Original Article

MOLECULAR METABOLISM

Lipid nanoparticle delivery of glucagon receptor siRNA improves glucose homeostasis in mouse models of diabetes

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ABSTRACT

Objective: Hyperglucagonemia is present in many forms of diabetes and contributes to hyperglycemia, and glucagon suppression can ameliorate diabetes in mice. Leptin, a glucagon suppressor, can also reverse diabetes in rodents. Lipid nanoparticle (LNP) delivery of small interfering RNA (siRNA) effectively targets the liver and is in clinical trials for the treatment of various diseases. We compared the effectiveness of glucagon receptor (Gcgr)-siRNA delivered via LNPs to leptin in two mouse models of diabetes.

Methods: Gcgr siRNA encapsulated into LNPs or leptin was administered to mice with diabetes due to injection of the β-cell toxin streptozotocin (STZ) alone or combined with high fat diet (HFD/STZ).

Results: In STZ-diabetic mice, a single injection of Gcgr siRNA lowered blood glucose levels for 3 weeks, improved glucose tolerance, and normalized plasma ketones levels, while leptin therapy normalized blood glucose levels, oral glucose tolerance, and plasma ketones, and suppressed lipid metabolism. In contrast, in HFD/STZ-diabetic mice, Gcgr siRNA lowered blood glucose levels for 2 months, improved oral glucose tolerance, and reduced HbA1c, while leptin had no beneficial effects.

Conclusions: While leptin may be more effective than Gcgr siRNA at normalizing both glucose and lipid metabolism in STZ diabetes, Gcgr siRNA is more effective at reducing blood glucose levels in HFD/STZ diabetes.

Keywords Type 1 diabetes; Type 2 diabetes; Glucose metabolism; Lipid metabolism; Glucagon; Leptin

1. INTRODUCTION

Hyperglucagonemia is present in type 1 [1,2] and type 2 [3,4] diabetes and contributes to elevated blood glucose by stimulating glycogenolysis, gluconeogenesis, and ketogenesis, while suppressing glycogen synthesis. Moreover, suppression of glucagon action by glucagon receptor (Gcgr) gene deletion, glucagon immunosuppression, or Gcgr antagonist can ameliorate diabetic symptoms in models of both type 1 and type 2 diabetes. To investigate the effect of suppressing glucagon action in a model of insulinopenic diabetes, Gcgr wildtype (WT) and knockout (KO) mice were injected with the β-cell toxin streptozotocin (STZ) [5,6]. GcgrWT mice become hyperglycemic, hyperketonemic, polyuric, and cachectic, while, remarkably, GcgrKO mice were protected from these diabetic symptoms [5,6]. In addition, immunoneutralization of glucagon using a monoclonal antibody reduced hyperglycemia in alloxan-diabetic rabbits [7]. Moreover, in STZ-injected mice, weekly treatment with a Gcgr antibody completely normalized blood glucose levels for up to 12 weeks, concomitant with an improvement in HbA1c levels [8]. Similarly, immunoneutralization of endogenous glucagon improved oral glucose tolerance and reduced hepatic glucose output in obese leptin-deficient *ob/ob* mice [9], and Gcgr antisense oligonucleotides or small interfering RNA (siRNA) diminished hyperglycemia and improved oral glucose tolerance in obese leptin receptor null *db/db* mice [10,11]. Finally, genetic deletion of Gcgr in diet-induced obese mice or *db/db* mice prevented obesity, hyperinsulinemia, and hyperglycemia [12]. Therefore, inhibiting glucagon action can improve diabetic symptoms in various models of diabetes.

The hormone leptin, well known for its role in body weight regulation, has also shown promise as a glucose-lowering therapy. In rodent models of type 1 diabetes, leptin monotherapy can potently reduce diabetic symptoms and normalize hyperglycemia [13—20]. Interestingly, leptin can reduce circulating glucagon levels and levels of hepatic p-CREB indicative of reduced Gcgr signaling [13,14,16], which has been thought to be important for the glucose-lowering mechanism of leptin. Moreover, in STZ-diabetic rodents, intracerebroventricular

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Abbreviations: AUC, area under the curve; FVII, factor VII; Gcgr, glucagon receptor; HFD, high fat diet; KO, knockout; LFD, low fat diet; LNP, lipid nanoparticle; siRNA, small interfering RNA; STZ, streptozotocin; WT, wildtype

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leptin reduces preproglucagon mRNA levels in the pancreas, glucagon content in the pancreas [15], and plasma glucagon levels [21], suggesting that leptin can act through the central nervous system to suppress glucagon production. However, in type 2 diabetes, leptin monotherapy appears to be less efficacious as an anti-diabetic therapy. Although leptin injections in a rat model of obese type 2 diabetes normalized fasting blood glucose [22], leptin treatment in humans with type 2 diabetes did not increase insulin-mediated stimulation of glucose disposal [23] nor meaningfully reduce HbA1c [24]. The failure of leptin to improve type 2 diabetes may be due to leptin resistance as many obese individuals are hyperleptinemic [25].

In this report, we investigated the efficacy of Gcgr siRNA delivered using lipid nanoparticle (LNP) technology and compared this treatment to leptin therapy in mouse models of type 1 and type 2 diabetes. LNPs are capable of effectively and safely delivering genetic drugs such as siRNA to target tissues, and they are the most clinically advanced delivery systems for siRNA, with multiple LNP-siRNAs in clinical trials for the treatment of various diseases [26]. In addition, LNPs effectively target the liver [27,28], where glucagon exerts most of its biological functions. Indeed mice with full-body or hepatocyte specific *Gcgr* gene deletion display a similar degree of improvement of fasting blood glucose levels and glucose tolerance highlighting the importance of glucagon action on the liver in regulating glucose metabolism [29,30]. We find that Gcgr siRNA can potently improve glucose metabolism in both STZ (a model of type 1 diabetes) and high fat diet (HFD)/STZ (a model of type 2 diabetes) diabetic mice. However, while leptin was able to improve both glucose and lipid metabolism in STZ-diabetic mice, no changes were observed in HFD/STZ-diabetic mice given leptin treatment.

2. RESEARCH DESIGN AND METHODS

2.1. Animals

Male C57BL/6J mice (stock 000664), C57BL/6J mice on 60% HFD (stock 380050) or C57BL/6J mice on 10% low fat diet (LFD) (stock 380056) were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and acclimatized on arrival for at least a week. Mice were housed on a 12-h:12-h light–dark cycle with *ad libitum* access to normal chow (Harlan Laboratories, #2918, Indianapolis, IN, USA), 60% HFD (Research Diets, Inc., D12492i, New Brunswick, NJ, USA) or 10% LFD (Research Diets, Inc., D12450Bi, New Brunswick, NJ, USA) and water. All experiments were approved by the University of British Columbia Animal Care Committee and carried out in accordance with the Canadian Council on Animal Care guidelines.

2.2. Generation of STZ-diabetic mice

STZ (Sigma–Aldrich, Oakville, Canada) was prepared in a pH 4.5 acetate buffer and 180 mg/kg STZ was administered i.p. to 9 week old C57BL/6J mice. Non-diabetic controls received an i.p. injection of acetate buffer alone.

2.3. Generation of HFD/STZ-diabetic mice

C57BL/6J mice were put on a 60% HFD (Research Diets, Inc., D12492i, New Brunswick, NJ, USA) at 6 weeks of age and remained on the diet until the end of the study. At 10 weeks of age, STZ (Sigma–Aldrich, Oakville, Canada) was prepared in a pH 4.5 acetate buffer and administered at a dose of 100 mg/kg. Controls were put on a 10% LFD (Research Diets, Inc., D12450i, New Brunswick, NJ, USA) at 6 weeks of age and did not receive an i.p. injection of acetate buffer.

2.4. Preparation and delivery of LNP-siRNA

siRNAs were purchased from Integrated DNA Technologies (Coralville, IA, USA); sequences are in Supplemental Table 1. LNP-siRNA systems were prepared as previously described [31]. For all studies, siRNA was delivered via the tail vein. Either 5 mg/kg or 10 mg/kg LNP encapsulating Gcgr siRNA 2 or 10 mg/kg LNP encapsulating an equal mix of Gcgr siRNA 1 and 2 were injected. LNP encapsulating FVII siRNA was injected at the same total dose of GCGR siRNA for each study.

2.5. Leptin therapy

STZ-diabetic mice at 10 weeks of age received 20 μg/day of recombinant mouse leptin (Peprotech, Rocky Hill, NJ, USA) prepared in sterile water and administered via Alzet 1004 mini-osmotic pumps (DURECT Corporation, Cupertino, CA, USA). HFD/STZ-diabetic mice at 12 weeks of age received 20 μg/day PEG-ylated leptin (Protein Laboratories Rehovot Ltd., Rehovot, Israel) prepared in sterile water by daily i.p. injection while control HFD/STZ mice received water (vehicle).

2.6. Plasma analyte analysis

All blood parameters were measured using samples collected from 4 h fasted mice. Hemoglobin A1c (HbA1c) levels were measured using a Siemens DCA 200 Vantage Analyzer (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) from whole blood. Plasma insulin (Mouse Ultrasensitive Insulin ELISA, ALPCO, Salem, NH, USA), glucagon (Glucagon ELISA, Mercodia, Salem, NC, USA), leptin (Mouse Leptin ELISA, Crystal Chem, Downers Grove, IL, USA), β-hydroxybutyrate (β-Hydroxybutyrate LiquiColor Test, Stanbio, Boerne, TX, USA), free fatty acids (HR Series NEFA HR[2] Kit, Wako Diagnostics, Richmond, VA, USA), triglycerides and glycerol (Serum Triglyceride Determination Kit, Sigma–Aldrich, Oakville, Canada), and cholesterol (Cholesterol E, Wako Diagnostics, Richmond, VA, USA) were measured from cardiac puncture blood samples. For samples from PEG-leptin injected mice, plasma leptin levels were analyzed using a PEG-leptin standard curve as the PEG-leptin was less potent in the assay than an equivalent amount of normal leptin.

2.7. Oral glucose tolerance tests

Mice were fasted for 4 h and given oral gavages of either 1.5 g/kg of 30% dextrose or 2 g/kg of 50% dextrose. Blood glucose was measured via the saphenous vein using a One Touch Ultra Glucometer (LifeScan) with a detection limit of 1.1–33.3 mM. During the oral glucose tolerance tests, if samples fell over the detection limit they were diluted with non-diabetic blood of known glucose concentration, re-assayed, and original blood glucose levels were calculated. Area under the curve (AUC) was measured starting at baseline (0 min) values.

2.8. Immunofluorescence and analysis of α-cell area

Pancreata were harvested from mice on day 7, fixed overnight in paraformaldehyde at 4°C, rinsed in 70% ethanol, embedded in paraffin, and sectioned by Wax-it Histology Services Inc. (5 μm thickness; Vancouver, BC, Canada). The antibodies used are detailed in Supplemental Table 2. Cell nuclei were counterstained with VECTASHIELD HardSet Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA, USA). Whole slide fluorescence scanning was performed using the ImageXpress Micro Imaging System (Molecular Devices Corporation, Sunnyvale, CA, USA). Individual images were stitched together to recreate the pancreas area and then quantified using MetaXpress software. For α-cell area, glucagon positive area was expressed relative to the pancreas area; 3 sections were quantified per animal, separated by 200 μm.

