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EFFECTS OF CARVEDILOL OR AMLODIPINE ON TARGET ORGAN DAMAGE IN L-NAME HYPERTENSIVE RATS: THEIR RELATIONSHIP WITH BLOOD PRESSURE VARIABILITY

Carvedilol vs amlodipine in hypertension

Julieta S DEL MAURO[a*], Paula D PRINCE[b*], Martín DONATO[c], Nahuel FERNANDEZ MACHULSKY[d], Marcela A MORETTÓN[e], Germán E GONZÁLEZ[c], Facundo M BERTERA[a,f], Andrea CARRANZA[a], Susana B GORZALCZANY[a], Diego A CHIAPPETTA[e], Gabriela BERG[d], Celina MORALES[c], Ricardo J GELPI[c], Carlos A TAIRA[a,f], Christian HÖCHT[a,f].

*Both authors contributed equally to this work

[a]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Farmacología. Buenos Aires. Argentina.

[b]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Cátedra de Físicoquímica, Instituto de Bioquímica y Medicina Molecular-Consejo Nacional de Investigaciones Científicas y Técnicas (IBIMOL-CONICET). Buenos Aires. Argentina.

[c] Universidad de Buenos Aires, Facultad de Medicina, Instituto de Fisiopatología Cardiovascular (INFICA), Departamento de Patología. Buenos Aires. Argentina.

[d]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Laboratorio de Lípidos y Aterosclerosis, Departamiento de Bioquímica Clínica, INFIBIOC. Buenos Aires. Argentina.

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[e]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Tecnología Farmacéutica, Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Buenos Aires. Argentina.

[f]Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Instituto de Fisiopatología y Bioquímica Clínica (INFIBIOC). Buenos Aires. Argentina.

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Correspondence and address:

Julieta Sofía Del Mauro,

Cátedra de Farmacología,

Facultad de Farmacia y Bioquímica,

Universidad de Buenos Aires,

Junín 956, (C1113AAD) Buenos Aires, ARGENTINA.

Phone: +(54-11)-4964-8265

e-mail: julietadelmauro@gmail.com

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Abstract

Objectives: To compare the effects of chronic oral treatment with carvedilol or amlodipine on blood pressure and blood pressure variability and target organ damage in N-nitro-l-arginine methyl ester (L-NAME) hypertensive rats.

Methods: Wistar rats were treated with L-NAME administered in the drinking water for 8 weeks together with oral administration of carvedilol 30 mg/kg (n=6), amlodipine 10 mg/kg (n=6) or vehicle (n=6). At the end of the treatment, echocardiographic evaluation, blood pressure and short-term variability measurements were performed. Left ventricular and thoracic aortas were removed to assess activity of metalloproteinase 2 and 9 and expression levels of transforming growth factor β, tumor necrosis factor α and interleukin-6. Histological samples were prepared from both tissues.

Results: Carvedilol and amlodipine induced a comparable reduction of systolic and mean arterial pressure and its short-term variability in L-NAME rats. The expression of transforming growth factor β, tumor necrosis factor α and interleukin-6 decreased in both organs after carvedilol or amlodipine treatment and the activity of metalloproteinase was reduced in aortic tissue. Treatment with carvedilol or amlodipine completely prevented left ventricular collagen deposition and morphometric alterations in aorta.

Conclusion: Oral chronic treatment with carvedilol or amlodipine significantly attenuates blood pressure variability and reduces target organ damage and biomarkers of tissue fibrosis and inflammation in L-NAME hypertensive rats.

Keywords:

β-blocker, blood pressure, calcium channel blocker, left ventricle, thoracic aorta.

Introduction

β-blockers have been the cornerstone in the treatment of arterial hypertension due to their ability to reduce cardiovascular-related mortality in clinical trials [1]. However, updated guidelines of the Eighth Joint National Committee do not further recommend β-blockers for the initial treatment of hypertension, considering the higher rate of the primary composite outcome of cardiovascular death, myocardial infarction, or stroke compared to the use of an angiotensin receptor blocker [2]. In addition, the National Institute for Health and Clinical Excellence (NICE) has recently downgraded the use of β-blockers from first-line agents for hypertension to fourth-line add-on therapy, based on the findings from meta-analyses that show a lack of benefit of β-blockers compared with placebo or other antihypertensive drugs [1].

The lack of clinical benefits of β-blockers in uncomplicated hypertension has been attributed to their lower ability to reduce central blood pressure (BP) and blood pressure variability (BPV) [3,4]. Findings from the Conduit Artery Function Evaluation (CAFE) study suggest that treatment with antihypertensive regimen containing atenolol is less effective than an amlodipine-based treatment on central aortic pressure reduction despite similar impact on brachial BP [3]. In addition, central pulse pressure was significantly associated with the degree of total cardiovascular events and the development of renal impairment, suggesting that the reduced ability of atenolol to reduce central BP might partially explain the lower protection from cardiovascular events [3].

Findings accumulated in the last decades have established that an increase in BPV contributes to the development of target organ damage (TOD) associated with hypertension [5]. It has been suggested that large BPV induces the activation of local

angiotensin II and mineralocorticoid receptor systems and chronic myocardial inflammation, resulting in cardiac hypertrophy and fibrosis [6]. Prospective clinical trials have revealed that antihypertensive agents may differ in their ability to control excessive BPV, suggesting that calcium channel blockers are more effective than other BP lowering drugs for the reduction of short-term, mid-term and long-term BPV [7]. A post-hoc analysis of the ASCOT-BPLA has demonstrated that amlodipine is significantly more effective than atenolol in the reduction of short-term and visit-to-visit BPV, and these effects explain the lower risk of stroke and coronary events in amlodipine treated patients with respect to subjects assigned to atenolol [4,8]. A recent review summarizing the effect of antihypertensive therapy on various types of BPV in hypertensive patients has concluded that calcium channel blockers are more effective than other antihypertensive agents for the attenuation of short-term and long-term BPV and may be considered a preferable treatment in reducing BPV measures in high-risk patients [9].

Although the lack of beneficial effects of β-blockers on central BP and BPV explains their lower ability to protect the hypertensive patient from cardiovascular events, it is important to point out that almost all the negative findings were obtained in clinical trials using atenolol, a second generation β-blocker with adverse effects on metabolic parameters and lack of effect on central BP [10]. Considering that β-blockers greatly differ in their pharmacokinetic and pharmacodynamic properties, the extrapolation of these results to third generation β-blockers, such as carvedilol and nebivolol, seems to be inappropriate. Recently, we have shown that acute administration of carvedilol or nebivolol induces a greater attenuation of short-term BPV than atenolol in sinoaortic denervated rats [11].

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Taking into account these previous findings, the aim of the present study was to compare the effects of chronic oral treatment with carvedilol or amlodipine on BP control, BPV and TOD in a N-nitro-l-arginine methyl ester (L-NAME) rat model of secondary hypertension.

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Materials and methods

Preparation of carvedilol and amlodipine formulation

Liquid formulations were prepared for oral administration of carvedilol and amlodipine. The formula of the carvedilol solution consisted of 0.5% (w/v) carvedilol (Droguerías Saporiti, Buenos Aires, Argentina), 10% (w/v) D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and 40% (v/v) propylene glycol. Amlodipine solution consisted of 0.5% (w/v) amlodipine (Droguerías Saporiti, Buenos Aires, Argentina) and 5% (w/v) TPGS. Vehicle solution was composed by 10% (w/v) TPGS and 40% (v/v) propylene glycol.

Animals and treatment

Animal experiments and animal care procedures were approved by the Animal Care Committee of the School of Pharmacy and Biochemistry, University of Buenos Aires (EXP-UBA N°0062949/2015) and were in line with the published Guide for the Care and Use of Laboratory Animals (NIH, 8º Ed., 2011). Animals were maintained on a 12-h light/dark cycle in a room at 22 ± 2 ºC with adequate air recycling. All animals were fed standard rodent diet (Asociación Cooperativas Argentinas, Buenos Aires, Argentina) with the following composition (w/w): 20% proteins, 3% fat, 2% fiber, 6% minerals and 69% starch and vitamin supplements, containing the same amount of calories. Male Wistar rats (220-250 g) were treated with L-NAME (Sigma Aldrich, St. Louis, MO, USA) administered in the drinking water during 8 weeks (30 mg/kg/day). The dose of L-NAME in the drinking water was selected taking into account that L-NAME reaches a maximal response at 10–15 mg kg/day inducing an increase in BP in the range of 30-40 mmHg [12]. Along with L-NAME intake, animals received 30

mg/kg carvedilol (n=6), 10 mg/kg amlodipine (n=6) or vehicle (n=6) by oral gavage once a day for 8 weeks. Male Wistar rats receiving tap water and administered with vehicle were used as the control normotensive group (n=6).

Determination of indirect BP and echocardiography

During the last two weeks of treatment, systolic arterial pressure (SAP) was measured by the indirect tail-cuff method in awake animals using a sphygmomanometer coupled to a Grass 7C polygraph (Grass Instrument Co., Quincy, MA, USA). The rats were trained to the procedure of SAP measurement at 1:00 PM 3 times a week for 2 weeks previous to the final measurement and under the same conditions. The final measurement was carried out 3 times a week for 2 weeks starting at 1:00 PM. Before SAP determination, rats were conditioned in a thermostatic (28 °C) and silent room for 60 min and then transferred to a standard setup with a heating pad (37 °C) acrylic restrainer, tail cuff and pulse sensor. Each day, SAP was calculated as the average of six separate measurements assessed during a period of 10 minutes in restrained animals. Intraday fluctuations of SAP related to short-term BPV, were calculated by assessing the standard deviation (SD) of consecutive BP measurements within the same day. Interday variation of SAP, linked to mid-term BPV, was assessed by the estimation of SD of mean SAP calculated for each day. All indirect measurements were performed by the same investigator, who was kept blind about the purpose of the study. Short-term BPV was assessed by the estimation of SD of six consecutive BP measurements, taking into consideration the recommendations for BP measurement in experimental animals from the Subcommittee of Professional and Public Education of the American Heart

Association Council on High Blood Pressure Research, which include the assessment of 3 up to 10 measurements in each recording session [13].

In the last week of treatment, rats were anesthetized with a mixture of ketamine (35 mg/kg) and xilazine (5 mg/kg), and an echocardiography was performed using ultrasonography (Acuson Sequoia C512) using a 14-MHz linear ultrasound transducer. The two-dimensional parasternal short-axis imaging plane was used to obtain M-mode tracings at the level of the papillary muscles. Left ventricular (LV) internal dimensions and LV wall thickness (LVWT) were determined at systole and diastole using leading-edge methods and guidelines of the American Society of Echocardiography [14]. Left ventricular end diastolic diameter (LVEDD) was measured at the time of the maximal LVEDD, while left ventricular end systolic diameter (LVESD) was assessed at the time of the most anterior systolic excursion of the posterior wall. In the same images, the diastolic posterior wall thickness (D. Post. WT) was measured. Ejection fraction (EF) and shortening fraction (SF) were also calculated and used as ejective indexes of systolic function. The E/A wave ratio (calculated from the ratio of the early (E) to late (A) ventricular filling velocities), and the duration of the isovolumic relaxation time (IVRT), were estimated by the Doppler-echo study.

Determination of direct hemodynamic parameters

At the end of the two-months treatment, animals were anesthetized with mixture of ketamine (35 mg/kg) and xilazine (5 mg/kg), and the left carotid artery was cannulated with a polyethylene cannula containing heparinized saline solution (25 U/ml). The cannula was tunnelled under the skin and externalized at the back of the neck. The measurements of direct pressure were performed in freely moving animals 24 h after the

cannula placement. The day of the measurement, the arterial cannula was connected to a Spectramed P23XL pressure transducer (Spectramed, Oxnard, CA, USA) coupled to a Grass 79D polygraph (Grass Instrument Co., Quincy, MA, USA). The polygraph was connected to a digital converter adaptor unit (Polyview, PVA 1, Grass-Astro Med, West Warwick, RI, USA), and 2-hour BP recordings were continuously assessed at a sampling rate of 500 Hz and stored for further analysis with a software program (Polyview 2.3 Astro-Med, West Warwick, RI). Mean arterial pressure (MAP), heart rate (HR), and short-term BPV were assessed. MAP was calculated as the sum of the diastolic pressure and one-third of the pulse pressure, HR was estimated tachographically by counting the pulsatile waves of arterial pressure recording and BPV was continuously estimated by determination of SD and spectral analysis of whole 3 min periods of BP recordings. As BPV largely depends from mean BP values, coefficient of variation (CV %) was estimated by relating average SD with the corresponding MAP [5]. In addition, beat-to-beat BPV was evaluated by the spectral analysis of the original data of whole 3-min MAP recordings using Fast Fourier Transform with a Hamming window as previously reported [15], whenever the quality of the MAP recording was visually considered to be satisfactory and free from artifacts and apparent cardiac arrhythmias. In this way, a complete 3-min segment of the original blood pressure recording was selected and used for power spectral analysis using Fast Fourier transform algorithm with a frequency resolution of 0.01 Hz. Spectral densities were calculated in the very low frequency (VLF) (0.1-0.2 Hz), low frequency (LF) (0.2-0.7 Hz) and high frequency (HF) (0.7-2.5 Hz) ranges. Although it is well-known that LF variability is subject to modulation of neural sympathetic vascular tone, the LF/HF ratio was used as an index of this activity, as the normalization procedure tends to reduce the effect of changes in the absolute values of BPV at the LF [16].

After the measurement of hemodynamic parameters, all animals were sacrificed by decapitation, and the thoracic aorta and left ventricle were removed to assess TOD. The left ventricular weight/body weight ratio was determined using a precision balance.

Western Blot assessment of aortic and ventricular expression levels of proinflammatory and profibrotic cytokines

Left ventricles and thoracic aorta tissue samples were homogenized in ice-cold homogenization buffer (150 mM NaCl, 50 mM Trizma-HCl, 1% (v/v) sodium deoxycholate, 1 mM EGTA, 1 mM NaF, 1 Mm phenylmethane sulfonyl fluoride and 1 mM sodium pervanadate (all reactives from Sigma Aldrich, St. Louis, USA) in the presence of 1X Halt Protease Inhibitor Cocktail (Thermo Scientific, Rockford, USA, pH 8.0). In order to obtain an adequate amount of sample, left ventricles and thoracic aorta were diluted in 3 vol (w/v) and 10 vol (w/v) of buffer, respectively. Then, each homogenate was centrifuged at 10,000 rpm for 10 min at 4 °C and the supernatants were used for the determinations. The protein content was measured by the Lowry method [17]. Samples were resuspended in a 6X solution of sample buffer (375 mM Tris-HCl buffer, pH 6.8 containing 12% (w/v) SDS, 50% (v/v) glycerol, 15% (v/v) β-mercaptoethanol and 0.06% (w/v) bromophenol blue) and heated at 95 ºC for 5 min. An equal amount of protein (50 μg) was loaded onto a 12% SDS-PAGE and transferred to PVDF membranes. After blocking for 1 h in 3% (w/v) nonfat milk in saline phosphate buffer (PBS), membranes were incubated overnight at 4°C with the corresponding primary antibodies (dilution 1:1000 in PBS): mouse anti-transforming growth factor β1 (TGFβ1), goat anti-tumor necrosis factor α (TNFα), goat anti-interleukin 6 (IL-6) and rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or rabbit anti-β-

tubulin. The blots were hybridized with a secondary antibody coupled to horseradish peroxidase (dilution 1:5000 in PBS). Complexes were visualized by chemiluminescence detection (Pierce ECL Western Blot Substrate). Densitometry analysis of the bands was performed using Image J (National Institute of Health, Bethesda, Maryland, USA). All antibodies were from Santa Cruz Biotechnology, Inc. Dallas, TX, USA.

Measurement of aortic and ventricular activity of matrix metalloproteinases MMP-2 and MMP-9

MMP-2 and MMP-9 activities were measured in ventricle and aorta by gelatinolytic zymography. Heart or aorta tissues were homogenized in 50 mM Tris buffer, pH 7.4, containing 5 mM $CaCl_2$, 1 µM $ZnCl_2$ and 1% (v/v) Triton X-100. 50 µg of protein were applied to a non-reduced sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis copolymerized with 0.1% (w/v) gelatin (G-8150, Sigma Aldrich, St. Louis, MO, USA), substrate for MMP-2 and MMP-9, and the zimography was carried out as previously described [18]. Gels were run in a Mini Protean-3 (Bio-Rad Laboratories, Hercules, CA, USA) and incubated for 18 h in 0.15 M NaCl, 10 mM $CaCl_2$, 50 Mm Tris HCl pH: 7.4 at 37°C. After staining with Coomassie blue R-250 (B-0149, Sigma Aldrich, St. Louis, MO, USA) and distained with acetic acid-methanol-water (1:3:6), enzyme activity was demonstrated by the absence of staining in areas where the gelatin had been degraded. Pro MMP-2 (72 kDa) and MMP-9 (84 kDa) were identified by molecular weight. Conditioned media from the promyelocyte U—937 cell lines was used as activity standard. The CV intra-assay was < 4.8%, and CV inter-assay < 8.6%. Because of the complexity of this assay, the CV is considered as quite satisfactory. Band intensities were quantified using Sion-Image J software (Scion

Corporation, National Institute of Standards and Technology Gaithersburg, MD, USA), and relative activity was expressed as a ratio to the internal standard.

Interstitial collagen determination in left ventricle and morphometry of aorta

Part of the left ventricle and thoracic aorta (from 5 to 10 mm above diaphragm) were sectioned and fixed in 10% (v/v) formaldehyde. Paraffin embedded sections measuring 5 μm were cut into slices. Aortas were stained with hematoxylin-eosin (HE), whereas left ventricles were stained with Picrosirius Red. Microscopic fields from each section were photographed at 400 X using a microscope (Olympus CX31microscope, Japan) and a digital camera (U-CMA D3 Olympus, Japan), and analyzed with a computerized image analyser software (Image Pro-Plus 3.0; Media Cybernetics, Silver Spring, MD, USA). The interstitial collagen fraction (ICF) was calculated as the ratio of the collagen area to the entire area of an individual section, which is the sum of the areas representing the myocyte and interstitial space [19]. For aorta sections, randomized microscopic fields under 50 X magnification were examined and aortic media wall thickness and lumen area were calculated using the Image Pro-Plus program according to Xiong X, *et al.* [20]. Sections from both tissues were evaluated under blind conditions.

Statistical Analysis

Regression analysis and statistical tests were performed using standard software (GraphPad Prism v. 6.01 for Windows; GraphPad Software, San Diego, CA, USA). Normal distribution of the data and variables were verified using the Kolmogorov–

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Smirnov test. Comparisons between groups were made with one way analysis of variance (ANOVA) followed by Tukey test. The correlation between the BP, BPV and parameters of myocardial damage were studied by means of Pearson's test. Results are expressed as means ± SEM. A probability value < 0.05 was considered statistically significant.

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Results

Effects of the treatment on indirect and direct BP

Table 1 shows the results of indirect and direct determination of BP in normotensive Wistar rats and L-NAME hypertensive rats chronically treated with carvedilol, amlodipine or vehicle. The analysis of tail-cuff BP measurements showed that both treatments reduced SAP compared to vehicle administration. Evaluation of direct hemodynamics parameters revealed that hypertension induced by L-NAME significantly increased HR and the treatment with carvedilol, but not amlodipine or vehicle were able to reduce HR. Central BP assessment at the carotid artery of cannulated animals showed that carvedilol and amlodipine induced a slight but significant reduction in MAP (**Table 1**).

Preliminary evaluation of the variability of SAP by a direct method revealed that chronic treatment with carvedilol or amlodipine reduced the intraday fluctuation of SAP when compared with vehicle, suggesting that both drugs are able to attenuate short-term BPV. Attenuation of short-term BPV induced by carvedilol or amlodipine was confirmed by direct BP measurement. In this context, both carvedilol and amlodipine significantly reduced SD and CV of MAP in comparison to vehicle treated L-NAME rats. On the other hand, whilst amlodipine significantly reduced interday fluctuations of SAP, the effects of carvedilol on mid-term BPV did not reach statistical significance (**Table 1**). LF/HF ratio was calculated from BP data points, considering the fact that LF/HF has been suggested as a marker of sympathetic vascular activity [16]. Spectral analysis of BP recordings established that chronic treatment with carvedilol or amlodipine, but not with vehicle, reduced LF/HF ratio in L-NAME hypertensive rats.

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Effects of the treatment on echocardiographic data of systolic and diastolic function

Table 2 shows the results of echocardiographic parameters of systolic and diastolic function. The analysis of the results revealed that hypertension induced by L-NAME increased D. Post. WT by 31%, and this effect was completely prevented by oral treatment with carvedilol and amlodipine, but not with vehicle (**Table 2**). Administration of carvedilol and amlodipine re-established the E/A wave ratio, which was reduced by 37% in the L-NAME hypertensive rats. No differences in the other echocardiographic parameters were observed between L-NAME hypertensive rats treated with carvedilol, amlodipine or vehicle.

Effects of the treatment on hypertrophy, heart fibrosis and morphometry of the aorta

Chronic administration of carvedilol and amlodipine resulted in a decrease in LV mass index expressed as the left ventricle weight/body weight ratio (LVW/BW). Treatment with vehicle did not modify the left ventricular mass index. Hypertension induced by chronic administration of L-NAME was associated with a significant increase of interstitial fibrosis in the left ventricle when compared to normotensive Wistar rats, evidenced by intensification in the ICF of analyzed fields of a 56%. Furthermore, carvedilol and amlodipine treatment reversed this alteration inducing a substantial decrease in the content of collagen in the left ventricle of L-NAME hypertensive rats (**Figure 1**). Morphological data obtained from sections of the aorta showed an increase in the media thickness of the aorta and in the media-to-lumen diameter ratio in L-NAME hypertensive rats treated with vehicle compared with Wistar rats ($p < 0.05$)

(**Figure 2**). Oral administration of carvedilol and amlodipine completely prevented these alterations in the aortic wall and significantly reduced media thickness and media-to-lumen diameter ratio compared to L-NAME rats ($p < 0.05$).

Effects of the treatment on biochemical markers of TOD

The expression and activity of molecular markers of TOD were assessed on left ventricle and thoracic aorta of normotensive Wistar rats and L-NAME hypertensive rats chronically treated with carvedilol, amlodipine or vehicle. We evaluated the expression of TGFβ as a marker of ventricular and aortic fibrosis, and the expressions of IL-6 and TNFα to determine the effect of these drugs on inflammatory processes. The results showed a double of TGFβ expression in hypertensive rats compared to normotensive animals. This increase in TGFβ expression was completely attenuated by oral treatment with carvedilol and amlodipine, re-establishing baseline levels of TGFβ in both left ventricle and aorta. L-NAME hypertensive rats chronically treated with vehicle showed higher levels of the inflammatory cytokines TNFα and IL-6 in both tissues (84% and 48% in left ventricle, respectively and 44% and 59% in thoracic aorta, respectively; $p < 0.05$ vs Wistar rats). Oral treatment with carvedilol and amlodipine reduced overexpression of TNFα in both tissues ($p < 0.05$ vs. L-NAME rats). Similar results were obtained in ventricular expression of IL-6 in rats treated with the third generation β-blocker or the calcium channel blocker. Amlodipine, but not carvedilol, completely attenuated the increase of IL-6 levels in aorta induced by L-NAME (**Figure 3**).

In accordance with the Western Blot analysis of pro-fibrotic and pro-inflammatory cytokines, L-NAME induced hypertension increased the activities of MMP-2 and MMP-9 in thoracic aorta with regards to normotensive Wistar rats. Carvedilol and amlodipine attenuated the increase in 72 kDa pro MMP-2 and 84 kDa MMP-9 activities

in L-NAME hypertensive rats. Conversely, we found no significant differences in the gelatinolytic activity of MMP-9 on the left ventricle of normotensive Wistar rats and L-NAME hypertensive rats chronically treated with carvedilol, amlodipine or vehicle (**Figure 4** and **Figure 5**). The activity of pro MMP-2 could not be determined in the left ventricle because of the sensibility of the method.

Relationships between BP, BPV, myocardial and aortic damage in L-NAME hypertensive rats

Relationships between BP, BPV, myocardial and aortic damage are shown in **Table 3**. A positive and significant correlation was found between LVW/BW and intraday SD of SAP, SD of MAP, CV% of MAP and MAP, but not with SAP or interday SD of SAP. A negative correlation between E/A ratio and intraday SD of SAP, SD of MAP, CV% of MAP was also found. Interstitial LV fibrosis was positively related to SAP, intraday SD of SAP, SD of MAP and CV% of MAP. Finally, both media thickness and media-to-lumen diameter ratio were positively correlated with BP and BPV values with the exception of CV% of MAP.

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Discussion

Chronic intake of L-NAME has been established as an experimental model of hypertension characterized by an increase of both BP and BPV [12, 21-24] and associated to TOD. Previous studies have shown that chronic blockade of nitric oxide synthase by oral intake of L-NAME increases wall thickness of the thoracic aorta, proinflammatory cytokines levels, TGFβ expression in coronary arteries and relative wall thickness of the left ventricle [21, 25-29]. Chronic oral treatment with carvedilol and amlodipine induced a slight reduction of peripheral indirect and carotid direct BP in L-NAME rats, although both treatments were not able to normalize BP when compared with normotensive control animals. Conversely, both carvedilol and amlodipine attenuated short-term BPV in L-NAME hypertensive rats, allowing the shift of intraday SD of indirect SAP and SD of direct carotid MAP to values detected in Wistar animals, reducing thereby CV of MAP in L-NAME hypertensive rats. These results suggest that carvedilol and amlodipine are more effective in reducing short-term BPV than in the control of mean BP levels. Moreover, the effect of chronic treatment with carvedilol on BP and its short-term variability was comparable to amlodipine, suggesting that vasodilating β-blockers provide similar hemodynamic benefits when compared with calcium channel blockers. Nowadays, the effect of carvedilol on BPV has been scarcely reported in animal models of hypertension. In previous studies, we showed that acute intravenous treatment with carvedilol induced an attenuation of short-term BPV in normotensive animals, spontaneously hypertensive rats and fructose-fed rats [30,31]. At the clinical setting, treatment with 25 mg carvedilol bid during 3 months has resulted in an attenuation of the coefficient of BP in mild-to-moderate essential hypertensive patients [32].

The analysis of mid-term BPV, estimated as interday SD of SAP from indirect BP measurement, revealed that amlodipine, but not carvedilol, is able to reduce the day-to-day BPV in L-NAME hypertensive rats. Day-to-day BPV is related to factors influencing the degree of BP control, including adequate dosing and titration of the antihypertensive treatment and the half-life of the elimination of antihypertensive agents [33,34]. Therefore, the greater ability of amlodipine to control the day-to-day BPV in L-NAME rats can be explained by its sustained antihypertensive effect. Clinical findings have shown a greater trough-to-peak ratio for SAP after administration of amlodipine once-daily in hypertensive patients compared with carvedilol [35,36].

In the present study, beat-to-beat BPV by spectral analysis of continuous direct BP was used to assess the effects of carvedilol and amlodipine on vascular sympathetic activity in L-NAME hypertensive rats. Identification of frequency components of BPV by power spectral analysis can potentially provide information about the mechanisms involved in BP regulation [37]. Whilst LF of beat-to-beat BPV variability is modulated by sympathetic vasomotor tone, HF variability is influenced by cardiac output [16]. Hypertensive state induced by chronic intake of L-NAME has been associated with an increase in LF/HF ratio when compared with control normotensive animals, indicating the contribution of the vascular sympathetic system in BP regulation in this experimental model. Similar findings were found in a previous study in two weeks L-NAME hypertensive rats, suggesting the maintenance of early hemodynamic alterations during the chronic stage of hypertension induced by nitric oxide synthase inhibition [11]. Chronic treatment with amlodipine and carvedilol induced the shift of LF/HF values to levels reported in Wistar normotensive rats, suggesting that both carvedilol and amlodipine are able to attenuate vascular sympathetic overactivity after 8 weeks. Although the effects of chronic administration of carvedilol on beat-to-beat BPV have

not been previously reported, a reduction in BPV at the LF has been demonstrated after acute intravenous administration of carvedilol in L-NAME hypertensive rats [38]. On the other hand, the decrease in LF induced by dihydropiridine calcium channel blockers has been attributed to the blockade of α_1-adrenoceptor-mediated vasoconstriction via blockade of store-operated Ca^{2+} channels [37]. In addition, Nobre *et al.* [39] found that oral treatment with amlodipine is able to normalize the elevation of LF BPV in in two-kidney, one-clip (2K1C) hypertensive rats.

A second objective of the present work was to compare the effects of chronic treatment with carvedilol or amlodipine on TOD at left ventricle and thoracic aorta of L-NAME hypertensive rats by means of morphological, echocardiographic, histological and biochemical investigations. It is a well-known fact that inflammatory cytokines, such as TNFα and interleukin 1β, contribute to TOD associated to hypertension by the modulation of matrix metalloproteinases expression [40,41]. In our study, both carvedilol and amlodipine were able to prevent the inflammatory state at left ventricle and aorta of L-NAME hypertensive rats, evidenced by a reduction in the expression of TNFα and IL-6. Previous studies have shown that carvedilol exerts anti-inflammatory actions in rats with myocardial infarction by the blockade of α_1- and β-adrenoceptors in cardiomyocytes [25]. In this context, it was found that β-adrenoceptor activation increases the IL-6 gene family expression in cardiac fibroblasts and enhances lipopolysaccharide induced TNFα expression in cardiomyocytes [42,43]. On the other hand, the blockade of calcium channel blockers with amlodipine is also able to reduce the proinflammatory state associated to hypertension. Navarro-Gonzalez *et al.* found that the treatment of hypertensive diabetic patients with amlodipine partially reduced the elevation of serum levels of cytokines, such as TNFα and IL-6 [44].

Chronic treatment with carvedilol or amlodipine was also able to prevent the overactivity of both MMP-9 and pro MMP-2 in thoracic aorta, which contributes to vascular remodeling associated to hypertension [25,45,46]. Our results are in line with the fact that carvedilol has been shown to reduce the activities of different metalloproteinases by its ability to modulate redox-related pathways [47]. In a previous study from other authors, treatment with amlodipine was also able to attenuate the increase MMP-2 activity in aorta of two-kidney, one-clip (2K1C) hypertensive rats [48]. In accordance with the actions of carvedilol and amlodipine on proinflammatory cytokines and MMP activity, both antihypertensive treatments exert protective effects on vascular and ventricular remodeling in L-NAME hypertensive rats evidenced by morphological, histological and echocardiographic findings. Chronic treatment with carvedilol or amlodipine of L-NAME rats induced a similar preventive effect of myocardial TOD, taking into account their ability to prevent the increase in the LV hypertrophy index, the enhancement of interstitial collagen deposition and the overexpression of the profibrotic biomarker TGFβ induced by L-NAME intake. Although the effects of carvedilol on cardiac hypertrophy have not been previously studied in this experimental model of hypertension, carvedilol was able to reduce myocardial hypertrophy and ventricular fibrosis in rats with acute myocardial infarction. Moreover, the amelioration of structural heart alterations promoted by carvedilol was accompanied by a reduction on the expression of the fibrogenic cytokine TGFβ [25]. More recently, Chen *et al*. [49] found that the early administration of carvedilol protected against doxorubicin-induced cardiomyopathy by reducing both myocardial expression of TGFβ and collagen deposition. Beneficial effects of amlodipine on ventricular alterations associated to hypertension were also described in previous

studies. In this way, chronic oral treatment with amlodipine 8 or 20 mg/kg/day prevented cardiac hypertrophy and fibrosis in spontaneously hypertensive rats [50].

In our study, the analysis of echocardiograms also indicated that hypertensive animals have ventricular hypertrophy evidenced by an increase in diastolic posterior wall thickness and diastolic dysfunction, demonstrated by an inversion of E/A ratio, which was lower than 1. This alteration in the E/A ratio may be caused by the increase in collagen deposition in the left ventricle that leads to an increased myocardial stiffness, which can lead to diastolic heart failure. Treatment with carvedilol or amlodipine was also able to prevent the echocardiographic alterations induced by L-NAME, demonstrating their ability to reduce left ventricular hypertrophy and improve diastolic dysfunction.

At the vascular level, chronic oral administration of carvedilol or amlodipine attenuated in a similar manner aortic thickening and overexpression of TGFβ induced by L-NAME hypertension. Previous studies in spontaneously hypertensive rats evidenced the ability of carvedilol and amlodipine to reduce vascular hypertrophy of mesenteric arteries [51,52]. In addition, amlodipine has demonstrated to reduce TGFβ expression in coronary arteries of chronic L-NAME hypertensive rats [29].

In order to establish the relative contribution of BP and BPV on the vascular and cardiac protective effects induced by carvedilol or amlodipine in L-NAME hypertensive rats, we correlated aortic and ventricular damage with different hemodynamic parameters using pooled data from the different therapeutic groups. Regression analysis of morphological parameters of abdominal aorta and hemodynamic variables of the pooled data revealed a statistical positive relationship between media wall thickness and media thickness/lumen diameter, with both BP and short-term BPV. The analysis showed that the LV hypertrophy index, E/A ratio and interstitial LV fibrosis showed a significant

correlation with the degree of short-term BPV. Conversely, in most comparisons, SAP and MAP did not show any correlation with the different markers of ventricular damage. These results suggest that both BP reduction and short-term BPV attenuation induced by carvedilol and amlodipine contribute to the prevention of TOD at abdominal aorta of chronic L-NAME hypertensive rats. However, attenuation of BPV, rather than reduction of BP, contributes to the TOD protection at the left ventricle induced by the chronic oral treatment with carvedilol or amlodipine. Some previous studies are in line with our results. In this context, Xie *et al.* [50] have found that long-term treatment with atenolol, nifedipine, irbesartan, or hydrochlorothiazide markedly reduced BPV, enhanced baroreflex sensitivity, and produced significant organ protection in spontaneously hypertensive rats. Compared with the BP level, the degree of BPV and the baroreflex sensitivity values showed a much closer relationship with TOD in treated hypertensive rats [50]. Moreover, by comparing TOD in sham-operated and sinoaortic-denervated Wistar Kyoto rats and spontaneously hypertensive rats, Miao *et al*. established a greater contribution of BPV than BP in LV hypertrophy, glomerular damage, and aortic hypertrophy [51]. At the clinical setting, a weak positive correlation was recently found between short-term BPV and LV mass index in a meta-analysis of cohort, cross-sectional or case–control studies [52].

It is important to recognize some limitations of our study. In first place, both the direct and indirect BP assessment methods used in this work are complicated by stress. Moreover, the indirect SAP measurement by tail-cuff is not a good methodology for the quantification of short-term BPV [13]. Nevertheless, the effects of carvedilol or amlodipine on SD of SAP assessed by sparse indirect BP measurement were in line with those obtained from the analysis of continuous MAP recording in cannulated animals. In second place, we do not assess the degree of nitric oxide synthase inhibition

in the different experimental groups. Previous studies have shown that carvedilol stimulates nitric oxide production via its "biased agonism" activity, and the increased levels of nitric oxide contribute in the antihypertensive response of this third generation β-blocker [53,54]. A remarkable point is the fact that carvedilol exerts similar hemodynamic and cardioprotective effects than amlodipine in an unfavorable animal model of hypertension, reinforcing the beneficial effects of third generation β-blockers. Further comparative studies in other models of hypertension will contribute to clarify the relative efficacy of carvedilol and amlodipine on the control of BPV and the prevention of TOD.

In conclusion, the results of the present study reveal that chronic oral treatment with carvedilol shows a comparable ability to reduce short-term BPV than amlodipine, providing similar TOD protection at the left ventricle and abdominal aorta. Regression analysis of the data suggests that attenuation of short-term BPV induced by carvedilol and amlodipine contributes in a greater manner than the reduction of BP in the prevention of myocardial damage in L-NAME hypertensive rats. Although further studies are needed, including a group of animals treated with atenolol, our results suggest that third generation β-blockers with pleiotropic actions, such as carvedilol, are superior to non-vasodilating β-blockers, providing similar cardioprotective benefits than calcium channel blockers, in part due to their ability to attenuate short-term BPV.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ACCEPTED MANUSCRIPT

References

1. Leonetti G, Egan CG. Use of carvedilol in hypertension: an update. *Vasc Health Risk Manag* 2012; 8:307-322.

2. James PA, Oparil S, Carter BL, Cushman WC, Dennison-Himmelfarb C, Handler J, et al. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). *JAMA* 2014; 311:507-20.

3. Williams B, Lacy PS, Thom SM, Cruickshank K, Stanton A, Collier D, et al. Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation* 2006; 113:1213-25.

4. Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, et al. Effects of beta blockers and calcium-channel blockers on within-individual variability in blood pressure and risk of stroke. *Lancet Neurol* 2010; 9:469-480.

5. Parati G, Ochoa JE, Lombardi C, Bilo G. Blood pressure variability: assessment, predictive value, and potential as a therapeutic target. *Curr Hypertens Rep* 2015; 17:537.

6. Kai H, Kudo H, Takayama N, Yasuoka S, Aoki Y, Imaizumi T. Molecular mechanism of aggravation of hypertensive organ damages by short-term blood pressure variability. *Curr Hypertens Rev* 2014; 10:125-133.

7. Hocht C, Del Mauro JS, Bertera FM, Taira CA. Drugs affecting blood pressure variability: an update. *Curr Pharm Des* 2015; 21:744-755.

ACCEPTED MANUSCRIPT

8. Höcht C, Bertera FM, Taira CA. Importance of blood pressure variability in the assessment of cardiovascular risk and benefits of antihypertensive therapy. *Expert Rev Clin Pharmacol* 2010; 3:617-621.

9. Eguchi K. Effects of Antihypertensive Therapy on Blood Pressure Variability. *Curr Hypertens Rep* 2016;18:75

10. Ripley TL, Saseen JJ. β-blockers: a review of their pharmacological and physiological diversity in hypertension. *Ann Pharmacother* 2014; 48:723-733.

11. Bertera FM, Del Mauro JS, Lovera V, Chiappetta D, Polizio AH, Taira CA, et al. Acute effects of third generation β-blockers on short-term and beat-to-beat blood pressure variability in sinoaortic-denervated rats. *Hypertens Res* 2013; 36:349-355.

12. Bertera FM, Del Mauro JS, Lovera V, Chiappetta D, Polizio AH, Taira CA, et al. Enantioselective pharmacokinetics and cardiovascular effects of nebivolol in L-NAME hypertensive rats. *Hypertens Res* 2014; 37:194-201.

13. Kurtz TW, Griffin KA, Bidani AK, Davisson RL, Hall JE; Subcommittee of Professional and Public Education of the American Heart Association. Recommendations for blood pressure measurement in humans and experimental animals. Part 2: Blood pressure measurement in experimental animals: a statement for professionals from the subcommittee of professional and public education of the American Heart Association council on high blood pressure research. *Hypertension* 2005;45:299-310.

14. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards,

Subcommittee on Quantitation of Two-Dimensional Echocardiograms. *J Am Soc Echocardiogr* 1989;2:358–67

15. Pladys P, Lahaie I, Cambonie G, Thibault G, Lê NL, Abran D, Nuyt AM. Role of brain and peripheral angiotensin II in hypertension and altered arterial baroreflex programmed during fetal life in rat. Pediatr Res 2004;55:1042-9.

16. Salman IM. Current Approaches to Quantifying Tonic and Reflex Autonomic Outflows Controlling Cardiovascular Function in Humans and Experimental Animals. *Curr Hypertens Rep* 2015;17:84.

17. Lowry Oh, Rosebrough Nj, Farr Al, Randall Rj. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193:265-275.

18. Muzzio ML, Miksztowicz V, Brites F, Aguilar D, Repetto EM, Wikinski R, et al. Metalloproteases 2 and 9, Lp-PLA(2) and lipoprotein profile in coronary patients. *ArchMed Res* 2009; 40:48–53.

19. González GE, Cassaglia P, Noli Truant S, Fernández MM, Wilensky L, Volberg V, et al. Galectin-3 is essential for early wound healing and ventricular remodeling after myocardial infarction in mice. *Int J Cardiol* 2014; 176:1423-5.

20. Xiong X, You C, Feng QC, Yin T, Chen ZB, Ball P, et al. Pulse width modulation electro-acupuncture on cardiovascular remodelling and plasma nitric oxide in spontaneously hypertensive rats. *Evid Based Complement Alternat Med* 2011; 2011:812160.

21. Sadek SA, Rashed LA, Bassam AM, Said ES. Effect of aliskiren, telmisartan and torsemide on cardiac dysfunction in l-nitro arginine methyl ester (l-NAME) induced hypertension in rats. *J Adv Res* 2015; 6:967-974.

ACCEPTED MANUSCRIPT

22. Zambrano S, Blanca AJ, Ruiz-Armenta MV, Miguel-Carrasco JL, Revilla E, Santa-María C, et al. The renoprotective effect of L-carnitine in hypertensive rats is mediated by modulation of oxidative stress-related gene expression. *Eur J Nutr* 2013; 52:1649-1659.

23. Tsuchiya K, Tomita S, Ishizawa K, Abe S, Ikeda Y, Kihira Y, et al. Dietary nitrite ameliorates renal injury in L-NAME-induced hypertensive rats. *Nitric Oxide* 2010; 22:98-103.

24. Wang DS, Xie HH, Shen FM, Cai GJ, Su DF. Blood pressure variability, cardiac baroreflex sensitivity and organ damage in experimentally hypertensive rats. *Clin Exp Pharmacol Physiol* 2005; 32:545-552.

25. Li B, Liao YH, Cheng X, Ge H, Guo H, Wang M. Effects of carvedilol on cardiac cytokines expression and remodeling in rat with acute myocardial infarction. *Int J Cardiol* 2006; 111:247-255.

26. Paulis L, Matuskova J, Adamcova M, Pelouch V, Simko J, Krajcirovicova K, et al. Regression of left ventricular hypertrophy and aortic remodelling in NO-deficient hypertensive rats: effect of L-arginine and spironolactone. *Acta Physiol* 2008; 194:45-55.

27. Bartunek J, Weinberg EO, Tajima M, Rohrbach S, Katz SE, Douglas PS, et al. Chronic N(G)-nitro-L-arginine methyl ester-induced hypertension: novel molecular adaptation to systolic load in absence of hypertrophy. *Circulation* 2000; 101:423-429.

28. Li L, Shigematsu Y, Hamada M, Hiwada K. Relative wall thickness is an independent predictor of left ventricular systolic and diastolic dysfunctions in essential hypertension. *Hypertens Res* 2001; 24:493-499.

ACCEPTED MANUSCRIPT

29. Kataoka C, Egashira K, Ishibashi M, Inoue S, Ni W, Hiasa K, et al. Novel anti-inflammatory actions of amlodipine in a rat model of arteriosclerosis induced by long-term inhibition of nitric oxide synthesis. *Am J Physiol Heart Circ Physiol* 2004; 286:H768-774.

30. Bertera FM, Del Mauro JS, Chiappetta D, Polizio AH, Buontempo F, Taira CA, et al. Enantioselective pharmacokinetic and pharmacodynamic properties of carvedilol in spontaneously hypertensive rats: focus on blood pressure variability. *Naunyn Schmiedebergs Arch Pharmacol* 2012; 385:325-335.

31. Bertera F, Di Verniero CA, Mayer MA, Chiappetta D, Buontempo F, Polizio AH, et al. Pharmacokinetic and pharmacodynamic properties of carvedilol in fructose hypertensive rats. *Xenobiotica* 2012; 42:206-219.

32. Crippa G, Venturi C, Carrara G. Effect of antihypertensive treatment with carvedilol or lercanidipine on ambulatory blood pressure variability. *Am J Hypertens* 2002; 15: 50A.

33. Parati G, Ochoa JE, Lombardi C, Bilo G. Assessment and management of blood-pressure variability. *Nat Rev Cardiol* 2013; 10:143-155.

34. Bouissou-Schurtz C, Lindesay G, Regnault V, Renet S, Safar ME, Molinie V, et al. Development of an Experimental Model to Study the Relationship Between Day-to-Day Variability in Blood Pressure and Aortic Stiffness. *Front Physiol* 2015; 6:368.

35. Hernández RH, Armas-Hernández MJ, Chourio JA, Armas-Padilla MC, López L, Alvarez M, et al. Comparative effects of amlodipine and nifedipine GITS during treatment and after missing two doses. *Blood Press Monit* 2001; 6:47-57.

36. Moser M, Frishman W. Results of therapy with carvedilol, a beta-blocker vasodilator with antioxidant properties, in hypertensive patients. *Am J Hypertens* 1998; 11:15S-22S.

37. Stauss HM. Identification of blood pressure control mechanisms by power spectral analysis. *Clin Exp Pharmacol Physiol* 2007; 34:362–388.

38. Di Verniero CA, Bertera F, Buontempo F, Bernabeu E, Chiappetta D, Mayer MA, et al. Enantioselective pharmacokinetic-pharmacodynamic modelling of carvedilol in a N-nitro-l-arginine methyl ester rat model of secondary hypertension. *J Pharm Pharmacol* 2010; 62:890-900.

39. Nobre F, da Silva CA, Coelho EB, Salgado HC, Fazan R. Antihypertensive agents have different ability to modulate arterial pressure and heart rate variability in 2K1C rats. *Am J Hypertens* 2006; 19:1079-1083.

40. Morillas P, de Andrade H, Castillo J, Quiles J, Bertomeu-González V, Cordero A, et al. Inflammation and apoptosis in hypertension. Relevance of the extent of target organ damage. *Rev Esp Cardiol* 2012; 65:819-825.

41. Siwik DA, Chang DL, Colucci WS. Interleukin-1beta and tumor necrosis factor-alpha decrease collagen synthesis and increase matrix metalloproteinase activity in cardiac fibroblasts in vitro. *Circ Res* 2000; 86:1259-1265.

42. Yin F, Li P, Zheng M, Chen L, Xu Q, Chen K, et al. Interleukin-6 family of cytokines mediates isoproterenol-induced delayed STAT3 activation in mouse heart. *J Biol Chem* 2003; 278:21070-5.

ACCEPTED MANUSCRIPT

43. Wang Y, Wang Y, Yang D, Yu X, Li H, Lv X, et al. β_1-adrenoceptor stimulation promotes LPS-induced cardiomyocyte apoptosis through activating PKA and enhancing CaMKII and IκBα phosphorylation. *Crit Care* 2015; 19:76.

44. Navarro-Gonzalez J, Mora-Fernandez C, Gomez-Chinchon M, Muros M, Herrera H, Garcia J. Serum and gene expression profile of tumor necrosis factor-alpha and interleukin-6 in hypertensive diabetic patients: effect of amlodipine administration. *Int J Immunopathol Pharmacol* 2010; 23:51-59.

45. Berg G, Miksztowicz V, Schreier L. Metalloproteinases in metabolic syndrome. *Clin Chim Acta* 2011; 412:1731-1739.

46. Martinez-Lemus LA, Galiñanes EL. Matrix metalloproteinases and small artery remodeling. *Drug Discov Today Dis Models* 2011; 8:21-28.

47. Wu TC, Chen YH, Leu HB, Chen YL, Lin FY, Lin SJ, et al. Carvedilol, a pharmacological antioxidant, inhibits neointimal matrix metalloproteinase-2 and -9 in experimental atherosclerosis. *Free Radic Biol Med* 2007; 43:1508-1522.

48. Marçal DM, Rizzi E, Martins-Oliveira A, Ceron CS, Guimaraes DA, Gerlach RF, et al. Comparative study on antioxidant effects and vascular matrix metalloproteinase-2 downregulation by dihydropyridines in renovascular hypertension. *Naunyn Schmiedebergs Arch Pharmacol* 2011; 383:35-44.

49. Chen YL, Chung SY, Chai HT, Chen CH, Liu CF, Chen YL, et al. Early administration of carvedilol protected against doxorubicin-induced cardiomyopathy. *J Pharmacol Exp Ther* 2015; 355:516-27.

ACCEPTED MANUSCRIPT

50. Sevilla MA, Voces F, Carrón R, Guerrero EI, Ardanaz N, San Román L, et al. Amlodipine decreases fibrosis and cardiac hypertrophy in spontaneously hypertensive rats: persistent effects after withdrawal. *Life Sci* 2004; 75:881-891.

50. Xie HH, Shen FM, Zhang XF, Jiang YY, Su DF. Blood pressure variability, baroreflex sensitivity and organ damage in spontaneously hypertensive rats treated with various antihypertensive drugs. *Eur J Pharmacol* 2006; 543:77-82.

51. Miao CY, Xie HH, Zhan LS, Su DF. Blood pressure variability is more important than blood pressure level in determination of end-organ damage in rats. *J Hypertens* 2006; 24:1125-1135.

52. Madden JM, O'Flynn AM, Fitzgerald AP, Kearney PM. Correlation between short-term blood pressure variability and left-ventricular mass index: a meta-analysis. *Hypertens Res* 2016; 39:171-177.

53. Andresen BT. A pharmacological primer of biased agonism. *Endocr Metab Immune Disord Drug Targets* 2011;11:92-8.

54. Afonso RA, Patarrao RS, Macedo MP, Carmo MM. Carvedilol action is dependent on endogenous production of nitric oxide. *Am J Hypertens* 2006;19:419-25.

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FIGURE LEGENDS:

FIGURE 1. Effects of the treatments on interstitial fibrosis. Panel A shows the value of collagen surface area (in percentage) in the left ventricle of Wistar rats (white bars) and L-NAME hypertensive rats after 8 weeks of treatment with vehicle (black bars), 30 mg/kg carvedilol (light gray bars) or 10 mg/kg amlodipine (gray bars). $^{a}p < 0.05$ versus Wistar rats; $^{b}p < 0.05$ vs L-NAME rats. Panel B shows representative images of left ventricular Picro-Sirius Red staining, showing interstitial fibrosis (Original magnification 400 X).

FIGURE 2. Effects of the treatments on aortic morphometry. Panel A shows media wall thickness (μm) and Panel B shows the media wall thickness/lumen diameter in Wistar rats (white bars) and L-NAME hypertensive rats after 8 weeks of treatment with vehicle (black bars), 30 mg/kg carvedilol (light gray bars) or 10 mg/kg amlodipine (gray bars). $^{a}p < 0.05$ versus Wistar rats; $^{b}p < 0.05$ vs L-NAME rats. Panel C shows representative images of thoracic aorta stained with hematoxylin and eosin (Original magnification 50 X).

FIGURE 3. Expression of TGFβ (panel A), TNF-α (panel B) and IL- 6 (panel C) connoted in percent respect to normotensive Wistar rats and their representative photographs of Western blot analysis of left ventricular and aorta homogenates of Wistar rats (white bars) and L-NAME hypertensive rats after 8 weeks of treatment with vehicle (black bars), 30 mg/kg carvedilol (light gray bars) or 10 mg/kg amlodipine (gray bars). $^{a}p < 0.05$ versus Wistar rats; $^{b}p < 0.05$ vs L-NAME rats. TGFβ: transforming growth factor β; TNFα: tumor necrosis factor α; IL-6: interleukin 6; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

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FIGURE 4. Activity of matrix metalloprotease MMP-9 in left ventricle (panel A) and aorta (panel B) homogenates of Wistar rats (white bars) and L-NAME hypertensive rats after 8weeks of treatment with vehicle (black bars), 30 mg/kg carvedilol (light gray bars) or 10 mg/kg amlodipine (gray bars). $^{a}p < 0.05$ versus Wistar rats; $^{b}p < 0.05$ vs L-NAME rats.

FIGURE 5. Activity of matrix metalloprotease pro MMP-2 in aorta homogenates of Wistar rats (white bars) and L-NAME hypertensive rats after 8 weeks of treatment with vehicle (black bars), 30 mg/kg carvedilol (light gray bars) or 10 mg/kg amlodipine (gray bars). $^{a}p < 0.05$ versus Wistar rats; $^{b}p < 0.05$ vs L-NAME rats.

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TABLE 1. Cardiovascular and morphometric parameters of normotensive Wistar rats and L-NAME hypertensive rats chronically treated with carvedilol, amlodipine or vehicle. Results are expressed as means ± SEM. Abbreviations: SAP: systolic arterial pressure; SD: standard deviation; MAP: mean arterial; CV: coefficient of variation; HR: heart rate; LF: low frequency; HF: high frecuency.

	Wistar (n=6)	L-NAME (n=6)	L-NAME Carvedilc (n=6)	L-NAME Amlodipine (n=6)
Indirect blood pressure (tail cuff)				
SAP (mmHg)	134±2	170±4[a]	156±2[b]	158±4[a]
Intraday SD of SAP (mml	3.38±0.52	7.36±0.67[a]	3.60±0.32[b]	3.80±0.28[b]
Interday SD of SBP (mml	3.04±0.75	14.25±1.95[a]	10.92±0.72[a]	5.96±1.05[b,c]
Direct Blood pressure (carotid artery)				
MAP (mmHg)	119±8	179±7[a]	150±5[b]	150±5[b]
SD of MAP (mmHg)	3.84±0.15	6.24±0.50[a]	3.67±0.41[b]	3.02±0.42[b]
CV of MAP (%)	2.78±0.20	3.92±0.28[a]	2.49±0.16	2.06±0.25
HR	324±7	400±13[a]	304±11[b,d]	376±30
LF/HF Ratio	4.96±0.43	8.00±0.48[a]	4.41±0,42[b]	4.08±0.28[b]
Left ventricle weight (mg	884.8±12.02	1020.0±8.3[a]	875.3±19.7[b]	874.8±16.2[b]
Body weight (g)	411±24	411±14	402±10	411±17
Left Ventric Hypertrophy Index (mg/g	2.17±0.10	2.44±0.05[a]	2.18±0.07[b]	2.15±0.10[b]

[a]p <0.05 vs. Wistar Rats

[b]p <0.05 vs. L-NAME Rats

[c]p <0.05 vs. L-NAME Carvedilol Rats

[d]p <0.05 vs. L-NAME Amlodipine Rats

TABLE 2. Echocardiographic data of systolic and diastolic function in normotensive Wistar rats and L-NAME hypertensive rats chronically treated with carvedilol, amlodipine or vehicle. Results are expressed as means ± SEM. Abbreviations: LVEDD: left ventricular end diastolic diameter; D.POST.WT: diastolic posterior wall thickness; LVESD: left ventricular end systolic diameter; EF: ejection fraction; SF: shortening fraction; E/A: ratio of the early (E) to late (A) ventricular filling velocities; IVRT: isovolumic relaxation time.

	Wistar (n=6)	L-NAME (n=6)	L-NAME Carvedilol (n=6)	L-NAME Amlodipine (n=6)
LVEDD	7.64±0.22	7.46±0.23	7.53±0.13	7.90±0.31
D.POST.WT	1.60±0.04	2.10±0.10[a]	1.70±0.04[b]	1.68±0.13[b]
LVESD	4.88±0.18	5.04±0.36	5.15±0.17	5.30±0.32
EF	71.48±1.56	66.60±4.39	64.98±2.21	66.80±3.69
SF	36.29±1.20	32.83±3.03	31.57±1.80	32.94±2.87
E/A	1.41±0.10	0.89±0.13[a]	1.48±0.04[b]	1.57±0.10[b]
IVRT	31.60±2.20	36.80±1.90	35.25±1.03	32.00±1.73

[a] $p < 0.05$ vs. Wistar Rats

[b] $p < 0.05$ vs. L-NAME Rats

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TABLE 3. Linear regression coefficient (Pearson r) between values of blood pressure, blood pressure variability and ventricular or aortic damage. Abbreviations: SAP: systolic arterial pressure; SD: standard deviation; MAP: mean arterial pressure; CV: coefficient of variation; E/A ratio: ratio of the early (E) to late (A) ventricular filling velocities

	Left Ventricle Hypertrophy Index (mg/g)	E/A ratio	Interstitial left ventricular fibrosis (%)	Media wall thickness (μm)	Media thickness/lumen diameter (μm/mm)
SAP (mmHg)	0.2120	0.04595	0.5414*	0.8052*	0.7431*
Intraday SD (mmHg)	0.5964*	-0.7171*	0.5588*	0.6865*	0.9090*
Interday SD (mmHg)	-0.05173	-0.2120	0.3528	0.5519*	0.6210*
MAP (mmHg)	0.6587*	-0.0666	0.1774	0.7239*	0.8767*
SD of MAP (mmHg)	0.5741*	-0.8893*	0.5403*	0.5792*	0.6054*
CV of MAP (%)	0.5216*	-0.8298*	0.5490*	0.3105	0.1732

*p<0.05

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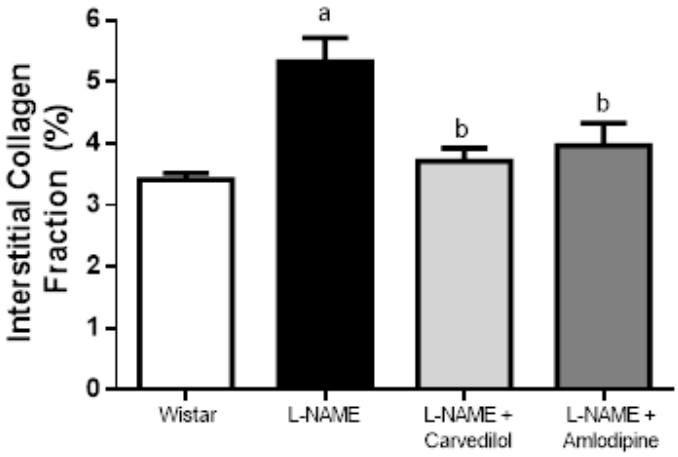

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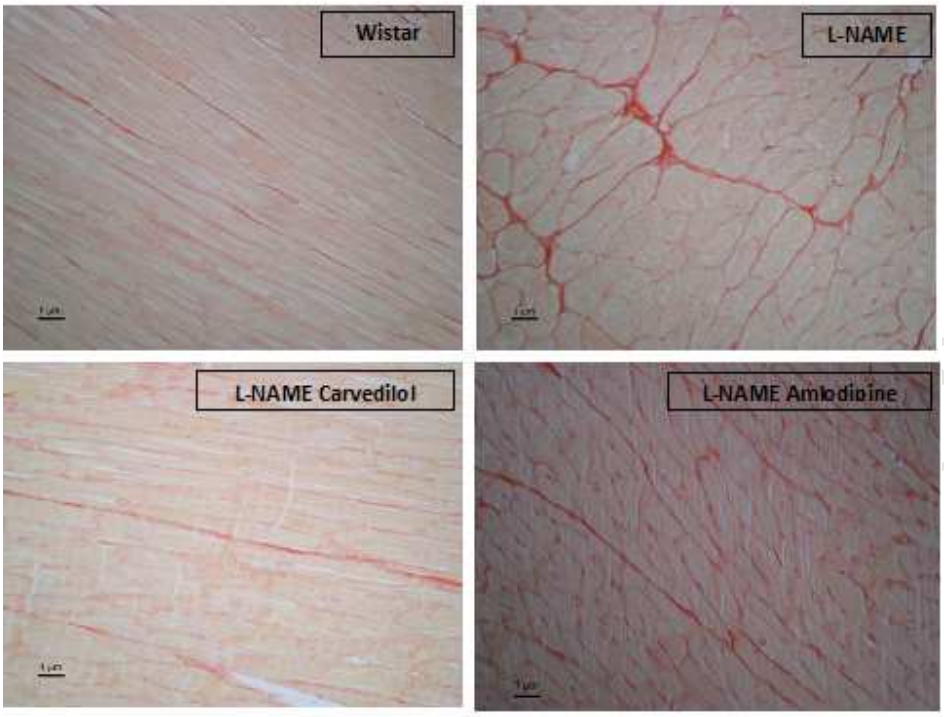

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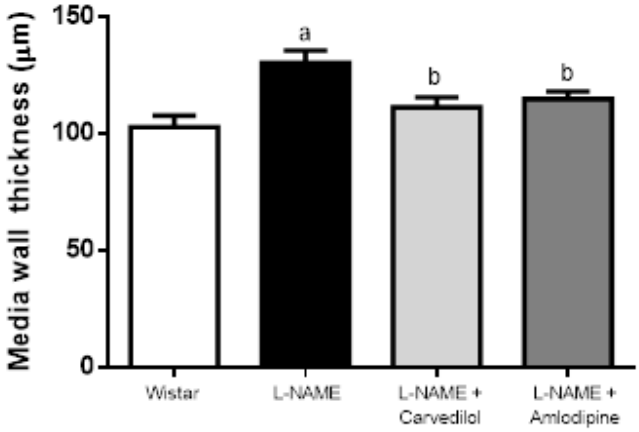

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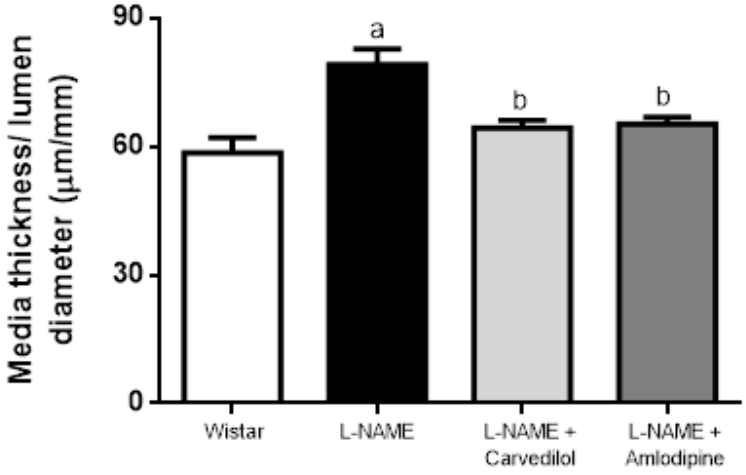

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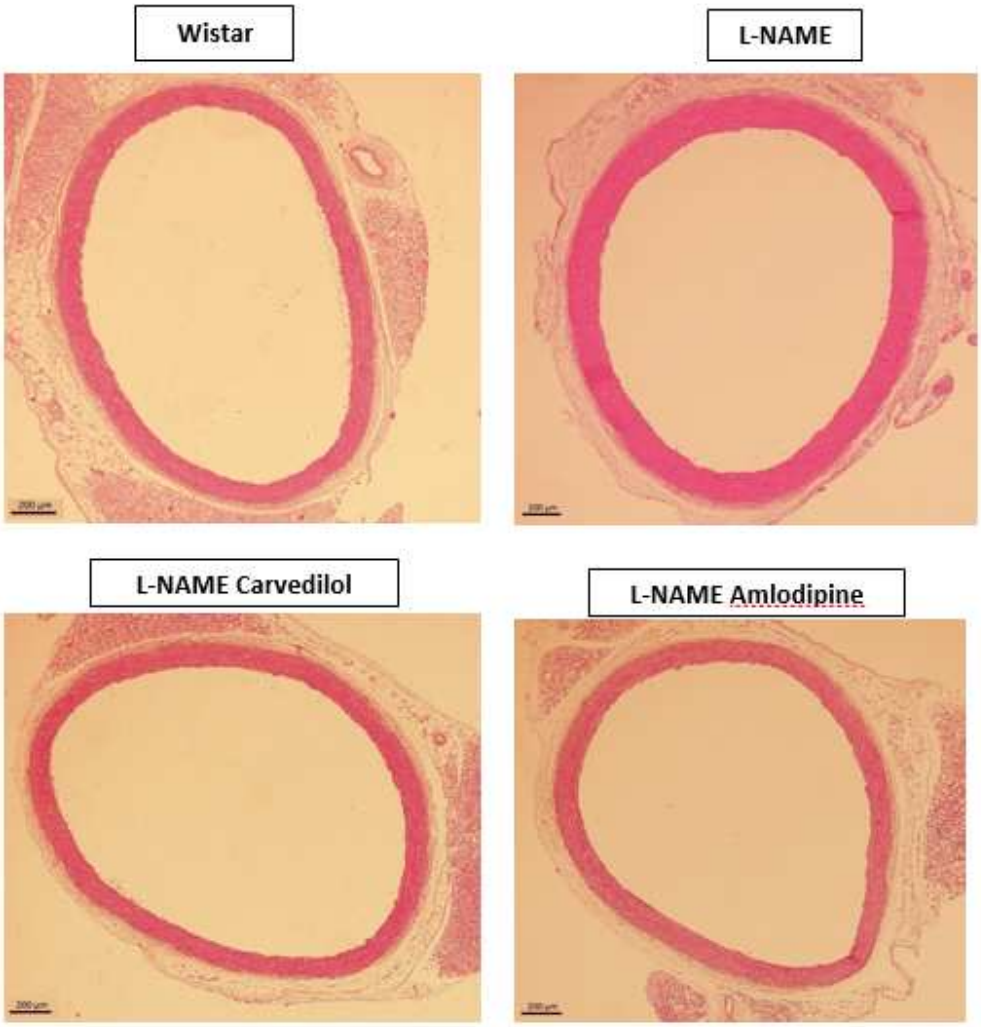

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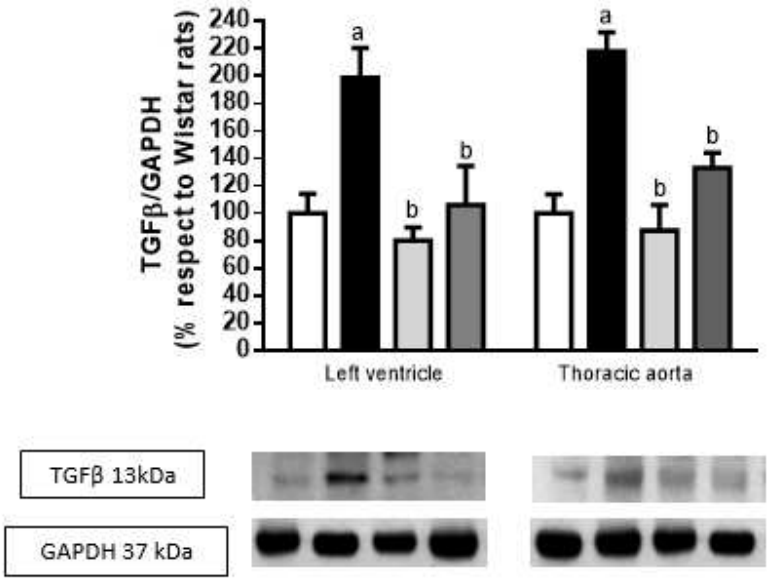

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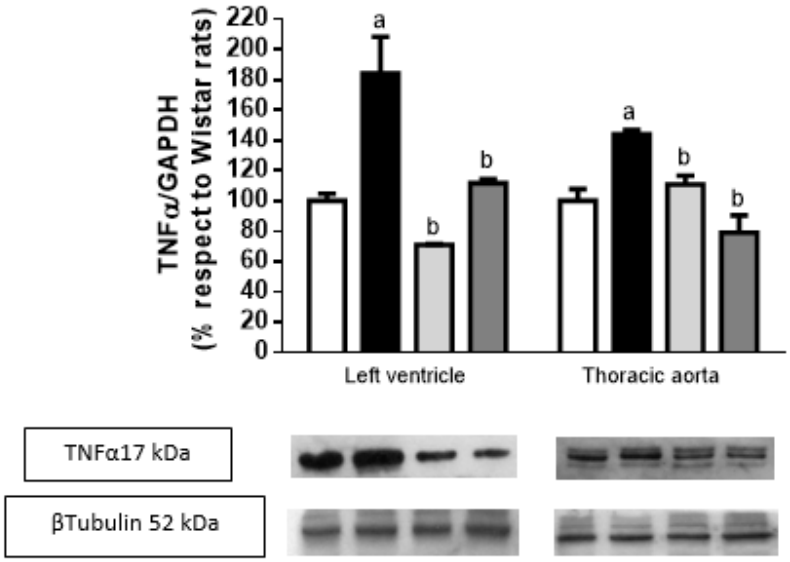

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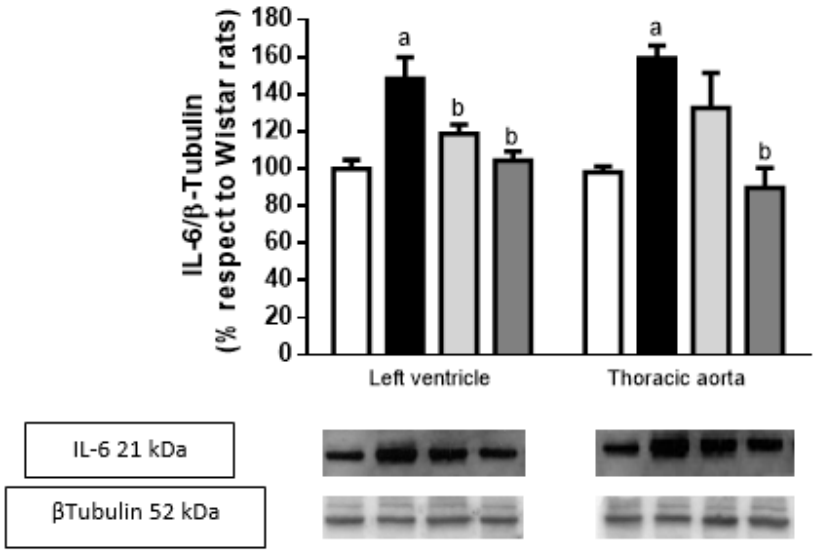

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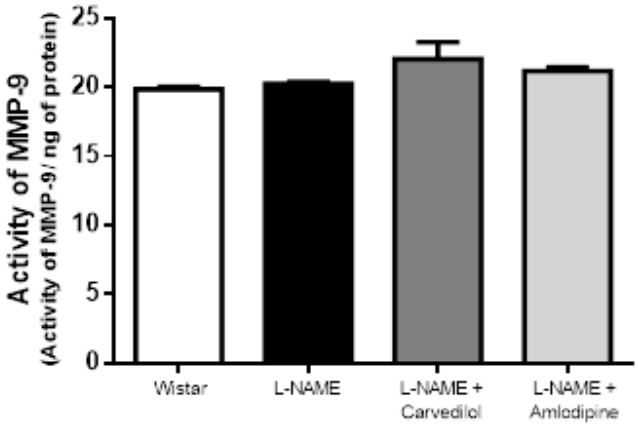

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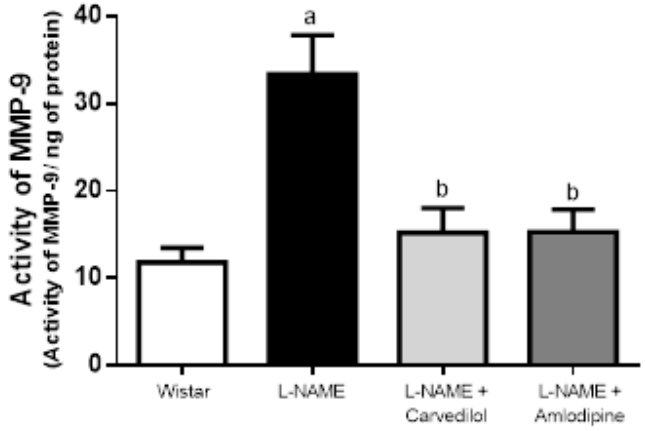

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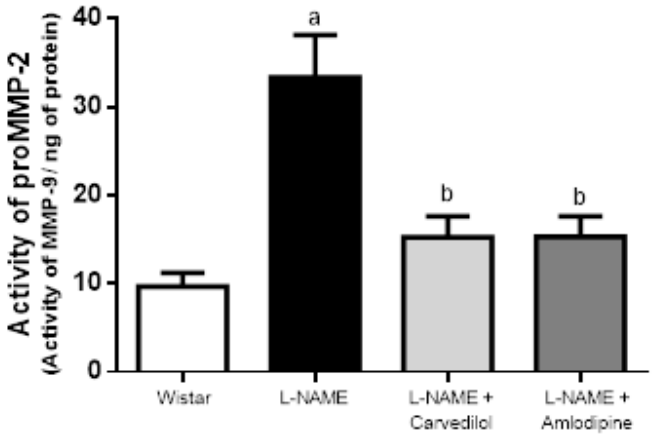

ACCEPTED MANUSCRIPT

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Highlights

- Carvedilol or amlodipine similarly attenuates short-term blood pressure variability.
- Carvedilol or amlodipine prevented target organ damage associated to hypertension.
- Attenuation of blood pressure variability contributes in the prevention of organ damage.

ACCEPTED MANUSCRIPT