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Fibroblast growth factor 21 prevents glycemic deterioration in insulin deficient mouse models of diabetes

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ABSTRACT

In type 1 diabetes, there is a rapid loss of glycemic control immediately after onset of the disease. We aimed to determine if the deterioration of glycemic control that occurs early after the onset of insulin-deficient diabetes could be blunted by treatment with recombinant fibroblast growth factor 21 (FGF21). Normal C57BL/6J mice made diabetic by a single high dose injection of streptozotocin (STZ) were randomized to receive twice daily subcutaneous injection of vehicle or recombinant human FGF21 at doses of 0.3 and 1.0 mg/kg for 10 days. Body weight was recorded daily and 5 h fasted glucose, insulin, glucagon, free fatty acids and ketones were determined at 6 and 10 days post-randomization.

The increase in fasting plasma glucose induced by STZ in untreated mice was prevented with FGF21 at 0.3 mg/kg BID. In contrast, at 1.0 mg/kg BID, FGF21 did not prevent the rise in plasma glucose after STZ. At the end of the study, plasma glucagon was significantly higher in the diabetic group treated with FGF21 1.0 mg/kg BID than in the untreated group. This was not seen for the group treated with FGF21 0.3 mg/kg BID. There were significant dose dependent reductions in plasma free fatty acids with FGF21 treatment but no significant change in plasma ketones (β-hydroxybutyrate). FGF21 treatment did not have significant effects on body weight in lean insulin deficient mice.

In conclusion, FGF21 prevents increases in glycaemia and has lipid lowering properties in mouse models of insulin deficient diabetes, although by increasing the dose increased glucagon levels are seen and hyperglycemia persists.

1. Introduction

Glycemic control in type 1 diabetes is currently maintained with the use of both short acting and long acting insulin analogs. However, despite the effectiveness of insulin therapy, some individuals still have difficulty maintaining good glycemic control (DeVries et al., 2012). Therefore, there remains interest in developing pharmacological therapies to be used in conjunction with insulin. One such potential novel therapy is fibroblast growth factor 21 (FGF21), which is a member of the FGF superfamily of peptides (Kliewer and Mangelsdorf, 2010). FGF21 has previously been demonstrated to lower body weight, blood lipids and alleviate hyperglycemia in multiple animal models (Kharitonenkov et al., 2005; Kim et al., 2013; Mu et al., 2012) and recently lower lipids in human subjects with type 2 diabetes (Gaich et al., 2013). FGF21 has also been demonstrated to increase glucose uptake into adipocytes and skeletal muscle in an insulin independent manner (Izumiya et al., 2008; Ogawa et al., 2007). We have, furthermore, recently demonstrated that FGF21 might contribute to the diabetes resistance seen in glucagon receptor knockout mice made completely insulin deficient by multiple high doses of the beta cell toxin streptozotocin (Omar et al., 2014). These properties led us to explore whether FGF21 could be utilized as a glucose lowering strategy for insulin deficient diabetes and in this study we have examined whether pharmacological intervention with recombinant native FGF21 would prevent declines in glycemic control in mice with insulin deficient diabetes induced by a single high dose injection of streptozotocin. The effects of pharmacological intervention with FGF21 were also investigated on plasma lipids and body weight.

2. Materials and methods

2.1. Preparation of recombinant human FGF21

Full length recombinant human metFGF21 was expressed in *Escherichia coli* and purified by chromatography at Novo Nordisk

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2.2. Animals

Six week old C57BL6/JBomTac female mice were purchased from Taconic Europe (Skensved, Denmark). The mice had free access to standard chow and water throughout the study. The mice were kept in a climate controlled room at 20 °C with a 12 h light dark cycle. Body weight was recorded daily at the beginning of the light period and at the time of the first subcutaneous injection of study drug or vehicle. All experimental procedures were approved by the regional Ethics committee for Malmö/Lund and the internal ethics committee for Novo Nordisk A/S.

2.3. Induction of diabetes with high dose streptozotocin injection (insulin deficient model)

Mice were fasted for 5 h and anesthetized with an intraperitoneal injection of midazolam 12.5 mg/kg (Dormicum; Roche, Basel Switzerland) and a combination of fluanison 25 mg/kg and fentanyl 0.78 mg/kg (Hypnorm; Janssen, Beerse, Belgium), then given a single intravenous injection in the tail vein of either streptozotocin (0.150 g/kg in 0.01 M sodium citrate buffer; pH 4.5) or of citrate buffer alone. After 7 days, blood samples were taken to determine the extent of hyperglycemia induced by streptozotocin treatment. Mice with fasting plasma glucose lower than 6.8 mmol/l were excluded and the remaining diabetic mice were divided into groups of eight so that mean fasting glucose was matched in each experimental group prior to randomization. An ethical endpoint of 20% bodyweight loss after streptozotocin injection was set and animals that lost more than this were euthanized.

2.4. Induction of diabetes with multiple low dose streptozotocin injections in diet induced obese mice (obese insulin deficient model)

Male C57BL6JBom Tac mice from Taconic Europe (Skensved, Denmark) were fed a high fat “western” diet containing 41% kcal from fat (Research Diets, D12079B, New Brunswick, New Jersey, USA) *ad libitum*. Five weeks after initiation of the high fat diet, mice were given multiple low dose (50 mg/kg) intraperitoneal injections of streptozotocin, daily for five days. Three weeks after the final streptozotocin injection, mice were given twice daily subcutaneous injections of recombinant human FGF21 at a dose of 0.3 or 1.0 mg/kg or vehicle, daily for 14 days. Blood glucose was monitored twice weekly from the last streptozotozin injection until study termination.

2.5. Pharmacological intervention with recombinant human FGF21

The pharmacological intervention was initiated at day 0, which was 7 days after diabetes induction with streptozotocin. The streptozotocin diabetic mice were randomized to one of three treatment arms: vehicle (50 mM sodium phosphate, 145 nM sodium chloride, 0.05% tween 80, pH 7.4), FGF21 0.3 mg/kg or FGF21 1.0 mg/kg. Non-diabetic control mice received vehicle. These two FGF21 doses were chosen as they have been shown to be glucose lowering in pilot studies using mouse models of type 2 diabetes (unpublished observation). Injections were given subcutaneously twice daily for 10 days. Blood samples were taken from the retrobulbar intraorbital sinus plexus in anesthetized mice after a 5 h fast at 0, 6 and 10 days after the start of treatment.

2.6. Biochemical analyses

Plasma was separated from whole blood and frozen at −20° for later analysis. Plasma glucose was determined by the glucose oxidation method with ABTS as the substrate. Insulin was measured by mouse specific ELISA, detection limit 0.2 μg/l (Mercodia, Uppsala Sweden). Glucagon was measured with a high specificity ELISA that has no cross-reaction to glicentin, oxyntomodulin or the major proglucagon fragment, detection limit 1.0 pmol/l, (Mercodia, Uppsala Sweden). Non-esterified free fatty acids were measured using a colorimetric detection kit (WAKO diagnostics, Richmond VA, USA). Plasma β-hydroxybutyrate was detected using a colorimetric detection kit (Sigma-Aldrich, St. Louis MO, USA).

2.7. Gene expression analysis

At study termination, liver and gonadal white adipose tissue, and pancreatic tissue samples were snap-frozen on liquid nitrogen and stored at −80 °C until analysis. Total RNA was extracted from the liver and white adipose tissue using Trizol (Invitrogen) and the RNeasy mini-kit (Qiagen) according to the manufacturer's instructions. cDNA was synthesized using iScript reverse-transcription kit BioRad). Quantitative real-time PCR was performed on an ABI 7900 Sequence Detection System (Applied Biosystems) using a locked nucleic acid probe-based system from Roche. Primers were designed using Primer3 software (bioinfo.ut.ee/primer3). All samples were run in duplicate, and expression was calculated using the ddCT method. Results are expressed as fold induction relative to vehicle-treated samples.

2.8. Statistical analysis

Data are presented as mean ± S.E.M. Statistical significance was determined by Student's unpaired *t*-test. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Body weight

At randomization, seven days after the injection of streptozotocin, body weight was significantly decreased in all experimental groups (Fig. 1A). After the initiation of vehicle and FGF21 injections, body weight was similar between all diabetic groups throughout the treatment period. There was no significant difference in body weight even in the diabetic group receiving FGF21 at a dose of 1.0 mg/kg BID (Fig. 2).

3.2. Plasma glucose

Plasma glucose was similarly increased by streptozotocin in all groups at the time of randomization (Fig. 1B). Thereafter, plasma glucose was monitored at 6 and 10 days after randomization. In the untreated diabetic group, plasma glucose rose slightly after 6 days and greatly after 10 days resulting in an increase of approximately 6 mmol/l (or 50%) compared to baseline, suggesting a progressive worsening of the diabetes. In contrast, the increase in fasting glycaemia seen in untreated diabetic controls, was totally prevented by treatment with FGF21 0.3 mg/kg BID (Fig. 3A). When comparing the glycemic development, diabetic mice treated with 0.3 mg/kg BID FGF21showed decreased plasma glucose already at day 6 and was significantly lower than that of untreated diabetic controls at day 10 (Fig. 3B). Diabetic mice treated with the higher dose of 1.0 mg/kg BID. FGF21 did not differ from untreated diabetic mice in their fasting plasma glucose profile throughout the study (Fig. 3A and B).