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Beneficial metabolic actions of a stable GIP agonist following pre-treatment with a SGLT2 inhibitor in high fat fed diabetic mice

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ABSTRACT

The purpose of the present study was to examine if a stable glucose-dependent insulinotropic polypeptide (GIP) agonist could exert beneficial metabolic control in diabetic mice which had been pre-treated with sodium-glucose-cotransporter-2 (SGLT2) inhibitor dapagliflozin (DAPA). High fat fed mice administered low dose streptozotocin (STZ) received vehicle, DAPA once-daily over 28 days, or DAPA once-daily for 14 days followed by (dAla^2)GIP once-daily for 14 days. Energy intake, body weight, glucose and insulin concentrations were measured at regular intervals. Glucose tolerance, insulin tolerance test, dual-energy X-ray absorptiometry (DEXA) and pancreatic histology were examined. Once-daily administration of (dAla^2)GIP for 14 days in high fat fed diabetic mice pre-treated with DAPA demonstrated significant decrease in body weight, blood glucose and increased insulin concentrations which were independent of changes in energy intake. Similarly, glucose tolerance, glucose-stimulated insulin secretion, insulin sensitivity and HOMA-β were significantly enhanced in (dAla^2)GIP-treated mice. DEXA analysis revealed sustained percentage body fat loss with no changes in lean mass, bone mineral content and density. Pancreatic immunohistochemical analysis revealed decreased islet number and increases in islet area, beta cell area and pancreatic insulin content. The DAPA-induced increase in alpha cell area was also reversed. Additional acute *in vitro* and *in vivo* experiments confirmed that the impaired action of (dAla^2)GIP under hyperglycaemic-induced conditions was significantly reversed by DAPA treatment. These data demonstrate that (dAla^2)GIP can exert beneficial metabolic control in high fat fed diabetic mice pre-treated with DAPA. The results highlight possibility of a targeted and personalized approach using a GIP agonist and SGLT2 inhibitor for the treatment of type 2 diabetes.

1. Introduction

Obesity is a major contributing factor to the development of insulin resistance and impaired glucose tolerance mediated through several pathways including hormone imbalance, increased cytokine production and inflammation (Ahrén, 2015; Igoillo-Esteve et al., 2010; López-Jaramillo et al., 2014). To overcome the resultant insulin resistance, pancreatic beta-cells elicit a compensatory hyperinsulinemic response but over time they become exhausted and unable to generate sufficient insulin leading to type 2 diabetes. Mechanisms which appear to be involved in beta-cell dysfunction include glucotoxicity, lipotoxicity, oxidative stress and amyloid formation (Saisho, 2015). Given the complex, progressive and variable pathogenesis of type 2 diabetes, there is an urgent need for new and improved treatment strategies.

Presently there are a number of anti-hyperglycemic agents available for patients with type 2 diabetes which target various facets of the disorder. These include: biguanides, sulfonylureas, meglitinides, DPP-4 inhibitors, GLP-1 receptor agonists, thiazolidinediones, alpha-glucosidase inhibitors, amylin analogues, insulin and more recently the sodium-glucose-cotransporter-2 (SGLT2) inhibitors (Bailey, 2015). SGLT2 is a high-capacity, low-affinity sodium glucose co-transporter which under normoglycemic conditions reabsorbs approximately 90% of glucose in the S1 segment of the promixal tubules (Hediger and Rhoads, 1994; Meng et al., 2008; Wright, 2001). Dapagliflozin (DAPA), a selective and potent oral

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SGLT2 inhibitor reduces renal glucose reabsorption which leads to dose-dependent increase in glucosuria and concomitant reduction in hyperglycemia (Han et al., 2008; Tahrani et al., 2013). Moreover, as the mode of action of DAPA has been shown to be insulin-independent, its efficacy will not be affected by insulin resistance and progressive beta-cell decline.

GIP is a 42-amino acid gut hormone released from K cells in response to nutrient ingestion (Elliott et al., 1993). Although GIP has been shown to exhibit potent glucose-lowering actions via stimulation of insulin secretion its therapeutic potential has been hindered by two main factors (Gault et al., 2003). Firstly, naturally-occurring GIP is rapidly degraded by the serine protease, dipeptidylpeptidase-4 (DPP-4), leading to a short half-life of approximately 5–6 min in humans (Deacon et al., 2000). However, various studies have shown that this aspect can be largely overcome by amino acid modifications and incorporation of moieties such as palmitic acid to increase renal stability (Irwin and Flatt, 2013). Secondly, the GIP-mediated arm of the incretin effect is severely compromised in patients with type 2 diabetes (Nauck et al., 1993). However, when hyperglycemia is corrected in patients with type 2 diabetes using sulfonylureas or insulin, GIP sensitivity can be restored (Aaboe et al., 2009; Højberg et al., 2009; Meneilly et al., 1993). Similarly, in rodent models the insulinotropic action of GIP is improved using phlorizin (Piteau et al., 2007), GLP-1 (Gault et al., 2011) and more recently the 25-amino acid peptide xenin (Martin et al., 2012). To date, no studies have examined metabolic actions and efficacy of a stable GIP agonist which has been introduced following pre-treatment regimen using an SGLT2 inhibitor. Therefore, this study evaluated a novel strategy whereby high fat diabetic mice were treated with the SGLT2 inhibitor DAPA for 14 days and treatment was subsequently switched to a stable GIP agonist, (dAla^2)GIP (Hinke et al., 2002) for a further 14 days.

2. Materials and methods

2.1. Animals

Male NIH Swiss mice at 6–8 weeks of age (Harlan, Oxon, UK) were housed in individual cages (22 ± 2 °C; 12:12 h light/dark cycle – 08:00 to 20:00 h). Mice had free access to high fat (HF) diet (45% AFE Fat; Product Code 824053; Special Diet Services, Witham, UK; total energy 26.15 kJ/g) containing lard and soya oil. An additional group of mice had free access to standard rodent chow (Teklad Global 18% Protein Rodent Diet; Product Code 2018S; Harlan, UK; total energy 13.0 kJ/g). All animals had free access to drinking water. All experiments were performed according to the Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) and UK Home Office Regulations (UK Animals Scientific Procedures Act 1986).

2.2. Experimental design

An experimental timeline for the study is displayed in Fig. 1. Briefly, mice on HF diet received STZ (Sigma–Aldrich, Dorset, UK) prepared in sodium citrate buffer (pH 4.5) on days −14 (50 mg/kg body weight; ip) and −7 (75 mg/kg body weight; ip). On day 0, mice commenced respective drug treatments as follows: Group 1 (HF control) – HF saline vehicle (0.9% wt/vol; po) once-daily for 28 days; Group 2 (DAPA) – HF dapagliflozin (1 mg/kg body weight; po; Selleck Chemicals; Stratech Scientific Ltd., Suffolk, UK; Catalog number S1548-SEL) once-daily for 28 days; Group 3 (DAPA → (dAla^2)GIP) – HF dapagliflozin (1 mg/kg; p.o.) once-daily for 14 days followed by (dAla^2)GIP (25 nmol/kg body weight; ip; GL Biochem Ltd., Shanghai, China) once-daily for 14 days; Group 4 (Lean control animals on normal diet without STZ injection) – lean saline vehicle (0.9% wt/vol; po) once-daily for 28 days. All treatments were administered at 14:00 h and mice remained on their respective diets for the duration of the study. Energy intake, body weight, non-fasting blood glucose and non-fasting plasma insulin concentrations were measured every 3–4 days. At the end of the study period, glucose tolerance (18 mmol/kg body weight; ip; 12-h fasted animals) and insulin tolerance (25 U/kg body weight; ip; non-fasted animals) tests together with DEXA analysis and gene expression were conducted (see below). The *in vivo* tests were performed at 10:00 h, 20 h after the last drug treatment. Pancreatic tissue was excised, weighed and stored for immunohistochemistry or processed for measurement of insulin content following extraction with ice-cold acid ethanol (750 ml ethanol, 235 ml H_2O and 15 ml concentrated HCl) as described previously (Gault et al., 2005).

2.3. Biochemical and DEXA analyses

Blood samples were collected (at the time points indicated in the Figures) from the tail vein of conscious mice into chilled fluoride/heparin micro-centrifuge tubes (Sarstedt, Numbrecht, Germany) and centrifuged at 12,000 rpm for 2 min (Beckman Instruments, Galway, Ireland). Glucose concentrations were measured using Ascencia Contour Blood Glucose Meter (Bayer Healthcare, Newbury, UK) and plasma/pancreatic insulin determined using a modified dextran-coated charcoal radioimmunoassay (Flatt and Bailey, 1981). HOMA-IR and HOMA-β were determined in 12-h fasted animals and values calculated as described previously (Gault et al., 2015). Measurement of body fat, lean mass, bone mineral content and bone mineral density was measured using dual-energy X-ray absorptiometry (DEXA) densitometry (Piximus Densitometer, Inside Outside Sales, USA).

2.4. Immunohistochemistry and image analysis

Pancreatic tissues were fixed in 4% paraformaldehyde for 48 h at 4 °C, processed using automated tissue processor (Leica TP1020, Leica Microsystems, Nussloch, Germany) and embedded in paraffin wax. Immunohistochemistry was performed as described previously (Moffett et al., 2015; Vasu et al., 2013). The following primary antibodies were used: mouse monoclonal anti-insulin antibody (ab6995, 1:1000; Abcam) and guniea-pig anti-glucagon antibody (PCA2/4, 1:200; raised in-house). Secondary antibodies were used as appropriate: Alexa Fluor 488 goat anti-guinea pig IgG – 1:400, Alexa Fluor 594 goat anti-mouse IgG – 1:400. Slides were viewed under FITC filter (488 nm) or TRITC filter (594 nm) using fluorescent microscope (Olympus system microscope, model BX51) and photographed using DP70 camera adapter system. Islet parameters including islet area, beta and alpha cell area were analysed in a blinded manner using Cell^F image analysis software (Olympus Soft Imaging Solutions, GmbH) and expressed as μm^2. Islet size distribution (small – islets < 10,000 μm^2; medium – >10,000 μm^2 and <25,000 μm^2; large – >25,000 μm^2) was determined using raw data set of total islet area computed using Cell^F image analysis software.

2.5. Gene expression

Whole kidney was excised, snap frozen in liquid nitrogen and mRNA extracted (Tripure Isolation Reagent; Roche Diagnostics, West Sussex, UK), quantified and purity determined by nanophotometer (Implen, Munich, Germany). cDNA was synthesized using Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics) and gene expression analysis was carried out by qPCR using Light Cycler 480 Probes Master (Roche Diagnostics) according