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C-terminal mini-PEGylation of glucose-dependent insulinotropic polypeptide exhibits metabolic stability and improved glucose homeostasis in dietary-induced diabetes

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ABSTRACT

Glucose-dependent insulinotropic polypeptide has been proposed as a potential therapeutic for type 2 diabetes, however, efforts to bring forward this drug have been hindered due to its short circulating half-life. We have adopted a novel strategy to increase potency and prolong GIP action through C-terminal mini-PEGylation (GIP[mPEG]). In contrast to GIP, GIP[mPEG] was resistant to dipeptidylpeptidase-IV (DPP-IV) up to and including 24 h. Both GIP[mPEG] and GIP concentration-dependently stimulated cAMP production (EC_{50} 6.6 and 0.7 nM, respectively) and insulin secretion ($p < 0.01$ to $p < 0.001$) in pancreatic BRIN-BD11 cells. Acute injection of GIP[mPEG] together with glucose to high fat fed mice significantly lowered plasma glucose ($p < 0.05$) and increased plasma insulin responses ($p < 0.05$). Furthermore, GIP[mPEG] markedly lowered plasma glucose when administered 4–24 h prior to a glucose load ($p < 0.05$). Daily administration of GIP[mPEG] for 20 days in high fat mice did not alter body weight, food intake or non-fasting plasma insulin, however, non-fasting plasma glucose concentrations were significantly lowered ($p < 0.05$). Moreover, glucose tolerance was significantly improved ($p < 0.05$) together with glucose-mediated plasma insulin responses ($p < 0.05$). Insulin sensitivity, pancreatic insulin content, triglyceride and adiponectin levels were not changed. In summary, these data demonstrate that C-terminal mini-PEGylation of GIP is a useful strategy to prolong metabolic stability and improve biological action thus representing a novel therapeutic option for type 2 diabetes.

1. Introduction

Glucose-dependent insulinotropic polypeptide (GIP) is an incretin hormone released from intestinal K-cells into the bloodstream following nutrient ingestion [1]. Although initially characterised for its effects on inhibiting the secretion of gastric acid [2], the major physiological role of GIP in terms of glucose homeostasis is in targeting beta-cells to enhance insulin secretion and thereby reduce postprandial hyperglycaemia [3]. Together with its sister incretin hormone, glucagon-like peptide-1 (GLP-1), GIP and GLP-1 are generally acknowledged to account for the total insulinotropic arm of the enteroinsular axis following meal ingestion [4].

In addition to actions on insulin secretion, GIP promotes the growth, differentiation, proliferation and survival of pancreatic beta-cells [5,6]. Furthermore, GIP elicits several physiologically important acute glucose-lowering actions through a variety of extrapancreatic mechanisms including effects on net glucose uptake in adipose [7], muscle [8] and liver [9]. Given this, the activation of GIP pathways represents an interesting approach for the treatment of diabetes including contributing to the antihyperglycaemic actions of

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DPP-IV inhibitors [10,11]. GIP also has a number of less well-characterised actions in other peripheral tissues including bone [12], brain [13] and blood vessels [14].

The potential of GIP-based therapeutics is compromised by three fundamental limitations. Firstly, insensitivity of the beta-cell to the insulin-releasing actions of GIP has been noted in type 2 diabetes [15,16]. However, recent studies indicate that this is a readily reversible phenomenon that can be negated by improvement of diabetes control using other glucose-lowering drugs [17,18]. Secondly, GIP undergoes proteolytic degradation by the enzyme dipeptidylpeptidase-IV (DPP-IV) within 3–5 min of secretion into the bloodstream producing the inactive major degradation product GIP(3-42) [19]. Thirdly, GIP and its metabolites are rapidly cleared from the kidney tubules within approximately 20 min [20].

To circumvent these difficulties, numerous DPP-IV resistant GIP analogues have been developed through selective N-terminal modification and several of these have been shown to possess markedly improved antihyperglycaemic and insulinotropic properties [1,10]. However, these analogues are still susceptible to renal filtration and clearance from the body. This has prompted further modification through conjugation of fatty acid moieties to GIP to delay renal clearance through binding to plasma proteins [1]. Indeed, several C-16 fatty acid derivatised analogues of GIP have been generated and shown to improve glucose tolerance and diabetic status in obese diabetic *ob/ob* mice [21–23]. Although such analogues proved effective, a recent study revealed that N-terminal modification of GIP exhibited similar biological efficacy to C-16 acylation [24]. Moreover, given the increased complexity, expense and possible immunogenicity of acylated peptides, their therapeutic potential remains uncertain.

In this study we have adopted a novel strategy to extend GIP action through simple addition of a mini polyethylene glycol [PEG] residue (~145 Da) to the C-terminus of GIP. This site was chosen as it is far distant from the N-terminus of GIP which is most critical for intact biological activity. PEGylation of peptides and proteins significantly prolongs plasma half-life through a combination of decreased renal clearance and enhanced proteolytic stability [25]. PEGylation has potential advantages over other methods to prolong biological half-life including decreased immunogenicity [26] and limited penetration of the blood brain barrier [27]. Indeed, PEGylation has been shown to improve the pharmacokinetic profile of other DPP-IV sensitive peptides [28], including GLP-1 [29]. To date only one study has been carried out to assess the potential of conjugation of a much larger PEG moiety (~40 kDa) to extend the *in vitro* half-life of a shortened form of GIP (GIP1-30) [30]. The effects of C-terminal mini-PEGylation of full length GIP on metabolic stability and biological actions have not yet been established. Therefore, in the present study we have characterised a novel GIP analogue, GIP[mPEG], with a mini-PEG residue conjugated to the C-terminus. Susceptibility to DPP-IV, as well as biological activities were examined *in vitro* and compared to the native hormone. Furthermore, the acute and sub-chronic actions of GIP[mPEG] on glucose homeostasis were assessed in mice with dietary-induced diabetes.

2. Materials and methods

2.1. Peptide synthesis

GIP[mPEG] and native GIP were purchased from Sigma Genosys (Cambridge, UK). GIP[mPEG] was generated by the simple addition (amide bond) of a 145 Da polyethylene glycol residue to the C-terminus of GIP. All peptides were characterised using matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry as described previously [21].

2.2. Degradation of GIP[mPEG] and GIP by DPP-IV

GIP[mPEG] and GIP were incubated at 37 °C in 50 mM triethanolamine-HCl (pH 7.8) with purified porcine DPP-IV (5 mU; Sigma Aldrich, Poole, Dorset, UK) for 0, 2, 4, 8 and 24 h. Degradation profiles were obtained using HPLC analysis as described previously [21] and HPLC peak area data used to calculate percentage intact peptide remaining at time points during the incubation.

2.3. In vitro biological activity

The effects of GIP[mPEG] and GIP on the stimulation of cAMP production and insulin secretion were measured using clonal pancreatic BRIN-BD11 cells as described previously [21]. For cAMP studies, BRIN-BD11 cells were seeded (30,000 cells per well) into 96-well plates (Nunc, Roskilde, Denmark) and washed with HBS buffer prior to incubation with various concentrations of GIP peptides in the presence of 1 mM IBMX (20 min; 37 °C). After incubation, medium was removed and cells lysed prior to measurement of cAMP using an HTS Immunoassay Kit (Millipore, Watford, UK). For insulin-release studies, BRIN-BD11 cells were seeded (100,000 cells per well) into 24-well plates and allowed to attach overnight at 37 °C. Following a 40 min pre-incubation (1.1 mM glucose; 37 °C), cells were incubated (20 min; 37 °C) in the presence of 5.6 mM glucose with a range of peptide concentrations. After 20 min incubation, buffer was removed from each well and aliquots (200 μL) stored at −20 °C prior to measurement of insulin using radioimmunoassay.

2.4. Animals

NIH male Swiss *TO* mice at 6–8 weeks of age were obtained from Harlan UK (Shaw's Farm, Blackthorn, UK). Animals were age-matched, divided into groups and housed individually in an air-conditioned room (22 ± 2 °C) with a 12 h light:12 h dark cycle. Animals had free access to drinking water and were fed a high fat diet composed of 45% fat, 20% protein and 35% carbohydrate (percent of total energy 26.15 kJ/g; Special Diet Services, Essex, UK). Prior to commencement of in vivo studies animals were maintained on the high fat diet for 140 days. Consistent with earlier observations [31,32], access to the high fat diet resulted in progressive body weight gain (54.8 ± 5.2 g *vs.* 38.9 ± 2.9 g; $p < 0.05$) and hyperglycaemia (8.8 ± 0.5 mM *vs.* 7.2 ± 0.4 mM; $p < 0.05$) compared with age-matched controls on normal laboratory chow. All animal experiments were carried out in accordance with the UK Animals (Scientific

Procedures) Act 1986. No adverse effects were observed following administration of any of the treatments.

2.5. Acute and persistent effects of GIP[mPEG] and GIP on glucose-lowering and insulin release in mice with dietary-induced diabetes

In a first series of experiments, overnight fasted mice received an intraperitoneal injection of glucose alone (18 mmol/kg body weight) or in combination with GIP[mPEG] or GIP (each at 25 nmol/kg body weight). In a second series of experiments, glucose (18 mmol/kg body weight; i.p.) was administered 4, 24 and 48 h after injection of GIP[mPEG], GIP (each at 25 nmol/kg) or saline (0.9% (w/v) NaCl). All test solutions were administered in a final volume of 8 ml/kg body weight.

2.6. Sub-chronic effects of once daily administration of GIP[mPEG] and GIP in mice with dietary-induced diabetes

Once daily intraperitoneal injections of either GIP[mPEG] (25 nmol/kg body weight) or saline vehicle (0.9% (w/v) NaCl) were administered at 16:00 h over a 20 day period. Food intake, body weight, plasma glucose and insulin were monitored at intervals of 2–4 days. Intraperitoneal glucose tolerance (18 mmol/kg body weight) and insulin sensitivity (25 U/kg body weight) tests were performed at the end of the study. At termination, blood for measurement of circulating triglycerides and adiponectin was taken and pancreatic tissues excised and processed for measurement of insulin following extraction with 5 mL/g ice-cold acid ethanol as described previously [31].

2.7. Biochemical analyses

Blood samples were collected from the cut tip of the tail vein of conscious mice at times indicated in the figures and immediately centrifuged using a Beckman micro centrifuge (Beckman Instruments, Galway, Ireland) for 30 s at 13,000 × *g*. Plasma glucose was assayed by an automated glucose oxidase procedure [33] using a Beckman Glucose Analyser II (Beckman Instruments, Galway, Ireland). Plasma and pancreatic insulin were assayed using a modified dextran-coated charcoal radioimmunoassay as described previously [34]. Plasma triglyceride levels were measured using a Hitachi Automatic Analyser 912 (Boehringer Mannheim, Germany). Plasma adiponectin concentrations were measured using an ELISA kit (Phoenix Pharmaceuticals Inc, Burlingame, CA, USA). All analyses were carried out according to the manufacturer's instructions.

2.8. Statistical analysis

Results are expressed as mean ± SEM and data compared using unpaired Student's *t*-test. Where appropriate, data were compared using repeated measures ANOVA or one-way ANOVA, followed by Student-Newman-Keuls *post-hoc* test. Incremental area under the curve (AUC) analyses for plasma glucose and insulin were calculated using the trapezoidal rule with baseline subtraction. Groups of data were considered to be significantly different if $p < 0.05$.

3. Results

3.1. Effects of GIP[mPEG] and GIP on in vitro DPP-IV stability, cAMP production and insulin secretion

Experimental masses obtained for GIP[mPEG] and GIP following HPLC purification and MALDI-TOF MS analysis were 5127.0 and 4982.9 Da, respectively. These data clearly demonstrate that the experimental masses obtained correlate closely with theore-

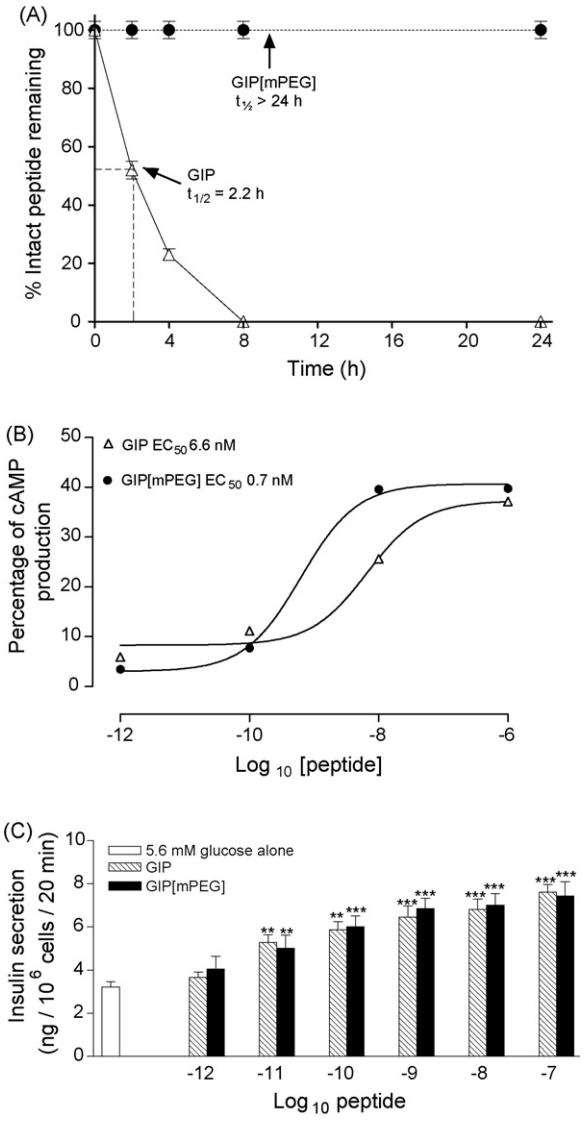

Fig. 1 – Effects of GIP[mPEG] and GIP on DPP-IV stability, cAMP production and insulin secretion. (A) Susceptibility of GIP[mPEG] and GIP to degradation by DPP-IV (5 mU) was measured ($n = 3$) following 0, 2, 4, 8 and 24 h incubations. Reaction products were subsequently analysed by HPLC and degradation expressed as a percentage of intact peptide relative to the major degradation fragment, GIP(3-42). BRIN-BD11 cells were exposed to various peptide concentrations for 20 min ($n = 8$) and (B) cAMP production or (C) insulin release measured. Values represent mean ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$ compared with 5.6 mM glucose control.

tical masses (5127.8 and 4982.4 Da, respectively), therefore confirming successful peptide synthesis. Fig. 1A demonstrates that GIP is progressively degraded by the action of DPP-IV to the major metabolite GIP(3-42). Indeed, GIP was completely degraded by 8 h with an estimated half-life of 2.2 h. In contrast, GIP[mPEG] remained fully intact throughout the 24 h incubation period (half-life > 24 h) as the presence of GIP(3-42) was not detected. Both GIP[mPEG] and GIP concentration-dependently stimulated intracellular cAMP production with EC_{50} values of 0.7 and 6.6 nM, respectively (Fig. 1B). In addition, GIP[mPEG] and GIP significantly stimulated insulin secretion (1.4–2.2-fold; $p < 0.01$ to $p < 0.001$) in a concentration-dependent manner compared with control incubations (Fig. 1C).

3.2. *Acute effects of GIP[mPEG] and GIP on glucose-lowering in mice with dietary-induced diabetes*

Fig. 2A depicts the plasma glucose responses to intraperitoneal administration of glucose alone or in combination with GIP[mPEG] or GIP (25 nmol/kg body weight). Administration of glucose alone resulted in a rapid and protracted rise in plasma glucose concentrations. The plasma glucose excursion following administration of GIP was not significantly different to animals receiving glucose alone. Administration of GIP[mPEG] exhibited a trend towards a lowered glycaemic response, but no significant differences in post-injection values were observed. However, area under the curve analysis (0–60 min) revealed significantly improved glucose-lowering actions of GIP[mPEG] (1.8-fold; $p < 0.05$). The plasma insulin response following administration of GIP[mPEG] or GIP was not significantly different to animals receiving glucose alone in terms of post-injection values (Fig. 2B). However, the overall plasma insulin response as judged by AUC demonstrated significantly improved insulinotropic activity (1.3-fold; $p < 0.05$) following administration of GIP[mPEG].

3.3. *Longer-term glucose-lowering effects of GIP[mPEG] in mice with dietary-induced diabetes*

As illustrated in Fig. 3A, glucose-lowering action of GIP[mPEG] was clearly evident when administered 4 h before an intraperitoneal glucose load. GIP[mPEG] significantly lowered plasma glucose at 30 min post-injection (1.9-fold; $p < 0.05$) compared with saline controls. Furthermore, AUC glucose values were significantly decreased by 58% ($p < 0.01$) and 48% ($p < 0.05$) compared to saline and GIP-treated groups, respectively. Notably, native GIP lacked significant effects on both post-injection and AUC glucose values. As shown in Fig. 3B, when GIP[mPEG] was administered 24 h prior to a glucose load plasma glucose concentrations were significantly lowered at 30 min post-injection (60%; $p < 0.05$). These results were further corroborated as illustrated by significantly decreased plasma glucose AUC values (30%; $p < 0.05$). After 48 h, GIP[mPEG] demonstrated a similar profile to saline controls (Fig. 3B).

3.4. *Effects of daily administration of GIP[mPEG] on body weight, food intake, non-fasting glucose and insulin concentrations in mice with dietary-induced diabetes*

Daily administration of GIP[mPEG] for 20 days had no effect on body weight and food intake compared to saline-treated controls (Fig. 4A and B). Similarly, non-fasting plasma insulin concentrations were not significantly different in treated and control mice at any time during the 20 day study (Fig. 4D). In

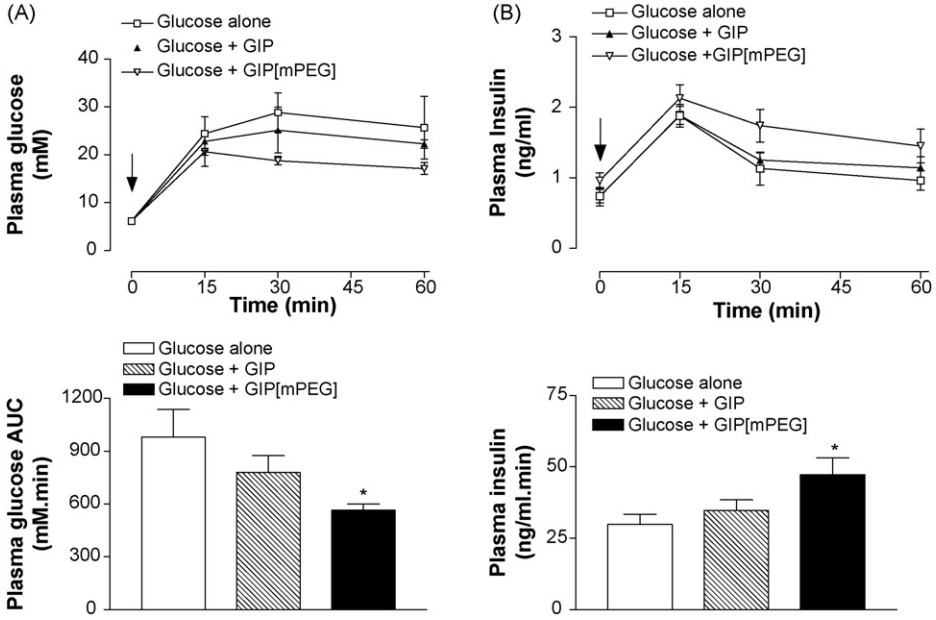

Fig. 2 – Acute effects of GIP[mPEG] and GIP on glucose-lowering and insulin release in mice with dietary-induced diabetes. (A) Plasma glucose and (B) plasma insulin concentrations were measured prior to and after intraperitoneal administration of glucose alone (18 mmol/kg body weight), or in combination with GIP[mPEG] or GIP (each at 25 nmol/kg body weight) in 18 h fasted mice previously fed a high-fat diet. Time of injection is indicated by the arrow (0 min). Plasma glucose and insulin area under the curve (AUC) values for 0–60 min post-injection are also included. Values represent mean ± SEM for 8 mice. *$p < 0.05$ compared to glucose alone.

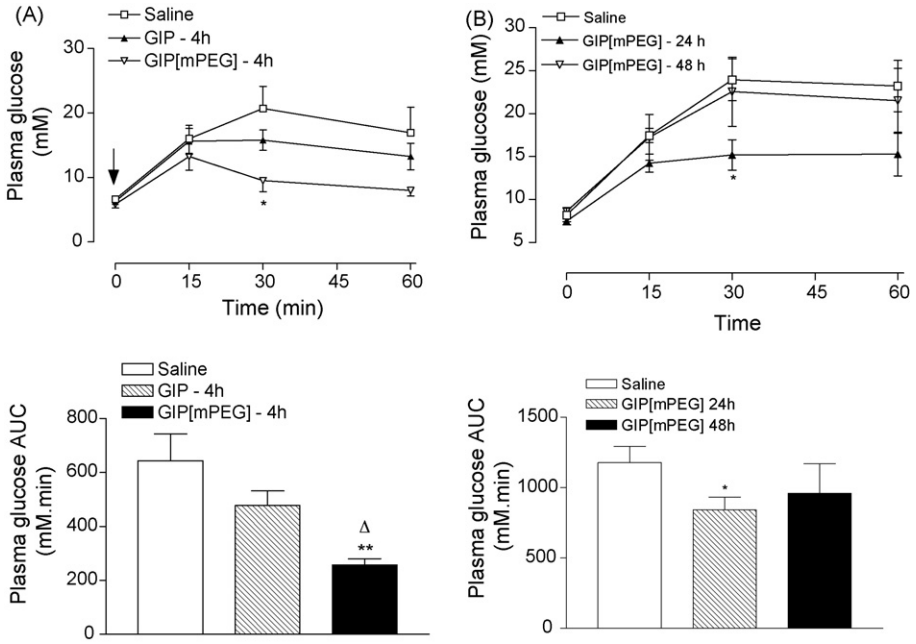

Fig. 3 – Persistence of glucose-lowering effects of GIP[mPEG] in mice with dietary-induced diabetes. Plasma glucose concentrations were measured prior to and after intraperitoneal administration of glucose (18 mmol/kg body weight) in mice injected (A) 4 h and (B) 24 and 48 h previously with GIP[mPEG], GIP (each at 25 nmol/kg body weight) or saline vehicle (0.9% (w/v) NaCl). Mice had previously been fed a high fat diet. Time of glucose injection is indicated by the arrow. Plasma glucose AUC values for 0–60 min post-injection are also shown. Values represent mean ± SEM for 8 mice. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to mice injected with saline. $^{\Delta}p < 0.05$ compared to mice injected with GIP.

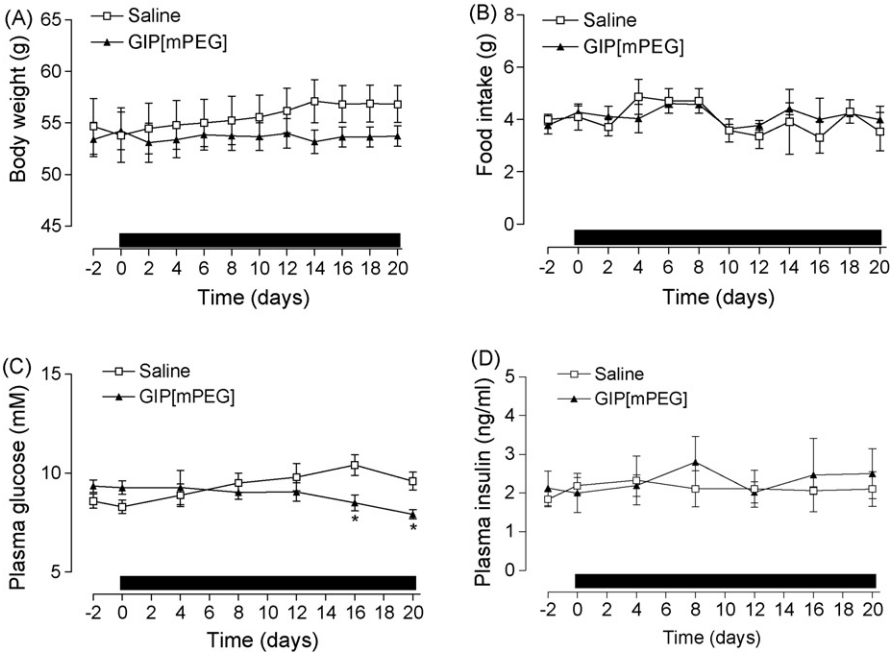

Fig. 4 – Effects of daily administration of GIP[mPEG] on (A) body weight, (B) food intake, (C) plasma glucose and (D) plasma insulin in mice with dietary-induced diabetes. Parameters were measured prior to and 20 days during treatment with GIP[mPEG] (25 nmol/kg body weight/day) or saline vehicle (0.9% (w/v) NaCl). Mice had previously been fed a high fat diet. Values represent mean ± SEM for 8 mice. $^{*}p < 0.05$ compared with saline-treated group.

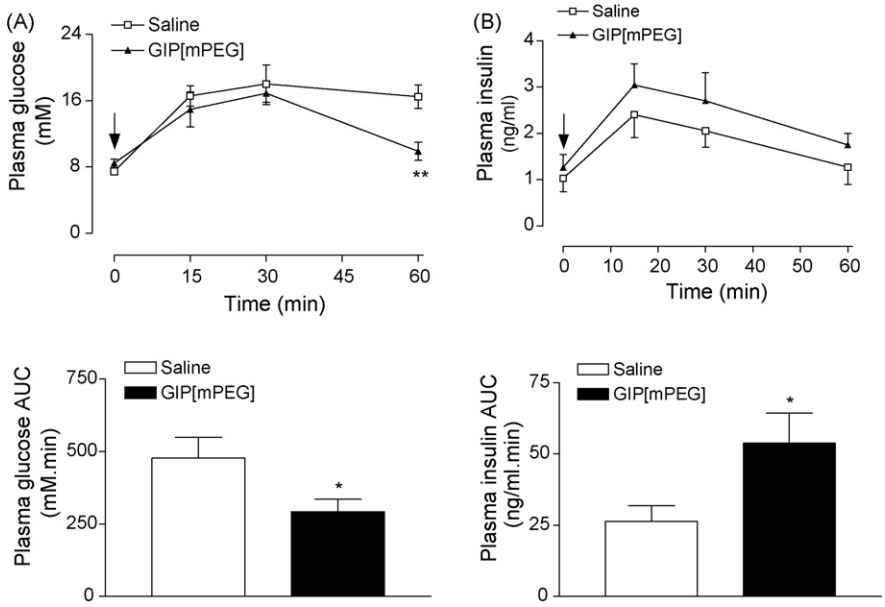

Fig. 5 – Effects of daily administration of GIP[mPEG] on (A) glucose tolerance and (B) plasma insulin responses to glucose in mice with dietary-induced diabetes. Tests were conducted after daily treatment with GIP[mPEG] (25 nmol/kg body weight/day) or saline vehicle (0.9% (w/v) NaCl) for 20 days. Glucose (18 mmol/kg body weight) was administered at the time indicated by the arrow. Mice had previously been fed a high fat diet. Plasma glucose and insulin AUC values for 0–60 min are also included. Values represent mean ± SEM for 8 mice. $^{*}p < 0.05$, $^{**}p < 0.01$ compared with saline-treated group.

contrast, non-fasting plasma glucose concentrations were significantly lower (1.2–1.3-fold; $p < 0.05$) in GIP[mPEG]-treated mice on days 16 and 20, respectively, compared to saline-treated controls (Fig. 4C).

3.5. *Effects of daily administration of GIP[mPEG] on glucose tolerance and insulin sensitivity in mice with dietary-induced diabetes*

As shown in Fig. 5, daily administration of GIP[mPEG] for 20 days resulted in an improved glycaemic response to an intraperitoneal glucose load with significantly decreased plasma glucose concentrations at 60 min post-injection (1.7-fold; $p < 0.01$). The beneficial effect of GIP[mPEG] was particularly evident from plasma glucose AUC values (0–60 min), which were significantly reduced (1.7-fold; $p < 0.05$) compared to saline-treated controls (Fig. 5A). Glucose-mediated plasma insulin concentrations were significantly elevated (1.9-fold; $p < 0.05$) after 20 days of GIP[mPEG] treatment compared to saline-treated controls (Fig. 5B). Plasma glucose concentrations were not significantly changed in 20 day treated GIP[mPEG] animals after administration of exogenous insulin (Fig. 6A). This was further corroborated by the overall plasma glucose AUC values (0–60 min) (Fig. 6B).

3.6. *Effects of daily administration of GIP[mPEG] on pancreatic insulin content, circulating triglycerides and adiponectin concentrations in mice with dietary-induced diabetes*

Pancreatic insulin content was not significantly different between treated and control groups (9.3 ± 1.3 μg/g *vs.* 8.5 ± 0.8 μg/g tissue) on day 20 of the study (pancreatic weights were not significantly different). Furthermore, daily administration of GIP[mPEG] had no effect on circulating triglyceride (441 ± 42 mg/dl *vs.* 413 ± 35 mg/dl) and adiponectin (9.6 ± 0.7 μg/ml *vs.* 10.1 ± 0.6 μg/ml) levels compared to controls.

4. Discussion

GIP is a physiological incretin that has recently received renewed attention as a potential antidiabetic agent [1]. The glucose-dependent nature of GIP is a particularly beneficial feature and GIP-based therapies may be more attractive than currently used, single action, non-endogenous drugs [10]. However, the therapeutic potential of GIP is severely hindered by rapid DPP-IV degradation and renal clearance [1,20]. N-terminal modifications of GIP and its sister incretin hormone GLP-1, have been successful in masking the potential binding site for DPP-IV, creating long-acting analogues [35]. Moreover, the first GLP-1-based pharmaceutical to reach the market and now currently being prescribed to type 2 diabetic patients was Byetta (Amylin/Eli Lilly), otherwise known as exendin-4(1-39), exenatide or AC-2993 [36]. Exenatide requires twice-daily administration and as such a longer-acting form of the drug known as exenatide-LAR (long acting release) is currently in phase III trials [37,38]. Other GLP-1-based pharmaceuticals including Liraglutide [39] and CJC-1131 [40] are currently in Phase III and Phase II clinical trials, respectively.

The progression of GIP-based therapies to the clinic has been somewhat thwarted due to a perceived inefficacy of GIP in type 2 diabetes [15,16]. However, recent knowledge that this

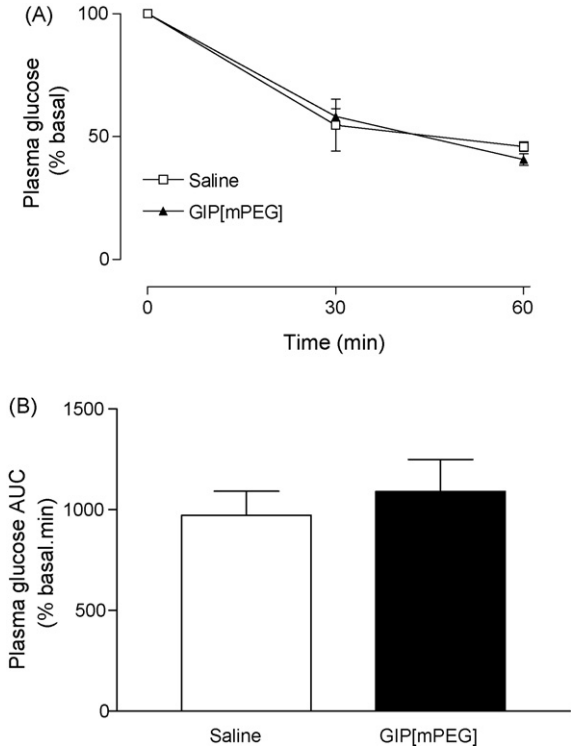

Fig. 6 – Effects of daily administration of GIP[mPEG] on insulin sensitivity in mice with dietary-induced diabetes. (A) Exogenous insulin (25 U/kg body weight) was administered by intraperitoneal injection in mice after daily injection of GIP[mPEG] (25 nmol/kg body weight/day) or saline vehicle (0.9% (w/v) NaCl) for 20 days. (B) Plasma glucose AUC values for 0–60 min post-injection. Mice had previously been fed a high fat diet. Values represent mean ± SEM for 8 mice.

is not a GIP specific defect, but represents just one aspect of a generalised beta-cell defect in type 2 diabetes, has rekindled interest in the therapeutic use of GIP [41]. Indeed, given that the major drawback of GLP-1-based therapies is a retardation of gastric emptying resulting in nausea and dizziness [42], the lack of effect of GIP on gastric emptying [43] strengthens its therapeutic applicability for type 2 diabetes. To date several second generation fatty acid derivatised GIP analogues have been synthesised and characterised [1]. Although these analogues impart markedly enhanced DPP-IV resistance and reduced kidney filtration giving rise to potent antidiabetic compounds, their complexity, increased expense and possible immunogenicity limits therapeutic use. Thus, the current study has examined conjugation of a mini-polyethylene glycol [mPEG] moiety to the C-terminus of GIP (GIP[mPEG]) as a novel means of prolonging biological activity.

As reported previously, native GIP was rapidly hydrolysed by DPP-IV with an *in vitro* half-life of 2.2 h [44]. In sharp contrast, GIP[mPEG] was completely resistant to enzymatic degradation. This is a surprising observation, but would suggest that the addition of the hydrophilic mini-PEG moiety inhibits DPP-IV action by altering GIP hydro-affinity for the serine protease, in a similar manner to that reported previously for GIP acylation [23]. Previous studies have demonstrated that distant regions of GIP affect DPP-IV cleavage [45], but given the three-dimensional structure of GIP [46,47], it is unlikely that a C-terminal mini-PEGylation could mask the DPP-IV binding site.

Irrespective of conformation change evoking DPP-IV resistance, mini-PEGylation of GIP had no adverse effect on post-receptor binding events in clonal pancreatic insulin-releasing cells. Thus, GIP[mPEG] exhibited a 10-fold reduction in EC_{50} for cAMP production indicating improved bioactivity compared to native GIP. This suggests retention of GIP-receptor affinity and activation of adenylate cyclase signal transduction pathways. *In vitro* studies also revealed that GIP[mPEG] stimulated insulin secretion from BRIN-BD11 cells in a manner similar to native GIP. The reason why GIP[mPEG] should elicit such a marked stimulation of cAMP compared to its modest actions on insulin secretion probably reflect the activation of other signal transduction pathways and potent permissive effect of glucose on GLP-1-induced insulin release. In agreement with these findings, GIP[mPEG] significantly reduced plasma glucose concentrations compared to the native peptide in an animal model of dietary-induced diabetes following conjoint administration with glucose. The longer-acting potential was further strengthened by the observed persistent glucose-lowering effect of GIP[mPEG] 4–24 h after administration. The development of a specific assay to directly measure GIP[mPEG] in plasma would provide more precise details of circulating half-life.

Results of these acute in vivo studies provided a strong basis for the subsequent 20 day study in high fat fed mice. Daily injection of GIP[mPEG] over this time period had no adverse or toxic effects. Body weight and food intake were similar to saline-treated controls, corroborating findings that GIP does not affect feeding activity [21]. Furthermore, lack of increased fat deposition by GIP[mPEG] treatment in high fat fed mice, does not accord with increased lipid synthesis and fat deposition observed for 8 weeks with [DAla^2]-GIP treatment [48]. However, more detailed NMR or spectroscopy studies are necessary to confirm this. Most importantly, non-fasting glucose concentrations were significantly reduced in the present study by GIP[mPEG] from day 16 and glucose tolerance was markedly enhanced. The observed improvement of glucose tolerance after 20 days treatment with GIP[mPEG] was accompanied by significantly increased plasma insulin responses. This confirms the long acting insulinotropic effects of GIP[mPEG] and its ability to overcome any associated pancreatic beta-cell defects in this high fat model [31]. Notably, non-fasting insulin concentrations were not changed, presumably reflecting the glucose-dependent insulinotropic nature of GIP. GIP[mPEG] induced improvement of glucose homeostasis was independent of any change in insulin sensitivity, similar to previous findings of fatty acid derivatised GIP analogues [21]. In contrast to some other studies using N-terminally modified GIP analogues [1,10], there were no changes in pancreatic insulin, plasma triglycerides levels or adiponectin although a more chronic treatment period may well improve these parameters. However, it is difficult to make direct comparisons to previous studies employing other animal models of diabetes-obesity.

In conclusion, this study demonstrates that C-terminally mini-PEGylated GIP displayed enhanced DPP-IV resistance and markedly improved bioactivity. This was evidenced through long-acting antihyperglycaemic and insulinotropic actions in an animal model of dietary-induced diabetes. These data clearly demonstrate that mini-PEGylation of GIP is a useful strategy to prolong metabolic stability and biological action and supports this approach as a potential future therapeutic option for the treatment of type 2 diabetes.

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