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The ACE 2 activator diminazene aceturate (DIZE) improves left ventricular diastolic dysfunction following myocardial infarction in rats

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ABSTRACT

Diminazene aceturate (DIZE) has been reported to enhance the catalytic efficiency of ACE-2 and presumably increases angiotensin 1–7 generation, interfering with cardiac remodeling after myocardial infarction (MI). Our aim was to investigate the chronic effects of DIZE on cardiac dysfunction post-MI. Male Wistar rats underwent myocardial infarction (MI) or SHAM surgery (SO) and were divided into groups treated with DIZE 15 mg/kg/day, *s.c.* or vehicle (Control). After 4 weeks, the hemodynamic variables were recorded by cardiac catheterism. Hearts were then arrested to obtain the left ventricular (LV) pressure-volume curves *in situ*. Cardiomyocyte hypertrophy and collagen content were determined by histology. DIZE prevented LV end-diastolic pressure increases in MI rats (MI: 26 ± 3.3 *vs.* MI-DIZE: 15 ± 1.6 mmHg, $P < 0.001$) without a significant effect on LV systolic pressure (LVSP). Moreover, DIZE improved LV contractility (+dP/dt, MI: 3014 ± 161 *vs.* MI-DIZE: 3884 ± 104 mmHg/s, $P < 0.001$) and relaxation (-dP/dt, MI: −2333 ± 91 *vs.* MI-DIZE: −2798 ± 120 mmHg/s, $P < 0.05$). Right ventricular SP was increased in the MI compared to that in the SO group (40 ± 0.6 *vs.* 30 ± 1.2 mmHg; $P < 0.01$), and DIZE partially prevented this augmentation. LV stiffness was reduced in MI-DIZE compared with that in MI (0.64 ± 0.01 *vs.* 0.78 ± 0.02 mmHg/mL; $P < 0.01$). DIZE treatment reduced the interstitial collagen content by 18% in the surviving LV myocardium. Cardiomyocyte hypertrophy remained unaffected by DIZE treatment. Our findings show that chronic DIZE treatment post-MI attenuates the morphofunctional changes induced by MI in rats. The effects on LV -dP/dt, chamber stiffness and collagen content suggest this drug can be used as a therapeutic agent to reduce interstitial fibrosis and diastolic dysfunction after MI.

1. Introduction

The ventricular dysfunction that develops post myocardial infarction (MI) is mainly consequence of pathological cardiac remodeling, which is an important factor in the development of heart failure during the chronic infarct phase [1]. Structural remodeling in the surviving noninfarcted myocardium includes left ventricular dilatation, cardiomyocyte hypertrophy and reactive fibrosis, which all contribute to the postinfarction remodeling. An important mechanism involved in this process is the activation of the deleterious axis of the renin-angiotensin system (RAS) by increasing the expression of or activating the angiotensin-converting enzyme (ACE) and AT1 receptors [2]. The main effector of this axis, the nonapeptide angiotensin II, activates subcellular pathways with proapoptotic, profibrotic and pro-hypertrophic properties [3]. Based on this *rationale,* ACE inhibitors and AT1 antagonists have been widely used to prevent heart failure development in infarcted patients [4].

More recently, another axis of RAS has gained attention, mainly due to its opposite effects on the ACE-Ang II-AT1 axis. Angiotensin-converting enzyme 2 (ACE2) is a central component of this axis because it can convert AngII into angiotensin (1–7), which *via* acting through the Mas receptor (MasR), exerts an antifibrotic, antihypertrophic and vasodilatory effect [5,6]. The balance between the deleterious (ACE/AngII/AT1R) and the protective (ACE2/Ang-(1–7)/MasR) axis of RAS seems to play an important role in cardiovascular homeostasis [7]. In cardiac ischemic injury, ACE2 has been considered to be a potential therapeutic target based on experimental and clinical reports showing that ACE2 levels increase after MI, probably acting *via* a compensatory

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mechanism [8,9]. Moreover, cardiac overexpression of ACE2 or angiotensin 1–7 improved cardiac function and remodeling after MI in rats [10,11].

Therefore, molecules able to activate ACE2 may represent a promising therapeutic approach in this field. A small molecule, XNT (1-[(2-dimethylamino)ethylamino]-4-(hydroxymethyl)-7-[(4-methylphenyl) sulfonyl oxy]-9H-xanthene-9-one), has been shown to be a potential activator of ACE2, being able to reverse cardiac and renal fibrosis in spontaneously hypertensive rats (SHR) [12]. More recently, a compound similar to XNT, diminazene aceturate (DIZE), has shown to exert off-target effects of activating ACE2 *in vitro* [13]. It is an aromatic diamidine used as a veterinary trypanocide and babesiacide [14,15] with advantages over XNT, including better bioavailability, pharmacological effects and physicochemical properties [16]. Qi and collaborators [17] demonstrated that chronic DIZE treatment in rats, initiated before MI, prevented cardiac dysfunctional changes by reducing the infarct size and cell death. The proposed mechanism is that DIZE enhances the catalytic efficiency of ACE-2 and presumably increases angiotensin 1–7 generation, thus interfering with cardiac remodeling. However, whether DIZE could be a therapeutic target in myocardial infarction remains unclear. Therefore, we sought to investigate the chronic effects of DIZE on cardiac dysfunction post-MI in rats.

2. Material and methods

2.1. Myocardial infarction

Male Wistar rats (12 weeks old) were divided into four experimental groups: 1) Sham-operated (SO); 2) SO - DIZE; 3) MI and 4) MI- DIZE. Rats were anesthetized (ketamine 50 mg/kg and xylazine 10 mg/kg, i.p.), the thorax was opened, and MI was induced by permanent ligation of the proximal descending branches of the left coronary artery (LAD) using a 6–0 polypropylene suture as previously described [18]. Successful coronary occlusion was confirmed by changes in the ST segment on an electrocardiogram recorded a few minutes after coronary ligature [19]. In the SHAM-operated animals (SO), the same surgical procedures except the coronary occlusion were performed. DIZE treatment (single daily injection, 15 mg/kg/day, s.c.) was initiated immediately after coronary ligation or sham surgery and continued throughout the four-week period. All animals were housed in a temperature-controlled room (~24 °C) and maintained on a 12:12-hour light:dark cycle with free access to water and food. All procedures involving experimental animals were approved by the Institutional Animal Care and Use Committee at the Department of Physiological Sciences, Federal University of Espírito Santo, Vitória.

2.2. Hemodynamic measurements

Cardiac function and hemodynamic measurements were assessed by catheterization four weeks after surgery. In brief, rats were anesthetized with the ketamine plus xylazine mixture (50/10 mg/kg, i.p.). Then, polyethylene catheters (PE-50) were inserted into the right common carotid artery and jugular vein and inserted into the left and the right ventricle, respectively [20]. For pressure measurements, the catheter was connected to a pressure transducer (TRI 21, Letica Scientific Instruments, Spain) and to a digital system (Powerlab/4sp ML750, ADInstrument, Australia). Data were analyzed using Chart 7.0 software. The parameters measured included systolic and diastolic arterial pressure (SBP and DBP), heart rate, right and left ventricular end diastolic pressure (RVEDP and LVEDP), right and left ventricular systolic pressure (RVSP and LVSP),and the rate of intraventricular pressure rise (+dP/dt)and decline (-dP/dt) [7].

2.3. In situ left ventricular pressure-volume relationship

After hemodynamic evaluation, the anesthetized rats had their hearts arrested (3 M KCl, i.v.). A double-lumen catheter (a PE-50 inserted into a PE-90) was introduced into the LV through the aorta to obtain the *in situ* left ventricular diastolic pressure-volume relationship [21]. In brief, the atrioventricular groove was occluded, and a small incision was made in the right ventricular free wall to avoid liquid accumulation inside this cavity and any compressor effect on the left ventricular chamber. Physiological saline (NaCl 0.9%) was pumped into the ventricular cavity at a constant rate (0.68 ml/min) (BI200, Insight Equipments, Brazil). Three curves were recorded in each heart over 10 min. For the analysis, the volume infused with fixed pressure values (0, 5, 10, 15, 20, 25 and 30 mmHg) was calculated, considering a volume of 0.05 mL equivalent to 0 mmHg.The curves were separated into two parts to exclude possible interferences of dilatation on the stiffness index [21]. In the first segment, from 0 to 5 mmHg, the pressure curve followed a linear tendency during volume infusion. The slope of this first linear segment was proportional to the left ventricular dilatation. In the second segment, from 5 to 30 mmHg, the pressure increase during volume infusion followed a monoexponential pattern ($P = V_O \times e^{kv}$), where V is the volume at 5 mmHg, k is the stiffness constant of the chamber, and v is the volume infused. To determine the stiffness constant in the 5–30 mmHg range without the interference of the first segment, the pressure scale was log-transformed. Therefore, the slope of the relationship between 5 to 30 mmHg of the pressure-volume curve indicates the stiffness of the left ventricular cavity [22].

2.4. Histological analysis

After pressure-volume recording, the heart was removed, and the ventricles were separated and weighed. The middle section of the LV was fixed in formalin and embedded in paraffin. The mid-ventricular sections were stained with picrosirius red to measure the infarct size and interstitial collagen, as they provide an adequate estimation of total LV infarct size [23]. Total infarct size was calculated as a fraction (%) of the LV circumference through digital planimetry. Interstitial fibrosis was determined *via* collagen fraction staining and thresholding automated analysis. Images were obtained from eight histological sections of each slice of the heart, captured with a video camera (AxioCam ERc 5 s, Carl Zeiss, Germany) coupled to an optical microscope (Olympus AX70, Olympus Corporation) under 400× magnification. Fifteen areas of high-power fields were chosen in the noninfarcted area of each heart, and the interstitial collagen content was measured through the percentage of the area stained by picrosirius red using the specific calibrated software [24,25]. To investigate the presence of hypertrophy, the cross-sectional area of each cardiomyocyte was measured in the hematoxylin/eosin stained slides by circumscribing cardiomyocytes positioned perpendicular to the section plane, with a visible nucleus and cell contours. A total of 40 to 50 cells were measured in the non-infarcted myocardium of each rat, excluding the border zone. In similar regions, cells were also measured in SO animals. Measurements were carried out using the ImageJ program (v. 1.43 u, National Institute of Health, USA) [2]. The lung water content was measured indirectly through the lung wet-to-dry weight ratio. In brief, the right and left lungs were harvested, cleaned of connective tissue, vessels and trachea, gently wiped and weighed immediately. The same samples were dried at 96 °C for 24 h. After drying, they were weighed again to calculate the ratio of wet to dry weight of lung tissue [26].

2.5. Statistical analysis

Data are expressed as the mean ± SEM and were analyzed by two way ANOVA followed by a Bonferroni post hoc test for multiple comparisons. Values of $P < 0.05$ were considered statistically significant. Analyses were performed using GraphPad Prism 5 software (GraphPad Prism Institute Inc.).