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Improvement of cardiac dysfunction by bilateral surgical renal denervation in animals with diabetes induced by high fructose and high fat diet

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ABSTRACT

Aims: Insulin resistance (IR) and sympathetic over-activation play a critical role in diabetic cardiomyopathy (DCM). Percutaneous renal sympathetic denervation (RDN) was tested to treat refractory hypertension. However, the benefits of RDN for DCM and IR still remain unknown. The present study aimed to investigate the effect and associated mechanisms of bilateral surgical RDN (bsRDN) on cardiac function and glucose metabolism in animals with diabetes.

Methods: Thirty-two male New Zealand white rabbits were randomly assigned to Chow ($n = 8$, normal diet) and TEST ($n = 24$, high-fructose fat diet [HFD]) groups. At 48 weeks after HFD feeding, animals in the TEST group were randomized to the Sham, HFD, and RDN subgroups and were fed a HFD for an additional 8 weeks. Repeated measurements of cardiac function, IR, apoptosis/autophagy, and histopathological assessment were performed at 48 and 56 weeks.

Results: HFD feeding for 56 weeks induced IR and diastolic cardiac dysfunction with hypertrophy in septum but well preserved eject fraction in the animals. Impaired IR further deteriorated over the time in the RDN group, featured by a more profound reduction in GLUT4 mRNA and its translocation to the plasma membrane. Successful denervation was associated with improvement of cardiac function via preventing myocardial fibrosis and over-expression of procollagen III, mammalian target of rapamycin, and cardiac apoptosis. Cardiac autophagy, assessed by either electron microscopy or Western blot, was enhanced by bsRDN.

Conclusions: Renal sympathetic denervation led to a significant improvement of HFD-induced cardiac dysfunction by shifting the cardiac apoptosis to autophagy, but worsening IR. Further study is required to identify the clinical benefits of RDN.

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1. Introduction

Diabetes mellitus (DM) carries a huge healthcare burden [1]. Type 2 diabetes (T2DM) accounts for 90–95% of all diagnosed DM in adults and results in increased mortality mainly due to developing atherosclerosis, hypertension, and diabetic cardiomyopathy (DCM). DCM was defined 40 years ago by Rubler and colleagues as ventricular dysfunction in the absence of coronary disease and hypertension [2], who reported post-mortem data from four patients with diabetes following death from heart failure. Prominent features of the diabetic myocardium are cardiac hypertrophy, myocardial interstitial fibrosis, myocardial apoptosis, and left ventricular dysfunction [3]. Multiple pathophysiological mechanisms are involved in the progress of DCM, and insulin resistance (IR) concomitant with T2DM is reported to be one of the most potential mechanisms of DCM [4]. Conventional strategies, including aggressive glycemic control and adjustment of lifestyle provide basic interventions in current guidelines [5,6].

Standard medications targeting sympathetic nerve (SN) hyperactivity for DCM, such as the renin–angiotensin–aldosterone system inhibitors and beta-blockers, are effective in improving cardiac function, but have recently been reported to fail to improve the long-term clinical outcome of DCM [3]. More recently, the novel percutaneous catheter-based renal artery sympathetic denervation (RDN) has been successfully used to treat drug-resistant hypertension [7]; however, controversy exists from a multicenter randomized study, at least partially driven by non-complete ablation of renal SN. Furthermore, results from the Simplicity Hypertension-3 (HTN-3) study failed to meet the primary efficacy end point, indicating that there was no significant difference in blood pressure reduction either in the office or as measured by ambulatory monitoring in the RDN group compared with the Sham group [8]. Nevertheless, Mahfoud et al. [9] demonstrated that the localized RDN procedure in humans improves indices of insulin action and glucose metabolism as well as cardiac diastolic function in patients with resistant hypertension, but not tested in the setting of DM.

Accordingly, we designed this study aiming to investigate the effect of surgical bilateral RDN (bsRDN, complete denervation) on cardiac function in New Zealand white rabbits with DM induced by HFD diet, an animal model reported to be accompanied by increased SN activity [10–12], and to analyze the underlying molecular mechanisms involved in bsRDN-derived benefits.

2. Materials and methods

2.1. Experimental animals and dietary treatments

Male New Zealand white rabbits age 12 weeks were purchased from the laboratory animal center of Jin Ling General Warren (SCXK Su 2007-0004). During the experiment, rabbits were housed individually in a room maintained at a constant temperature (20–24 °C) and humidity (40–70%), 12-h light/12-h-dark cycle (lights on at 08:00 h), and with free access to water and food. Thirty-two male rabbits were randomly (ratio of 1:3) assigned into Chow (normal diet, $n = 8$) and TEST ($n = 24$, diet consisting of 30% sucrose and 10% fat for up to 48 weeks) groups.

2.2. Bilateral renal surgical pharmacological denervation (bsRDN)

At 48 weeks, animals in the TEST groups were randomized (ratio of 1:1) into Sham ($n = 8$, renal SN was surgically isolated but not injured), RDN ($n = 8$, bsRDN was performed), and HFD ($n = 8$, no surgical operation at all) subgroups. bsRDN consisted of mechanical and chemical procedures and has been described previously [13]. In brief, all nerves visible along renal arteries and veins, from the aorta to the hilum of each kidney, were isolated and carefully stripped and nerves then sectioned (mechanical denervation). Chemical denervation was performed by quickly painting the renal artery with a solution of 10% phenol in 95% ethyl alcohol [14]. The same surgery was performed for sham denervation, but the renal artery and vein were not isolated and the nerves were left intact and were not painted with alcohol.

After the bsRDN, animals in three subgroups were fed the HFD diet for an additional 8 weeks. At 56 weeks, rabbits were sacrificed by an overdose of air injection through an ear vein. The hearts, livers, skeletal muscle, kidneys, and renal arteries were collected and preserved in either 10% formalin or liquid nitrogen. The experimental protocols were approved by the Nanjing Medical University Laboratory Animal Administration Committee and performed according to the Nanjing Medical University Guidelines for Animal Experimentation.

2.3. In vivo measurements

Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), and glucose were quantitated using commercially available kits (Rongshen Biotech, Shanghai, China). Plasma insulin was measured using a radioimmunoassay kit (Academy of Atomic Energy, Beijing, China). Homeostasis model assessment for IR (HOMA-IR) and beta cells (HOMA-β) was calculated using the following equations: [basal insulin (in mU/l) × (glucose (in mg/dl)/18))/22.5] and [20 × basal insulin (in mU/l)]/[glucose (in mg/dl)/18–3.5] [15], respectively. Serum levels of noradrenaline, adrenaline, and calcitonin gene-related peptide (CGRP) were measured using commercial enzyme-linked immunosorbent assay kits (Titer-Zyme Kits, Assay Design, Ann Arbor, MI) at prior-to bsRDN (pre-bsRDN) and 8 weeks after bsRDN (post-bsRDN-8w), according to the manufacturer's specifications.

2.4. Intravenous glucose tolerance test

At 48 weeks, rabbits were fasted overnight and an IVGTT performed. Rabbits were injected with 50% glucose solution (0.6 g/kg) into an ear vein, completed within 30 s. At time 0, 5, 15, 30, 60, and 120 min, blood glucose levels from an ear artery were measured with a glucose analyzer (ACCU-CHEK® Advantage, Roche, Mannheim, Germany) [16]. The bsRDN was repeated at 2, 4, and 8 weeks after the IVGTT.