at scientific congresses. TV, TK, OS, SM and HL have been paid for attending symposiums, organizing education, funds for research and consulting by various companies (Eli Lilly, Merck, Sharp & Dohme, Novartis and Novo Nordisk). MZ and TL-T are employed by Novo Nordisk A/S and hold stocks in the company. BB, HP, KL and KK have no competing interests to declare.

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