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Early intervention with liraglutide improves glucose tolerance without affecting islet microcirculation in young Goto–Kakizaki rats

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ABSTRACT

Liraglutide, an analog of glucagon-like peptide-1 (GLP-1), is an effective anti-diabetic agent with few side effects. Since native GLP-1 exerts vascular effects, we investigated changes in pancreatic islet blood flow using a non-radioactive microsphere technique, as well as insulin concentration and glucose tolerance after 17 day treatment with liraglutide in 6-week-old Goto–Kakizaki (GK) rats. Compared to saline-treated control GK rats, liraglutide limited body weight gain, decreased glycemia, improved glucose tolerance and lowered serum insulin concentration. Neither pancreatic or islet blood flow, nor pancreatic insulin content, was affected by liraglutide treatment. We conclude that early intervention with liraglutide decreases glycemia and improves glucose tolerance, thus halting the natural progression towards diabetes, without affecting islet microcirculation or pancreatic insulin content in young female GK rats.

1. Introduction

Despite substantial efforts made to achieve and maintain long-term glycemic control in type 2 diabetic patients, the natural progressive course of diabetes has proven difficult to halt, although effects of bariatric surgery appear hopeful so far [1,2]. Generally, glycemic control deteriorates over time and the risk of patients experiencing micro- and macrovascular complications increases. Currently available therapies are often associated with undesirable side effects, *e.g.*, hypoglycemia and weight gain, which compromise patients' adherence to treatment. Effective therapies addressing the unmet needs of type 2 diabetes are urgently needed.

The incretin system has attracted substantial focus of research in combating diabetes, especially when considering its multiple physiological actions. Studies on patients and animals consistently confirm that liraglutide, a GLP-1 analog which shares 97% sequence identity of human native GLP-1 [3], is an attractive option in the treatment of diabetes. The structural modification prolongs its half-life, making it resistant to degradation and suitable for clinical use [4,5].

It is now clear that an intact islet vascular system is crucial for maintaining normal islet β-cell function [6], and β-cell dysfunction is a requirement for the development of both type 1 and type 2 diabetes [7]. Changes in pancreatic islet microcirculation may impede insulin output in diabetic subjects, and could contribute to the pathogenesis of the disease. Islet hyper-perfusion and abnormal modulation of islet blood flow (IBF) after glucose challenge have been observed in various animal models of glucose intolerance and diabetes [8–12]. One notable characteristic of GLP-1 is its protective effects on the vascular system: GLP-1 was found to relax pulmonary and conduit arteries [13,14], reduce myocardial infarction [15] in rodents, and improve left ventricular function [16] and endothelial dysfunction in humans [17]. Furthermore, a single i.v. bolus of native GLP-1 has been shown to decrease basal IBF in GK rats and prevent IBF increase induced by glucose in Wistar rats [10].

To our knowledge, no previous study has been published concerning the vascular modulating effect of several days use of liraglutide on islet microcirculation in rodent models. We therefore injected GK rats, a widely used model of non-obese type 2 diabetes, with liraglutide for 17 consecutive days and observed the changes of pancreatic blood flow (PBF) and IBF, as well as insulin levels and glucose tolerance, to explore whether a vascular effect of liraglutide plays a part in its glucose lowering effect.

2. Materials and methods

2.1. Animals and groups

The study was approved by the local animal ethics committee at Karolinska Institutet. Six-week-old female GK rats, obtained from

Abbreviations: ABF, adrenal blood flow; fIBF, fraction IBF out of PBF; GK, Goto–Kakizaki; GLP-1, glucagon-like peptide-1; IBF, islet blood flow; KBF, kidney blood flow; MABP, mean arterial blood pressure; PBF, pancreatic blood flow.

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our KISÖS breeding colony (Animal Department, Stockholm South Hospital, Stockholm, Sweden), were divided randomly into two groups: 1) Liraglutide group (n = 11): subcutaneous (s.c.) injections of liraglutide (kindly donated by Dr. Lotte B. Knudsen at Novo Nordisk A/S, Bagsværd, Denmark) were given twice a day (in escalating doses to improve tolerability). 2) Control group (n = 10): only saline was injected s.c. twice a day. During the experiments, all rats were kept under controlled temperature, humidity, and 12-h light cycle, and had free access to commercial pelleted food (Type R34; ScanBur, Sollentuna, Sweden) and tap water.

2.2. Dosage escalation of liraglutide

Body weight of each animal was determined weekly. Rats were given s.c. injections (1 ml/kg) of saline or liraglutide twice a day, with a minimal interval of 9 h in between. Liraglutide was dissolved in saline. Injections were given in the abdominal area and intentionally varied every time to avoid fibrosis development and drug absorption problems. To improve tolerance to the drug, the dosage of liraglutide began at 0.1 mg/kg body weight, with an increase of 0.025 mg/kg per day, until the maximum dosage of 0.4 mg/kg at the 13th day and then continued for 5 days.

2.3. Intraperitoneal glucose tolerance tests (IPGTT)

On the 14th day, after the morning injection, rats were injected intraperitoneally with 30% (wt/vol) D-glucose solution (2 g/kg body wt). Blood samples were drawn from the tail vein immediately before and 10, 30, 60, and 120 min after glucose administration. Blood glucose concentrations were measured with test strips using a One-Touch glucometer (LifeScan, Sollentuna, Sweden) based on the glucose oxidase method. Area under the curve for the IPGTT was calculated according to the formula:

$$\mathrm{AUCg(mmol/L\cdot h)} = (G0 + G10)/12 + (G10 + G30)/6 + (G30 + G60)/4 + (G60 + G120)/2$$

2.4. Blood flow measurements

Three days after IPGTT and after the morning injection, pancreatic and islet blood flow was measured using a non-radioactive microsphere technique [18,19]. In short, rats were fully anesthetized with an intraperitoneal injection of sodium thiobutabarbital (180 mg/kg body weight; Inactin, Research Biochemicals International, Natick, MA), tracheotomized and placed on a heated operating table to keep the body temperature at 37 °C. Rats were tracheotomized because our previous experience is that anesthetized rats are otherwise very prone to develop acid–base disturbances (mainly respiratory acidosis) as determined by arterial blood gas analyses. Blood was drawn from the tail vein and blood glucose concentrations were measured using test strips as described above. Polyethylene catheters of different diameters were then inserted into the trachea, the ascending aorta via the right carotid artery, and the left femoral artery, respectively. The cranial catheter was connected to a pressure transducer (model PDCR 75/1, Druck Ltd., Groby, Leicestershire, UK) to constantly monitor the mean arterial blood pressure (MABP). After the blood pressure became stable, $1.5–2.0 \times 10^5$ black non-radioactive microspheres (IMT, Stason Labs, Irvine, CA) with a mean diameter of 10 μm were injected within 10 s via the carotid catheter. Starting 5 s before the microsphere injection, and continuing for 60 s, an arterial blood sample (the reference sample) was collected from the catheter in the femoral artery into a preweighed tube. The exact withdrawal rate was then determined in each animal by weighing the sample. After securing the reference sample, another arterial blood sample was drawn to measure glycemia by a glucometer (see above) and then centrifuged. Sera were stored at −20 °C pending measurements of serum insulin and liraglutide concentrations. The animals were decapitated immediately afterwards. Pancreas and adrenal glands, as well as samples from left kidney (including both cortex and medulla), were taken out, blotted and weighed. The whole pancreas was carefully dissected and freed of fat and lymph nodes under a stereomicroscope (Leica MZ6, Leica, Switzerland). Each pancreas was divided into two parts and weighed. The tail of the pancreas was cut into small pieces and placed between object slides and then treated with the freeze-thawing technique [18,19], which allows visualization of microspheres and pancreatic islets under microscope (Olympus BX40, Japan). The microsphere content of arterial reference sample was determined by transferring the sample to glass microfiber filters and counting the microspheres under the same microscope. The other piece of the pancreas was extracted for insulin measurements (see below).

The blood flow values were calculated according to the formula $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$ where Q_{org} is organ blood flow (ml/min), Q_{ref} is withdrawal rate of the reference sample (ml/min), N_{org} is number of microspheres present in the organ and N_{ref} is number of microspheres in the reference sample [18]. The microsphere contents of the adrenal glands were used as a control to confirm an even distribution of the microspheres in the arterial circulation. A difference <10% in blood flow values between the adrenal glands was used to confirm adequate mixing of the spheres in the circulation.

The purpose of the 3-day delay between IPGTT and blood flow measurements was to minimize stress imposed by i.p. injection of a highly concentrated glucose solution and drawing blood, which is known to influence islet blood flow [6]. To avoid a carry-over effect, we also wanted to ascertain that the metabolic effects of the glucose injection had subsided by the time of blood flow measurements.

Five out of 21 rats died during the course of the study. Apart from a deformity in the carotid artery, 4 rats died after anesthetization (two from the liraglutide group and two from the control group).

2.5. Serum liraglutide and insulin concentrations and pancreatic insulin content

Immediately after the dissection, heads of pancreas were weighed and homogenized in 1 ml acid ethanol (0.18 M HCl, 95% ethanol), kept on ice and frozen at −70 °C. After complete ultrasonication, samples were centrifuged at 3700 × *g* (Multifuge 3S-R, Heraeus, Hanau, Germany) for 10 min at 4 °C, and supernatants were stored at −70 °C for assay. Serum insulin concentration and pancreatic insulin content were determined with a rat insulin ELISA kit (Mercodia, Uppsala, Sweden). Non-fasting liraglutide concentrations were determined in sera 2–3 h after the final injection of subcutaneous injection of liraglutide with a GLP-1 ELISA kit (Millipore, Billerica, MA). Since there is a 97% sequence homology between liraglutide and native GLP-1 [3], this ELISA fully crossreacts with liraglutide (Dr. Lotte B. Knudsen, personal communication). Pancreatic protein was determined with Micro BCA Protein Assay Kit (Thermo Scientific, Pierce Biotechnology, Rockford, IL).

2.6. Statistics

Values are expressed as the mean ± SEM. Differences between two groups were evaluated for statistical significance by Student's unpaired *t* test. $P<0.05$ was deemed significant.

3. Results

3.1. Liraglutide limits body weight gain

There was an insignificant ($P = 0.13$) difference in baseline body weights between the two groups because two rats that were two weeks older than the others were randomized into the liraglutide group. On the 7th day of treatment body weight of the liraglutide group rats significantly decreased compared to baseline ($P<0.001$), whereas in the control group body weight steadily increased (Fig. 1A).