Life Sciences 92 (2013) 266–275

Contents lists available at SciVerse ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

The nonpeptide ANG-(1–7) mimic AVE 0991 attenuates cardiac remodeling and improves baroreflex sensitivity in renovascular hypertensive rats

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ARTICLE INFO

Article history:
Received 12 June 2012
Accepted 21 December 2012

Keywords:
AVE 0991
Angiotensin-(1–7)
Hypertension
Baroreflex sensitivity
Cardiac remodeling

ABSTRACT

Aims: The nonpeptide Ang-(1–7) analog, AVE 0991, is recognized as having beneficial cardiovascular effects similar to those induced by Ang-(1–7). In this study, we evaluated the effects of AVE 0991 on cardiovascular functions and on cardiac and renal remodeling in rats with 2K1C renovascular hypertension.
Main methods: Fisher rats underwent surgery to induce 2K1C renovascular hypertension and were then treated with AVE 0991 (1 or 3 mg/kg) for 28 days. At the end of treatment, the blood pressure (BP), heart rate (HR), and baroreflex sensitivity were evaluated, in conscious animals. The rats were then euthanized and the heart and kidneys removed for subsequent histological analysis.
Key findings: Treatment with AVE 0991 in 2K1C rats restored the baroreflex sensitivity of both bradycardic and tachycardic components to levels comparable to those of normotensive SHAM rats. At a higher dose (3 mg/kg), AVE 0991 was also anti-hypertensive in 2K1C rats. Furthermore, AVE 0991 reduced the heart weight, thickness of myocardial fibers, number of inflammatory cells, and area of collagen deposition in the hearts of 2K1C rats compared to SHAM rats. The inflammatory process and tissue area of collagen deposition were decreased in the clipped kidney of AVE 0091-treated 2K1C rats.
Significance: Our data showed that oral treatment with AVE 0991 reduces blood-pressure cardiac remodeling and improves baroreflex sensitivity in 2K1C renovascular hypertensive rats.

Introduction

Hypertension is characterized by increased activity of the sympathetic nervous system and overactivity of the renin angiotensin system (RAS), accompanied by reduced sensitivity of baroreflex control of arterial pressure (Korner et al., 1974; McCubbin et al., 1956), cardiac hypertrophy and remodeling, and renal lesion (Frohlich et al., 1999; Grobe et al., 2006; Sadjadi et al., 2005a,b).

Cardiac hypertrophy when accompanied by fibrosis may lead to loss of function (Frohlich et al., 1999; Grobe et al., 2006; Schaper, 1998). In addition to affecting cardiac function, hypertension may cause kidney damage, compromising the blood vessels, glomeruli and renal interstitium, causing fibrosis and loss of renal function (Klag et al., 1996; Ljutić and Kes, 2003; Soares et al., 2011). The hyperactivity of some components of the RAS is important in determining the progression of this disease. Angiotensin (Ang) II participates in cardiac remodeling by stimulating myocyte hypertrophy and fibroblast proliferation (González et al., 2002; Kawano et al., 2000). Conversely, the antitrophic and antifibrotic activity of Ang-(1–7) counteracts these effects (Grobe et al., 2007; Pei et al., 2010; Tallant et al., 2005).

Loot et al. (2002) found that in rats with heart failure, chronic treatment with Ang-(1–7) preserved cardiac function, coronary perfusion and aortic endothelial function. Iwata et al. (2005) demonstrated that in cardiac fibroblasts of adult rats, Ang-(1–7) inhibited collagen synthesis and decreased mRNA expression of growth factors, suggesting an important role of Ang-(1–7) in the regulation of cardiac remodeling. Additionally, Grobe et al. (2007) showed that chronic infusion of Ang-(1–7) prevented myocardial cell hypertrophy of the left ventricle (LV) and interstitial fibrosis induced by infusion of Ang II; and Pei et al. (2010) showed that treatment with Ang-(1–7) in SHR attenuated cardiac hypertrophy and deposition of collagen during hypertensive states.

The renovascular hypertension two-kidney one-clip (2K1C) model is characterized by a hyperactivity of the RAS. In this model, the levels of Ang II increase in both kidneys, clipped and unclipped, thereby increasing plasma levels of Ang II, aldosterone and sympathetic tone, and consequently the blood pressure (BP) (Navar et al., 1998; Von Thun et al., 1994; Zou et al., 1996). Additionally, renal

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0024-3205/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.lfs.2012.12.008

production of Ang II modulates the transport of salt and water by the renal tubules as well as the glomerular filtration process and collagen deposition (Ferrario and Varagic, 2010).

An important advance in studies of the effects of Ang-(1–7) was the discovery of non-peptide analog AVE 0991 (Wiemer et al., 2002). Several studies have shown that AVE 0991 acts as a Mas receptor agonist in the kidneys and blood vessels. AVE 0991 also induces release of nitric oxide (Lemos et al., 2005; Santos and Ferreira, 2006) and has a cardioprotective effect by improving cardiac function, reducing atherosclerosis, cardiac hypertrophy and remodeling (Benter et al., 2006; Ferreira et al., 2007b; Wiemer et al., 2002). In addition, recent studies have shown that treatment with AVE 0991 prevented myocardial hypertrophy induced by Ang II (He et al., 2010) and attenuated ventricular remodeling in rats with myocardial infarction (Zeng et al., 2012) through inhibition of inflammatory markers such as signaling TGF-β1/TNF-α and TGF-β1/Smad2.

Given these considerations, this study evaluated the effect of chronic administration of AVE 0991 on BP, HR, sensitivity of the reflex control of HR, cardiac remodeling and renal injury in conscious rats with 2K1C renovascular hypertension.

Materials and methods

Animals

Experiments were performed on male Fisher rats from ENUT, Universidade Federal de Ouro Preto, Brazil. The animals were housed in separate cages in groups of four (2K1C or SHAM), with free access to rat chow and tap water in a temperature- and light-controlled room. All animal procedures were in accordance with the Guidelines for Ethical Care of Experimental Animals, and were performed as approved by the Institutional Ethics Committee of the Federal University of Ouro Preto (Protocol # 2010/55).

Induction of renovascular hypertension

Goldblatt renovascular hypertension was induced as described by Goldblatt et al. (1934). Briefly, the rats (weighing 150–180 g) were anesthetized with a mixture of ketamine and xylazine (50 mg/kg and 10 mg/kg respectively, *ip*), and a silver clip (0.20 mm ID) was placed around the left renal artery through a midline incision (Goldblatt renovascular hypertension, 2-kidney, 1-clip model; 2K1C). Other rats were submitted to similar procedures but without the renal-artery clip placement (SHAM group or normotensive rats). Cardiovascular and histological measurements were carried out 30 days after the surgery.

Arterial pressure measurements

2K1C and SHAM rats were anesthetized with a mixture of ketamine and xylazine (50 mg/kg and 10 mg/kg respectively, *ip*) and a polyethylene catheter was inserted into the abdominal aorta through the femoral artery, for arterial pressure measurement, and another catheter was inserted into the inferior cava vein through the femoral vein, for drug injections in order to evaluate the baroreflex sensitivity. Pulsatile arterial pressure was monitored by a Gould pressure transducer (PM-1000, CWE) coupled to a blood pressure signal amplifier (UIM100A, Powerlab System). Mean arterial pressure (MAP) and heart rate (HR) were determined from the arterial pressure wave. All variables were continuously recorded with a PowerLab digital acquisition system (PowerLab 4/20, ADInstruments) with an 800 Hz sampling rate.

Intragastric treatment with AVE 0991 or vehicle

Three days after the surgery to induce 2K1C or SHAM, intragastric administration of AVE 0991 at a dose of 1 mg/kg or 3 mg/kg, or of the Vehicle (KOH, 1 ml of 10 mM KOH added to 9 ml of distilled water) by gavage was started and continued daily for 28 days, always at the same time of day. The animals received around 0.2 ml solution of AVE 0991 (1 or 3 mg/kg) or vehicle.

Evaluation of baroreflex sensitivity

The sensitivity of the baroreflex control of HR was determined by recording reflex HR changes in response to transient increases (baroreflex bradycardia) or decreases (baroreflex tachycardia) in MAP produced by repeated bolus injections of graded doses of phenylephrine (0.5 to 50.0 μg, iv) or sodium nitroprusside (0.5 to 50.0 μg, iv), respectively, in conscious rats. Evaluations of the sensitivity of reflex bradycardia or tachycardia were performed randomly, with a 15-min interval between them. The HR was converted to pulse interval (PI, ms) by the formula: 60,000/HR. A best-fit regression line was drawn from MAP and HR changes obtained with the different doses of phenylephrine or sodium nitroprusside for each animal. The slope of the regression line was used as an index of baroreflex sensitivity (baroreflex gain), as in previous studies (Alzamora et al., 2006).

Analysis of cardiac and renal structures

For the histopathological analysis, hearts and kidneys were collected and fixed in 10% neutral-buffered formalin solution. After 72 h of fixation, the hearts and kidneys were dehydrated, cleared, and embedded in paraffin. The paraffin block was cut into 4–5-μm

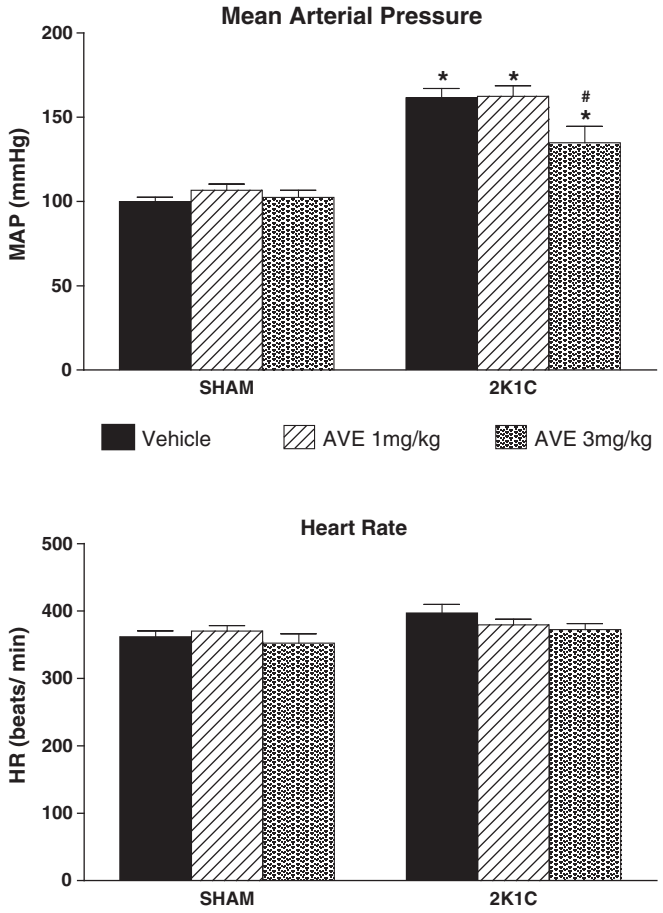

Fig. 1. Baseline levels of mean arterial pressure (MAP, mm Hg) and heart rate (HR, beats/minute) in normotensive rats (SHAM, n = 5–7) and hypertensive rats (2K1C, n = 7–9) treated, per gavage, for 28 days with vehicle or AVE 0991 (1.0 mg/kg or AVE 3.0 mg/kg). *p<0.05 compared to SHAM vehicle group; #p<0.05 compared to 2K1C vehicle group (two-way ANOVA, followed by Bonferroni).