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Down-regulation of fibroblast growth factor 2 and its co-receptors heparan sulfate proteoglycans by resveratrol underlies the improvement of cardiac dysfunction in experimental diabetes

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Abstract

Cardiac remodeling in diabetes involves cardiac hypertrophy and fibrosis, and fibroblast growth factor 2 (FGF2) is an important mediator of this process. Resveratrol, a polyphenolic antioxidant, reportedly promotes the improvement of cardiac dysfunction in diabetic rats. However, little information exists linking the amelioration of the cardiac function promoted by resveratrol and the expression of FGF2 and its co-receptors, heparan sulfate proteoglycans (HSPGs: Glypican-1 and Syndecan-4), in cardiac muscle of Type 2 diabetic rats. Diabetes was induced experimentally by the injection of streptozotocin and nicotinamide, and the rats were treated with resveratrol for 6 weeks. According to our results, there is an up-regulation of the expression of genes and/or proteins of Glypican-1, Syndecan-4, FGF2, peroxisome proliferator-activated receptor gamma and AMP-activated protein kinase in diabetic rats. On the other hand, resveratrol treatment promoted the attenuation of left ventricular diastolic dysfunction and the down-regulation of the expression of all proteins under study. The trigger for the changes in gene expression and protein synthesis promoted by resveratrol was the presence of diabetes. The negative modulation conducted by resveratrol on FGF2 and HSPGs expression, which are involved in cardiac remodeling, underlies the amelioration of cardiac function.
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Keywords: Resveratrol; Diabetic rats; FGF2; HSPGs; Diastolic dysfunction

1. Introduction

Diabetes is one of the main risk factors for cardiovascular disease. Diabetic patients can develop heart failure in the absence of hypertension and coronary heart disease, the so-called *diabetic cardiomyopathy*. Initially, diastolic dysfunction may progress to systolic dysfunction which has been associated with a worse prognosis [1].

Resveratrol (RSV; 3,5,4′-trihydroxystilbene), a polyphenolic antioxidant found in red wine, seems to protect the cardiovascular system through inhibitory action on apoptosis and acts against reperfusion injury of the ischemic myocardium, atherosclerosis and ventricular arrhythmia [2,3]. It also has antiinflammatory activity, acting on the adhesion molecules reducing their amount in the organism and suppressing the expression of tissue factor and cytokines in vascular cells [4,5]. Experimental studies in rats with diabetes induced by streptozotocin (STZ) showed hypoglycemic and lipid-lowering effects of RSV [6,7]. In addition to the cardioprotective effect already mentioned, RSV also reportedly promotes the improvement of cardiac dysfunction in cardiomyopathy in STZ-diabetic rats [8,9].

Cardiac remodeling in diabetes involves cardiac hypertrophy and fibrosis. Data from the literature suggest that fibroblast growth factor 2 (FGF2) is an important mediator of cardiac hypertrophy [10]. FGF2 is retained by the lower affinity receptors heparan sulfate proteoglycans (HSPGs) in the extracellular matrix and cell surface. After proper stimuli, FGF2 activates its high affinity cell receptor, probably FGFR1 (predominant FGFR in cardiomyocytes). The FGF2-FGFR1 pathway activates mitogen-activated protein kinase signaling by promoting cardiac remodeling. In addition, FGF2-FGFR1 signaling has been linked to the activation of transcription factors that regulate hypertrophy-induced genes [11,12].

Abbreviations: A, Late diastolic mitral inflow; A', late diastolic peak velocity; AMPK, AMP-activated protein kinase; BMI, Body mass index; E, Early diastolic mitral inflow; E', early diastolic peak velocity; FGF, Fibroblast Growth Factor; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase; HSPGs, Heparan sulfate proteoglycans; IVRT, Isovolumic relaxation time; LV, Left ventricular; N-A, Naso-anal length; PPARα, Peroxisome proliferator-activated receptor alpha; PPARγ, Peroxisome proliferator-activated receptor gamma; RSV, Resveratrol; S', systolic peak velocity; STZ, Streptozotocin; UBF, Upstream binding factor.

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HSPGs are highly charged macromolecules found on the surface of virtually every cell type. The main cell surface proteoglycans carrying heparan sulfate in mammalian cells are syndecans and glypicans. They interact with a wide variety of molecules, participating as regulators of biological processes, ranging from embryogenesis to hemostasis [13,14].

Syndecan-4 is described as essential to the development of heart failure in response to pressure overload [15], but little information exists about the role of Syndecan-4 and Glypican-1 in dysfunctional heart muscle in diabetic cardiomyopathy. However, a previous study from our group using SZT-diabetic rats showed increased expression of the proteoglycans Syndecan-4 and Glypican-1 in cardiac tissue [16,17]. These changes were parallel to the onset of diastolic dysfunction, an early event of diabetic cardiomyopathy, demonstrated by changes in peak velocity in early diastole and isovolumic relaxation time (IVRT).

Assuming the importance of FGF2 to cardiac remodeling, and that cardiac function is improved by treatment with RSV in diabetic rats, our proposal was to verify the effect of RSV on gene and protein expression of cell membrane HSPGs in the cardiac muscle in the presence and absence of diabetes and whether it corresponds to changes in FGF2 expression. We also aimed to investigate the impact of such changes on cardiac function.

2. Methods

Thirty-two Wistar rats obtained from the central animal facility of the University of São Paulo Medical School weighing 250–350 g were randomly divided into four groups of 8 animals including the following: control (C), RSV control (RC), diabetic (D) and diabetic treated with RSV (RD). At the end of 8 weeks, all animals were sacrificed by decapitation and the heart removed, washed in ice-cold 0.9% NaCl solution, weighed. Tissue slices were prepared for histological analysis while the rest of the tissue was frozen in liquid nitrogen and stored at−80 °C. All experimental procedures were performed in accordance with the 2011 Guide for the Care and Use of Laboratory Animals (Eighth edition, http://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) and approved by the institution's ethics committee.

2.1. Diabetes induction

Type 2 diabetes was induced by STZ (50-mg/kg IV, Sigma-Aldrich, St. Louis, MO, USA) dissolved in citrate buffer (0.01 M, pH 4.5) and followed after 15 min of an intraperitoneal injection of 100 mg/kg of nicotinamide (Sigma-Aldrich) [18]. The dose of nicotinamide was established after experiments with 50 mg of STZ and 100 (n=3 rats), 150 (n=3 rats) or 230 mg/kg (n=6 rats) of nicotinamide. The rats were followed by 15 days, and at the end of this period, the only group of rats that maintained a high level of glucose was the group of 100 mg/kg of nicotinamide. In the other two groups, the glucose returned to the basal level.

The rats were kept in cages with adequate ventilation and with a 12-h light/dark cycle and stable temperature (21–24 °C). After 15 days of induction, rats with glucose ≥180 mg/dl were considered to have diabetes. Glucose was measured with a portable glucometer (Accu-Chek, Roche, Basel, Swiss) before induction and 3, 7 and 14 days after induction and then weekly until completing 8 weeks.

After 2 weeks, rats of groups RC and RD received a daily portion (22.04 mg/kg) [19] for 6 weeks of RSV diluted in 12% ethanol and administered by gavage. In the control and diabetic groups without resveratrol (C and D), the rats received only 12% ethanol.

Water bottles were used for measuring daily water intake. The average of daily water consumption was calculated for each of the four animals housed in each cage during the study.

2.2. Echocardiography

After 8 weeks, before the sacrifice, transthoracic echocardiography was performed in all rats after anesthesia with 50 mg/kg of Ketalar (Park-Davies, Detroit, MI, USA) and 12-mg/kg xylazine (Bayer, Leverkusen, Germany) using Sequoia 512 (Acuson, Mountain View, CA, USA) equipped with a 13-MHz linear-array transducer [20]. Left ventricular end-systolic and end-diastolic diameter, septum and posterior wall thickness, both in diastole, were measured at the level of the papillary muscles on the short-axis view using 2-D guided M-mode imaging [20,21]. Three representative cardiac cycles were analyzed and averaged for each measurement.

The left ventricular (LV) dimensions including LV mass index, LV end-diastolic and LV end-systolic diameter were normalized to body weight in grams. The relative wall thickness was also measured. The peak velocity of early (E) and late (A) diastolic mitral inflow and E/A ratio, E wave deceleration time and IVRT were obtained from the mitral inflow recordings [20]. Early (E') and late diastolic (A') and systolic (S′) peak velocities from tissue Doppler imaging were measured with a sample volume placed at the septal side of the mitral annulus in the apical 4-chamber view. The myocardial performance index (MPI) is the ratio of isovolumic contraction time and isovolumic relaxation time to the ejection time (ET) measured from mitral inflow and LV outflow time intervals.

2.3. Clinical, cardiovascular and biochemical measurements

At the beginning and end of the study, all animals were weighed and the naso-anal length (N-A) was taken and used to calculate BMI (body weight [g]/length^2 [cm^2]).

For direct measurements of arterial pressure and drug administration, two catheters filled with saline solution were implanted into the femoral artery and femoral vein (PE-10) respectively, in anesthetized rats using isoflurane anesthetic gas 1 mL/mL 2.5% (Cristalia, Itapira, SP, Brazil) [22]. The recorded data were analyzed on a beat-to-beat basis to quantify changes in systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR).

Biochemical analyses were performed with blood samples collected from the venous plexus at the beginning and end of the study after decapitation. Glucose and triglycerides were analyzed using specific reagents for Dimension RxL, Siemens Healthcare Diagnostics Inc., Newark, DE, USA. Insulin was measured with an ELISA kit (Mercodia, Uppsala, Sweden). The quantification of NEFA was performed using a colorimetric kit (Randox Laboratories Ltd., Crumlin, County Antrim, UK).

2.4. Insulin tolerance test

The animals received an intravenous injection of insulin (0.75-U/kg body weight). Glucose was measured using a portable glucose meter at 0, 4, 8, 12 and 16 min after insulin injection. The rate constant for plasma glucose disappearance (KITT) was calculated using the formula $0.693/t^{1/2}$ with $t^{1/2}$ obtained from the slope inclination of the least squares analysis of plasma glucose concentrations for 4 to 14 min after the intravenous injection [23].

2.5. RT-qPCR

Total RNA was isolated from the heart apical region using TRIzol Reagent (Thermo Scientific, Waltham, MA, USA) as described in the manufacturer's protocol. RNA integrity was determined by agarose gel electrophoresis [24], and quantified by NanoDrop (Thermo Scientific) adopting values between 1.8 and 2.0 of 260/280 ratio.

The cDNA was synthesized using 5-μg total RNA by the Superscript II Transcriptase Reverse kit (Thermo Scientific). RT-qPCR was

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