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An orally active angiotensin-(1–7) inclusion compound and exercise training produce similar cardiovascular effects in spontaneously hypertensive rats

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ABSTRACT

Low angiotensin-(1–7) (Ang-(1–7)) concentration is observed in some cardiovascular diseases and exercise training seems to restore its concentration in the heart. Recently, a novel formulation of an orally active Ang-(1–7) included in hydroxy-propyl-beta-cyclodextrin (HPB-CD) was developed and chronically administered in experimental models of cardiovascular diseases. The present study examined whether chronic administration of HPB-CD/Ang-(1–7) produces beneficial cardiovascular effects in spontaneously hypertensive rats (SHR), as well as to compare the results obtained with those produced by exercise training. Male SHR (15-week old) were divided in control (tap water) or treated with HPB-CD/Ang-(1–7) (corresponding to 30 $\mu g\,kg^{-1}\,day^{-1}$ of Ang-(1–7)) by gavage, concomitantly or not to exercise training (treadmill, 10 weeks). After chronic treatment, hemodynamic, morphometric and molecular analysis in the heart were performed. Chronic HPB-CD/Ang-(1–7) decreased arterial blood pressure (BP) and heart rate in SHR. The inclusion compound significantly improved left ventricular (LV) end-diastolic pressure, restored the maximum and minimum derivatives (dP/dT) and decreased cardiac hypertrophy index in SHR. Chronic treatment improved autonomic control by attenuating sympathetic modulation on heart and vessels and the SAP variability, as well as increasing parasympathetic modulation and HR variability. Overall results were similar to those obtained with exercise training. These results show that chronic treatment with the HPB-CD/Ang-(1–7) inclusion compound produced beneficial effects in SHR resembling the ones produced by exercise training. This observation reinforces the potential cardiovascular therapeutic effect of this novel peptide formulation.

1. Introduction

The renin–angiotensin system (RAS) is a major cardiovascular and renal control mechanism. Based on many recent studies this system is composed of two effector arms: the classical one angiotensin-converting enzyme (ACE)/angiotensin II (Ang II)/angiotensin II receptor type 1 (AT1) which is responsible for most of the known actions of the RAS and the counter-regulatory arm ACE2/angiotensin-(1–7) (Ang-(1–7))/Mas receptor, which opposes a variety of the Ang II effects [10,11,39]. Ang-(1–7) is a heptapeptide primarily formed from angiotensin I by ACE-independent pathway and from angiotensin II cleavage by ACE2 [39]. Ang-(1–7) is present in the heart, and it contributes to preserve the cardiac function and the coronary perfusion in experimental models of cardiovascular diseases [22]. The cardioprotective effect of Ang-(1–7) is mainly mediated through G protein-coupled Mas receptor activation, also expressed in the heart, and modulated under physiological and pathological conditions [40]. Abnormal

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activation of the ACE/Ang II/AT1 receptor is observed in cardiovascular diseases, stimulating maladaptive cardiac responses and directly contributing to the progression to heart failure [1,44].

The spontaneously hypertensive rat (SHR) has many similarities with the human essential hypertension, including high blood pressure (BP), cardiac remodeling and target-organ damage [43]. The ACE/Ang II/AT1 axis appears as a key mechanism stimulating cardiac hypertrophy and fibrosis in this model [37]. Cardiac structural changes are also accompanied by left ventricular (LV) dysfunction and the impairment of the autonomic control of the BP in this model [4,20]. The association of LV dysfunction, high BP and impaired autonomic control in SHR determines the progression to heart failure, and in humans, it is considered an important predictor of mortality and morbidity caused by cardiovascular diseases [21].

Beneficial effects of exercise training on managing high BP have been extensively described in humans [28,31] and animal models [8,20] in the last decades. In SHR model, our group and others have previously shown that exercise training reduces BP mainly by improving the autonomic control and reducing sympathetic modulation in the heart and vessels [4], but also by decreasing overall cardiovascular oxidative stress and enhancing endothelial vasodilator function [3,33]. Although direct effects of exercise training on RAS are not well understood, some studies show upregulation of AT1 receptor in the heart after resistance training in normotensive rats [2], as well as increased Mas receptor expression and Ang-(1–7) concentration in SHR hearts after swim training [15]. In SHR, exercise training might therefore modulate the balance between both RAS axes which may contribute to prevent or reduce hypertension-induced cardiovascular dysfunction.

In addition to exercise, pharmacological approaches targeting the ACE/Ang II/AT1 axis are nowadays widely used in the treatment and handling of hypertension and heart failure. It is well established in the literature that AT1 receptor blockade in SHR reduces left ventricular (LV) mass and improves coronary hemodynamics [34]. However, the activation of the counteracting axis ACE2/Ang-(1–7)/Mas has been currently considered an alternative therapeutic strategy to control Ang II effects and to circumvent adverse responses observed with drugs blocking the Ang II axis. Thus, Mas agonists such as AVE 0991 or orally active formulations of Ang-(1–7) should be considered as a putative new class of cardiovascular drugs. Our group has recently described a new formulation of Ang-(1–7) which could allow the oral administration of this peptide, reducing its degradation by the digestive tract [23]. This is an inclusion compound composed of hydroxy-propyl-beta-cyclodextrin (HPB-CD), and Ang-(1–7). The structural arrangement of the inclusion compound protects the internal (guest) molecule, in this case Ang-(1–7), against proteolytic enzymes, and once HPB-CD is enzymatically degraded by bacterial enzymes at the distal portions of the digestive tract it releases the peptide which is absorbed mainly in the colon. We therefore aim to assess in this study the potential therapeutic effect of the Ang-(1–7)-inclusion compound (HPB-CD/Ang-(1–7)) on BP values and heart function, as well as to compare its effects with the ones of exercise training on autonomic control of BP and heart rate (HR) in SHR. For this purpose, we hereby compare the effect of chronic administration of HPB-CD/Ang-(1–7) with exercise training, as well as the association of these two interventions.

2. Methods

2.1. Animals

Male SHR (15-week old, 250–300 g) were obtained from the Fundação Universitária de Cardiologia (Porto Alegre, Brazil) and divided in four experimental groups: sedentary treated with tap water ($n = 8$), sedentary treated with oral administration of HPB-CD/Ang-(1–7) inclusion compound (72 $\mu g\,kg^{-1}\,day^{-1}$, corresponding to 30 $\mu g\,kg^{-1}\,day^{-1}$ of Ang-(1–7))($n = 8$), exercise-trained treated with tap water ($n = 9$) and exercise-trained treated with HPB-CD/Ang-(1–7) ($n = 8$). Treatment was administered by gavage once per day during 10 weeks, in the morning, concomitantly with the exercise training protocol. Animals received standard laboratory chow and water ad libitum and were housed in temperature-controlled rooms (22 °C) with a 12:12-h dark–light cycle. A moderate-intensity exercise protocol was performed in treadmill, 5 days/week during 10 weeks. Exercised rats progressed toward a speed of 20 m/min for 1 h, prescribed based on lactate threshold assessment, as previously described by Bertagnolli et al. [3]. One week before starting the exercise protocol, all animals were adapted to the procedure. All animal protocols and procedures used were in accordance with the Guidelines for Ethical Care of Experimental Animals and the US National Institutes of Health or European Commission guidelines, and were approved by Fundaçao Universitaria de Cardiologia de Porto Alegre Ethical Committee.

2.2. Acute Ang-(1–7) absorption protocol

Additional male SHR were allotted to an acute experiment to test the absorption of HPB-CD/Ang-(1–7) inclusion compound. Twenty-four hours before the experiment, a polyethylene catheter filled with saline was inserted in all rats femoral artery (under ketamine 90 mg/kg and xylazine 20 mg/kg anesthesia) to allow the collection of blood samples. SHR were divided in three groups ($n = 5$/group): control treated with tap water, treated with Ang-(1–7) not included (Bachem), and treated with HPB-CD/Ang-(1–7) inclusion compound (72 $\mu g\,kg^{-1}$ corresponding to 30 $\mu g\,kg^{-1}$ of Ang-(1–7)) by gavage. Blood samples were collected at 2, 4, 6, 8 and 24 h after treatment for measuring plasma Ang-(1–7) concentrations.

2.3. Hemodynamic measurements

Forty-eight hours after the last treatment day (10 weeks), rats from the chronic experiment were anesthetized with intramuscular injection of ketamine (90 mg/kg) and xylazine (20 mg/kg) for intra-arterial catheterization. Two polyethylene catheters (PE-10, Biocorp Australia, Huntingdale, Victoria, Australia) filled with heparinized saline solution, connected to a strain gauge transducer (NarcoBiosystem Pulse Transducer RP-155, Houston, TX, USA) linked to a pressure amplifier (HP 8805C, Hewlett Packard, USA), were inserted into the femoral artery and vein for direct measurements of systolic, diastolic, mean arterial and pulse pressures (SAP, DAP, MAP and PP, respectively), heart rate (HR), and drug administration, respectively. The intra-arterial measurements were performed 48 h after the surgical procedure. After the conscious pressure recordings, rats were anesthetized through femoral vein catheter with sodium pentobarbital (40 mg/kg, i.v., Cristalia, SP, Brazil) and a PE-50 catheter was inserted through the right carotid artery and advanced into the LV for recording intraventricular systolic (LVSP) and end-diastolic (LVEDP) pressures, as well as the maximum rate of LV pressure rise and fall ($+dP/dT$ and $-dP/dT$). The obtained pressure recordings were taken using a microcomputer equipped with an analog-to-digital conversion board (Windaq, 2 kHz sample rate, Dataq Instruments, Inc., Akron, OH, USA). Rats have their BP and HR constantly monitored during pentobarbital anesthesia to control the adequacy of the anesthesia. Intraventricular catheterization was only performed when BP and HR values were similar to those obtained with conscious intra-arterial recordings. An additional group of normotensive Wistar–Kyoto rats (25-week old) was added to the experiment to