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Differential effects of angiotensin receptor blockers on pancreatic islet remodelling and glucose homeostasis in diet-induced obese mice

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ABSTRACT

Obesity leads to adverse endocrine pancreas remodelling, reduced islet lifespan and early type 2 diabetes onset. AT1R blockade shows beneficial pleiotropic effects. This study sought to compare the effects of losartan and telmisartan on pancreatic islets remodelling and glucose homeostasis in diet-induced obese mice. High-fat diet yielded overweight, insulin resistance, islet apoptosis and hypertrophy. Suitable insulin levels and preserved endocrine pancreas structure were correlated to adequate AKT-FOXO1 pathway functioning in losartan-treated animals. Conversely, telmisartan yielded enhanced PDX1 and GLP-1 islet expression along with greater GLP-1 levels, with the consequent better islet glucose sensing and uptake. Greater islet vascularisation coupled with reduced apoptosis and macrophage infiltration seems to underlie the beneficial findings in both treatments. In conclusion, these results provide compelling evidence that two antihypertensive drugs (telmisartan and losartan) ameliorate pancreatic islet structure, glucose handling, and vascularisation in obese mice. Although only telmisartan countered overweight, both drugs yielded reduced apoptosis and islet preservation, with translational potential.

1. Introduction

Obesity has reached epidemic proportions and frequently stems from increased fat intake and physical inactivity (Seidell and Halberstadt, 2015). Adipose tissue capacity to buffer excessive exogenous or endogenous free fatty acids (FFA) is limited and the resulting increased leptin release from enlarged adipocytes triggers hyperinsulinemia, which is related to adverse pancreatic islet remodelling (Gustafson and Smith, 2015).

The pancreas is one of the most affected organs by lipotoxicity (van Raalte et al., 2010). It has been previously reported that high-fat feeding leads to enlarged pancreatic islets as an attempt to keep normal values of blood glucose at the expense of hyperinsulinemia (Janikiewicz et al., 2015; Souza-Mello et al., 2010). As lipotoxicity becomes chronic, glucose intolerance takes place and, at this stage, pancreatic islets present with altered metabolic pathways, besides a structural disarrangement (Del Guerra et al., 2005; Souza-Mello et al., 2011). These alterations threaten the survival of pancreatic islets, which tend to become dysfunctional due to a highly demanding for insulin and glucagon synthesis (Cerf, 2007).

Insulin resistance precedes type 2 diabetes onset in at least ten years. During this period, pancreatic islet undergoes a series of structural, functional and metabolic remodelling. Pancreatic duodenal homeobox 1 (PDX1) islet expression is essential to induce adequate expression of glucokinase (GK) and glucose transporter 2 (GLUT2), which mediate glucose sensing and uptake by the islet and ensures beta cell function and islet preservation (Cerf, 2007; Stitzel et al., 2015).

The use of angiotensin receptor blockers (ARBs) has unravelled pleiotropic effects on pancreatic structure and glucose homeostasis (Souza-Mello et al., 2010; Zhao et al., 2016). The inhibition of angiotensin type 1 receptor (AT1R) in endocrine pancreas favours angiotensin type 2 receptor (AT2R) actions, which seems to cause islet preservation (Shao et al., 2013). Besides a better coupling between glucose sensing and uptake by beta cells, antiapoptotic effects and greater islet vascularisation play a major role in islet preservation (Bonora, 2008). In this context, unlike losartan (a pure ARB), telmisartan is also a partial peroxisome proliferator-activated receptor (PPAR) gamma agonist, which is suggestive of additive beneficial effects on islet morphology and physiology (Bhatia and

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Viswanathan, 2006; Younis et al., 2012).

The present study sought to compare the effects of losartan (pure ARB) and telmisartan (ARB and partial PPAR-gamma agonist) on pancreatic islets remodelling and preservation, focusing on glucose sensing by the endocrine pancreas, glucose homeostasis, islet vascularisation and apoptosis in diet-induced obese mice.

2. Methods

2.1. Ethical approval

The Ethics Committee for Animal Experimentation of the State University of Rio de Janeiro approved the study protocol (Protocol Number CEUA/013/2015), and all procedures were conducted according to the recommendations contained in the Guide for the Care and Use of Laboratory Animals of National Institutes of Health (NIH Publication number 85-23, revised 1996).

2.2. Animals and diets

Male C57Bl/6 mice were maintained under controlled conditions (20 ± 2° C, humidity 60 ± 10%, and 12 h/12 h dark/light cycle) in pathogen-free cages, and had free access to food and water. At 3 months of age, they were randomly divided into two dietary groups: control (C: 14% protein, 10% fat, and 76% carbohydrates, total energy 15.960 kJ/kg) or high-fat diet (HF: 14% protein, 50% fat and 36% carbohydrates, total energy 21.000 kJ/kg), for 10 weeks (n = 40 per group). After that, treatment started with telmisartan (T, 10 mg/kg/day, Micardis; Boehringer, Ingelheim) or losartan (L, 10 mg/kg/day, Cozaar, Merck), for five weeks and the drug was mixed into the diet. Also, to isolate the effects of telmisartan from the consequences of a reduced energy intake, previously reported after the treatment with telmisartan (Penna-de-Carvalho et al., 2014; Souza-Mello et al., 2010), all groups treated with telmisartan had a pair-feeding (PF) group. Briefly, the amount of food (in grams) administered to C-T/PF and an HF-T/PF group was calculated to contain the same energy as those ingested by the C-T and HF-T groups on the day before. Of note, it was not necessary to include pair feeding groups to losartan-treated animals as they presented with similar energy intake to their counterparts, as previously showed in the literature (Frantz et al., 2013). In this way, C group resulted in four subgroups, as well as the HF group, totalizing eight experimental groups (n = 10, each), as follows:

a) C group: untreated, fed C diet;
b) C-L group: C diet treated with losartan;
c) C-T group: C diet treated with telmisartan;
d) C-T/PF group: untreated, fed C diet according to C-T group intake (pair feeding);
e) HF group: untreated, fed HF diet;
f) HF-L group: HF diet treated with losartan;
g) HF-T group: HF diet treated with telmisartan;
h) HF-T/PF group: untreated, fed HF diet according to HF-T group intake (pair-feeding);

The diets were produced by the PragSolucoes company (Jau, Sao Paulo, Brazil) based on the recommendations of the American Institute of Nutrition (AIN-93M) (Reeves et al., 1993) and the doses administered to the treated groups were used in previous studies in rodents (Chu et al., 2006; Penna-de-Carvalho et al., 2014).

2.3. Food intake, body mass, and systolic blood pressure

Food intake (grams) was controlled daily, and energy intake was measured as the product of food consumption by the energy content of the diet. Both body mass (BM) and systolic blood pressure (SBP) were evaluated weekly. SBP was assessed by tail-cuff plethysmography in conscious mice (Letica LE 5100, Harvard/Panlab, Barcelona, Spain). Before the SBP measurement, the animals were trained for four weeks in constraint conditions to minimize their stress.

2.4. Blood analysis

An oral glucose tolerance test (OGTT) was performed one week before euthanasia in animals fasted for 6 h. Then, received a glucose load (25% glucose solution, at a dose of 1 g/kg BM) by orogastric gavage and blood glucose concentrations were measured using an Accu-Chek Go glucometer (Roche Diagnostics, Basel, Switzerland).

Blood samples were collected from the caudal vein before and at 15, 30, 60 and 120 min after glucose administration. The area under the curve (AUC) was calculated for OGTT from 0 to 120 min using the trapezoid rule (GraphPad Prism version 6.05 for Windows, GraphPad Software, La Jolla, CA, USA) to assess glucose intolerance.

The plasma concentration of insulin and GLP-1 were analysed in duplicate using the enzyme-linked immunosorbent assay kit (Rat/Mouse Insulin ELISA kit Cat. #EZRMI-13K and multi-species GLP-1 ELISA Kit Cat. #EZGLP1T-36K, Millipore, Missouri, USA), using TP-READER Thermoplate equipment (Bio Tek Instruments, Inc Highland Park, USA).

The homeostasis model assessment of insulin resistance (HOMA-IR), was calculated as fasting glucose (mmol/L) x fasting insulin level ((μIU/L)/22.5 and the HOMA of beta cell function (HOMA-BETA) (a marker of basal insulin secretion by pancreatic beta cells) was calculated as 20 × fasting insulin level ((μIU/L)/fasting glucose (mmol/L) - 3.5 (Matthews et al., 1985).

2.5. Sacrifice and tissue extraction

After five weeks of treatment, the animals fasted overnight and were deeply anesthetized (sodium pentobarbital 150 mg/kg). Blood samples were obtained by cardiac puncture and centrifuged at room temperature to obtain plasma (120 g for 20 min), which was stored individually on −20 °C until assay.

The pancreas was dissected (n = 5), weighed and then rapidly fixed in a freshly prepared fixative solution [4% (w/v) formaldehyde and 0.1 M phosphate buffer, pH 7.2 for 48 h at room temperature]. Afterward, the material was embedded into Paraplast plus (Sigma-Aldrich, St Louis, MO, USA) for routine histology. Quickly right tibia was dissected to expose the two ends, and the length of the tibia was measured from the tibia plateau to the inner malleolus. This measure was used to correct the mass of the pancreas (because it is more appropriate than making a correction for body mass in cases of obesity) (Yin et al., 1982).

In another group of animals, the islets were isolated by collagenase digestion (n = 5) (Lacy and Kostianovsky, 1967). For this procedure, the pancreas was cannulated and inflated with cold Hanks' solution (supplemented with foetal bovine serum 1 mg/mL) containing 0.8 mg/mL collagenase (C9263, Sigma-Aldrich, St. Louis, USA). Next, the pancreas was removed and incubated in a 37° C water bath for 15 min to allow the digestion of the exocrine tissue. Subsequently, the tubes were vigorously shaken for approximately 15 s. The collagenase digestion was terminated by the addition of cold Hanks' solution. The islets were manually collected under a stereomicroscope (Luxeo 4D Stereozoom Microscope, Labomed, CA, USA) with a Pasteur pipette and immediately homogenized in extraction buffer (urea, 7 M; EDTA, 5 mM; Triton X-100, 1%; protease and phosphatase inhibitors).