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Cardioprotective effects of diminazene aceturate in pressure-overloaded rat hearts

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ABSTRACT

Aims: Angiotensin-converting enzyme 2 (ACE2) is a key modulator of the renin-angiotensin system. Recent studies have shown that diminazene aceturate (DIZE) acts as an ACE2 activator. The aim of this study was to evaluate the cardiac effects of chronic treatment with DIZE in pressure-overloaded rats.
Main methods: Male Wistar rats were divided into 4 groups: (1) sham; (2) aortic banded rats (AB); (3) AB + DIZE (1 mg/kg, gavage); and (4) AB + DIZE + A-779 (120 μg/day, osmotic mini-pumps). Cardiac hypertrophy was evaluated by ventricular mass index and myocyte cross-sectional area. mRNA expression of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and transforming growth factor beta 1 (TGF-β) was quantified by RT-PCR. Cardiac function was assessed according to the Langendorff technique. The ACE2 and Mas protein expression was examined by western blot analysis.
Key findings: DIZE treatment prevented the cardiomyocyte hypertrophy promoted by AB and A-779 inhibited this effect. Also, DIZE induced the expression of ANP and BNP mRNA in cardiac tissue from AB rats and attenuated the impairment in left ventricular end-systolic pressure and left ventricular developed pressure, + dP/dt and − dP/dt caused by AB. These effects were blocked by A-779. Moreover, DIZE prevented the increase in the expression of TGF-β mRNA in AB hearts, but it did not change the ACE2 and Mas protein expression.
Significance: These results showed that DIZE was efficient in preventing the cardiomyocyte hypertrophy and attenuated the left ventricular contractile impairment induced by pressure overload. However, further studies are necessary to confirm whether these effects were due to ACE2 activation.

1. Introduction

Cardiac hypertrophy is a response to increased mechanical and neurohormonal stimuli [1,2]. The renin-angiotensin system (RAS) has a vital role in the development and progression of the cardiac hypertrophy [3]. Components of the RAS, such as angiotensinogen [4], angiotensin-converting enzyme (ACE), and angiotensin (Ang) II [5] are overexpressed in ventricles in response to pressure overload. In addition, the cardioprotection achieved by blocking the RAS provides further evidence for the role of the ACE/Ang II/AT1 receptor axis in the development of pressure overload-induced cardiac hypertrophy [6].

Previous studies have established a new regulatory axis within the RAS [ACE2/Ang-(1–7)/Mas axis], in which Ang-(1–7) is produced mainly from Ang II by the catalytic activity of ACE2 [7,8]. Thus, ACE2 has emerged as a negative regulator of the RAS, playing an opposite role to the ACE/Ang II/AT1 receptor axis [9]. Loss of ACE2 accelerated the cardiac hypertrophy and shortened the transition period to heart failure in response to pressure overload [10], and exacerbated the development of systolic and diastolic dysfunction and impaired the vascular function in a diabetic mice model [11]. Pharmacological inhibition of ACE2 exacerbated the cardiac hypertrophy and fibrosis in Ren-2 hypertensive rats [12]. Instead, overexpression of ACE2 prevented the cardiac hypertrophy and fibrosis in spontaneously hypertensive rats (SHR) and in Ang

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II-treated rats [13,14]. Also, it was able to exert protective effects in hearts during myocardial infarction [15].

Overall, these studies indicate a crucial role of ACE2 in the cardiac structure and function. Thus, activation of this enzyme might be an important therapeutic strategy against cardiovascular diseases. In this context, Hernandez Prada et al. in 2008 [16] described the small-molecule ACE2 activator 1-[(2-dimethylamino)ethylamino]-4-(hydroxy-methyl)-7-[(4-methylphenyl)sulfonyloxy]-9H-xanthene-9-one (XNT). For instance, it has been reported that this compound decreases blood pressure, improves cardiac function and reverses myocardial and perivascular fibrosis in SHR [16,17], prevents pulmonary hypertension in monocrotaline-treated rats [18], and attenuates thrombus formation and reduces the platelet attachment to vessels in hypertensive rats [19].

The diminazene aceturate (DIZE), another ACE2 activator, holds better physicochemical characteristics when compared to XNT [19]. Treatment with DIZE increased the ACE2 activity in the renal cortex and medulla of rats submitted to subtotal nephrectomy [20], in plasma and cardiac tissue of infarcted rats [21], and in serum of stroked rats [22]. Recent studies have demonstrated beneficial cardiovascular effects of ACE2 activation using DIZE. This compound significantly attenuated the cerebral infarct size and neurological deficits in a model of cerebral ischemia [22,23]. In monocrotaline-treated rats, it prevented the development of pulmonary hypertension [24], enhanced the cardiac function [24], and improved the autonomic nervous system modulation [25]. Also, DIZE decreased the intraocular pressure of glaucomatous rats [26]. Qi et al. [21] demonstrated that DIZE was able to decrease the infarcted area and attenuated the left ventricular remodeling post-myocardial infarction in rats [21]. However, no data are available on the ability of this compound to prevent the left ventricular hypertrophy and dysfunction induced by pressure overload. Therefore, the aim of this study was to evaluate the cardiac effects of chronic treatment with DIZE in pressure-overloaded rats.

2. Material and methods

2.1. Ethical approval

Forty-three male Wistar rats weighing 200 to 350 g were provided by the animal facilities of the Federal University of Goiás, Brazil. All animals were kept in temperature-controlled rooms with a 12/12 h light/dark cycle and had free access to water and food. The protocols used in this work were approved by the ethics committee of the Federal University of Goiás (protocol number: 057/2012) and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Hypertrophic heart model and treatments

Cardiac hypertrophy was induced by abdominal aortic banding (AB), as described previously [27]. Briefly, a left laparotomy was performed and the descending aorta was isolated above the celiac artery after anesthesia with a mixture of ketamine and xylazine (70 mg/kg and 30 mg/kg, respectively, i.p.). Then, a bent 21-gauge needle was placed along with the isolated aortic segment and a suture was tied around the needle. After ligation, the needle was quickly removed. Sham-operated control rats were submitted to the same procedure, but without constriction of the aorta. AB rats were then randomly shared into three subgroups: aortic banded treated with vehicle (tap water, gavage); aortic banded treated with DIZE (Sigma, USA) (1 mg/kg/day, gavage) and aortic banded treated with DIZE plus A-779 (120 μg/day, osmotic mini-pumps). Twenty-one days after the surgery, the left ventricular mass index (VMI) was calculated through the ratio between the left ventricular wet weight and tibia length.

2.3. Cardiomyocyte morphometry

The middle part of the hearts were fixed in 10% buffered paraformaldehyde and embedded in paraffin. Samples were sectioned at a thickness of 5 μm and stained with hematoxylin and eosin following standard methods. Myocyte cross-sectional area (MCSA) was evaluated in two heart sections from each animal (an average of 50 cardiomyocytes for each section) using an ocular micrometer calibrated with a stage micrometer adapted to a light microscope (BX 51, Olympus) at 400 x magnification. The diameter of each myocyte was measured across the region corresponding to the nucleus. Approximately 100 cardiomyocytes were analyzed for each animal.

2.4. Blood pressure measurement

At the end of the treatments, mean arterial pressure (MAP) was recorded. One day before euthanasia, rats were anesthetized (ketamine 70 mg/kg and xylazine 30 mg/kg, i.p.) and a cannula (PE-50) was inserted into the right carotid artery and exteriorized through the back of the neck. The cannula was linked to a pressure transducer connected to a signal amplifier ETH-400 (CB Sciences, Inc.). These signals were registered by the ADInstruments PowerLab system at a sampling frequency of 1000 Hz. Blood pressure was recorded over 30 min in conscious rats 24 h after recovery from anesthesia. The MAP value of each animal was obtained by the average of 7 values (each value collected at 5 min interval).

2.5. Isolated heart preparation

At the end of the treatments, the rats were decapitated 10 to 15 min after an intraperitoneal injection of 200 IU heparin and the hearts were perfused accordingly to the Langendorff technique. Briefly, the heart was carefully dissected and perfused with Krebs-Ringer solution (KRS) containing (in mmol/L) NaCl (118.4), KCl (4.7), KH_2PO_4 (1.2), $MgSO_4 \cdot 7H_2O$ (1.2), $CaCl_2 \cdot 2H_2O$ (1.25), glucose (11.7), and $NaHCO_3$ (26.5). The perfusion pressure was maintained constant (70 mm Hg) at 37 °C and constant oxygenation (5% CO_2 and 95% O_2). A balloon was inserted into the left ventricle through the left atrium for isovolumetric recordings of the left ventricular pressure. The heart rate (HR) was derived from the intraventricular pressure wave. Coronary flow was measured by collecting the perfusate over a period of 1 min at regular intervals. After 20 to 30 min of stabilization, the hearts were perfused for an additional 20 min for cardiac function analysis. The values of each animal were obtained by the average of 5 values collected at 5-min intervals. Data were analyzed online using a data acquisition system (Biopac).

2.6. Isolated aortic ring preparation

Isolated aortic rings were used to evaluate if DIZE was able to prevent endothelium dysfunction induced by pressure overload. The thorax was opened, descending thoracic aorta was isolated, and adherent tissues were removed. The aorta was put into Krebs-Henseleit solution composed by (in mmol/L) NaCl (118.06), KCl (4.6), $NaHCO_3$ (24.9), $MgSO_4 \cdot 7H_2O$ (2.4), $CaCl_2 \cdot 2H_2O$ (3.3), KH_2PO_4 (0.9) and glucose (11.1) and sectioned into 4-mm long fragments. Aortic rings were held up by two parallel L-shaped wires and immersed into an organ bath containing gassed (95% O_2 and 5% CO_2) Krebs-Henseleit solution at 37 °C. The rings were kept under a tension of 1.5 g for 1 h to equilibrate. Thereafter, aortic rings were pre-constricted with phenylephrine at concentration of 10^{-7} mol/L and acetylcholine (Ach) was added to the bath in increasing cumulative concentrations (10^{-9} to 10^{-5} mol/L). Mechanical activity was recorded isometrically by a force transducer fed to an amplifier-recorder and analyzed using a data-acquisition system (Dataq Instruments). After this, a cumulative concentration-response (% contraction) curve was plotted.