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Left ventricular hypertrophy does not prevent heart failure in experimental hypertension☆

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ABSTRACT

Background: Left ventricular hypertrophy (LVH) secondary to hypertension has been accepted to prevent heart failure (HF) while paradoxically increasing cardiovascular morbi-mortality.
Objectives: To evaluate whether antihypertensive treatment inhibits LVH, restores beta-adrenergic response and affects myocardial oxidative metabolism.
Methods: Ninety spontaneously hypertensive rats (SHR) were distributed into groups and treated (mg/kg, p.o.) with: losartan 30 (L), hydralazine 11 (H), rosuvastatin 10 (R), carvedilol 20 (C). Hypertension control group comprised 18 normotensive rats (Wistar-Kyoto, WKY). Following euthanasia at 16 months, contractility was measured in 50% of rats (Langendorff system) before and after isoproterenol (Iso) 10^{-9} M, 10^{-7} M and 10^{-5} M stimulation. Left ventricular weight (LVW) was measured in the remaining hearts, and normalized by BW. Expression of thioredoxin 1 (Trx-1), peroxyredoxin 2 (Prx-2), glutaredoxin 3 (Grx-3), caspase-3 and brain natriuretic peptide (BNP) was determined.
Results: Systolic blood pressure (mm Hg): 154 ± 3 (L), 137 ± 1 (H), 190 ± 3 (R)*, 206 ± 3 (SHR)*, 183 ± 1 (C)**, and 141 ± 1 (WKY) (*$p < 0.05$ vs. L, H, WKY, **$p < 0.05$ vs. L, H, WKY, SHR). LVW/BW was higher in SHR and R ($p < 0.05$). Groups SHR, R and C evidenced baseline contractile depression. Response to Iso 10^{-5} M was similar in WKY and L. Expression of Trx-1, Prx-2 and Grx-3 increased in C, H, R and L ($p < 0.01$).
Conclusions: Present findings argue against the traditional idea and support that LVH might not be required to prevent HF. Increased expression of thioredoxins by antihypertensive treatment might be involved in protection from HF.

1. Introduction

Left ventricular hypertrophy (LVH) and heart failure are major causes of high morbidity and mortality [1]. Classically, LVH secondary to hypertension was initially regarded as a compensatory response aimed to counterbalance systolic wall stress and prevent heart failure [2]. However, the currently accepted concept is that of pathological hypertrophy with depressed ventricular function that leads to heart failure [3]. Therefore, the presence of LVH is a strong and independent risk factor for poor outcome in cardiac disease, paradoxically associated with increased cardiovascular morbidity and mortality [4,5].

Consequently, the suppression of cardiac hypertrophy has emerged as a possible strategy for mitigating pressure overload-induced end-organ damage [6]. Of note, experimental evidence suggests that myocardial hypertrophy is not necessary to maintain normal function under hypertensive stress [7].

The controversy arguing virtually opposite concepts, v.g.: "inhibition of hypertrophy, may not be a good therapeutic strategy in ventricular pressure overload" [3] vs. "inhibition of hypertrophy is a good therapeutic strategy in ventricular pressure overload" [7], has been recently discussed and revised by Crozatier & Ventura-Clapier [3] and Schiattarella & Hill [7].

Both groups pointed to a robust epidemiological literature, coupled with a large body of preclinical evidence, indicating that load-induced ventricular hypertrophy is maladaptive [6]. Both teams also agreed that several signaling cascades that trigger LVH can be interrupted effectively without consequences, even with some beneficial effects, while others although a few, claimed that interruption is not well tolerated [7].

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Key points in the discussion were the role of the β-adrenergic pathway and renin-angiotensin-aldosterone system (RAS). Taking into account that management of LVH has proved to be beneficial in a series of studies, and detrimental in others, we decided to perform the present investigation.

Therefore, the aim of this study was to evaluate whether chronic treatment of spontaneously hypertensive rats (SHR) with drugs which have different mechanisms of action, might inhibit development of LVH and reverse the impaired β-adrenergic and/or RAS response independently of blood pressure normalization. The hypothesis that the observed effects might be associated with changes in myocardial oxidative metabolism was also addressed [8,9]. Oxidative status was assessed by thioredoxin immunostaining supported by previous reports. Upregulation of the thioredoxin system TRX was found in endomyocardial biopsy samples of patients with severe LVH and other cardiomyopathies [10]. Also, inhibition of endogenous thioredoxin in the heart increases oxidative stress and cardiac hypertrophy [11].

2. Materials and methods

2.1. Animals

Male spontaneously hypertensive rats (SHR, 2-month old) were randomly distributed into six groups (n = 18 per group) and assigned to oral administration of (mg/kg) with: losartan 30 (L), hydralazine 11 (H), rosuvastatin 10 (R), carvedilol 20 (C), or water (control treatment). Normotensive male (Wistar-Kyoto, WKY, 2-month old) rats (n = 18) were used as controls. All animals were housed with 12–12 h light–darkness cycles, at a temperature of 21 ± 2 °C and fed a balanced extruded chow containing normal sodium and 16–18% proteins (Cooperación- Argentina). All procedures were performed according to the Canadian Council on Animal Care (Guide to the care and use of experimental animals, 1980–1984, 2 vol., Ottawa, Ont: CCAC) recommendations. Body weight was measured on a weekly basis. Every two weeks, blood pressure was recorded by tail plethysmography in awake animals, using a NIBP controller module in conjunction with a Power Lab system (AD Instruments, USA). Recordings were stored and analyzed using Lab Chart software (AD Instruments).

2.2. Biochemical determinations

At 0 and 16 months of treatment, plasma aliquots of blood were collected from the tail vein after 4-hour fasting. Concentration of glucose, total- and HDL-cholesterol fractions, creatinine and triglycerides, were determined using commercially available kits for enzymatic colorimetric assays (Sigma-Aldrich, USA).

2.3. Echocardiographic evaluation

Transthoracic echocardiograms were obtained in awake, gently restrained rats at 0, 6, 12 and 16 months of treatment using an ATL 3000 HDI (Bethold, WA, USA) echocardiographic system equipped with a 10.5 MHz transducer. Bi-dimensional and M-mode echocardiography images were acquired in short axis views at the level of the papillary muscle. Septal end-diastolic thickness (IVST) and left ventricular end diastolic posterior wall thickness (PWT) were determined at the parasternal long axis at the midchordal level. Left ventricular diastolic dimension (LVDD) and left ventricular end-systolic posterior dimension (LVPW) were measured perpendicularly to the long axis of the ventricle also at the midchordal level. Shortening fraction (SF %) was calculated as: 100 x (LVDD - LVPW)/LVDD. Left ventricular mass (LVM, mg) was determined using the standard cube method [12] as LVM = (LVDD + IVST + PWT)^3 − (LVDD)^3 – 1.04 and it was normalized by body weight (LVM/BW, mg/g). Other parameters were calculated as follows: Relative posterior wall thickness (RWTh) = (PWT + IVST/LVDD); end diastolic volume (EDV, mL) = 0.85 − (LVDD)^3; end-systolic volume (ESV, mL) = 0.85 − (LV systolic dimension)^3; cardiac output (CO, mL/min) = (EDV − ESV) × heart rate (HR); cardiac index (mL/g): CO/body weight; systolic volume (SV, mL) = EDV − ESV. Ejection Fraction (EF, %) was calculated as (EDV-ESV / EDV) × 100. Echocardiographic images and HR were simultaneously recorded. End-systolic posterior wall thickness (mm) (SPWT) was determined as an expression of cardiac contractile function.

2.4. Euthanasia

At the end of the 16-month treatment, all animals were administered an overdose of pentobarbital (40 mg/kg, i.p.). Fifty per cent of the hearts were mounted in a Langendorff system in order to measure contractility (see below). The remaining hearts were perfused with saline solution through the aorta and weighed, and the atria and right ventricle (RV) were cut away from the left ventricle (LV). Left ventricle and lungs were weighed (respective weights: LVW and LW) and the values were normalized by body weight (BW). Left ventricle was cut longitudinally, fixed in a phosphate buffered 10% formaldehyde solution (pH = 7.2), and processed for histology and immunohistochemistry using conventional techniques.

2.5. Contractile function response to beta-adrenergic stimulation

Fifty percent of the hearts were mounted in a modified Langendorff perfusion system as mentioned above.

Briefly, hearts were rapidly excised and mounted by the aortic root on a Langendorff apparatus in <1 min [13]. Each heart was perfused with Krebs-Henseleit buffer containing NaCl 118.5 mM, KCl 4.7 mM, $NaHCO_3$ 24.8 mM, KH_2PO_4 1.2 mM, Mg SO_4 1.2 mM, $CaCl_2$ 1.5 mM and glucose 10 mM, bubbled with 95% O_2–5% CO_2 gas mixture at 37 °C, and final pH was adjusted to 7.2–7.4. Two electrodes were secured to the epicardial surface and connected to a pacemaker at a constant heart rate of 275 beats/min. A saline-filled latex balloon connected by a catheter to a pressure transducer (Deltram II, Utah Medical System) was inserted into the left ventricle. The volume of the balloon was adjusted to achieve 8–10 mm Hg end-diastolic pressure. Coronary perfusion pressure (CPP) was also recorded through a pressure transducer connected to the perfusion line and coronary flow was adjusted to reach around 70 mm Hg CPP during the initial stabilization period. This flow was kept constant throughout the experiment. Left ventricular developed pressure (LVDP) and maximal rate of rise of left ventricular pressure ($LV + dP / dt_{max}$) were calculated in order to evaluate contractility in response to isoproterenol (Iso) 10^{-9}, 10^{-7} and 10^{-5} M.

2.6. Histological and immunohistochemical procedures

The resulting LV pieces were embedded in paraffin according to standard protocols, for light microscopy examination. Sections (3 μm thick) were cut and stained with hematoxylin and eosin and Masson's trichrome.

Following deparaffinization and rehydration, sections were washed in phosphate buffered saline solution (PBS) for 5 min. Immunohistochemical conventional techniques were performed using a modified avidin–biotin–peroxidase complex protocol. Immunolabeling was expressed as (positive area / total area) × 100. In order to estimate the degree of oxidative stress and evaluate the functional status of myocardium, specific antiredoxin antibodies were used: anti-thioredoxin-1, anti-glutaredoxin-3 and anti-peroxiredoxin-2 (anti-Trx-1, anti-Grx-3, anti-Prx-2, Abcam Inc., Cambridge, Ma, USA). Nuclear Trx-1 and Grx-3 expression and interstitial Prx-2 expression were quantified as indicators of antioxidant response. Immunopositivity for caspase-3 (apoptosis) and brain natriuretic peptide (BNP) was also determined. Control sections were incubated with non-immune normal rabbit serum.

2.7. Morphological analysis

For light microscopy stereology, a Nikon Eclipse 50i microscope (Nikon Corporation, Tokyo, Japan), equipped with a digital camera (Nikon Coolpix S4) and the Image-Pro Plus image processing software version 6.0 (Media Cybernetics, Silver Spring, Maryland, USA) were used. Forty fields of view were obtained by uniform systematic random sampling from the epicardium to the subendocardium of the LV, with the sole criterion being cross-sectional orientation of myocytes and capillaries [14]. Thin sections (3 μm width) were cut from paraffinized tissue blocks and stained with Masson's trichrome. An orthogonal grid with 300 test points, projected onto the fields of view that represented an area of 6.7 $10^4 \mu m^2$ (objective lens magnification: 40×) was used. The number of points hitting structures of interest was counted. The count number of points hitting myocardium, interstitium and coronary microvessels was used to estimate volume densities of these compartments according to: $V_V = Pp / Pt$, where V_V is volume density of a structure related to a reference volume, Pp is the number of points hitting the structure and Pt is the total number of points hitting the reference volume. Volume density of: Vv myocardium (Vv myo), Vv interstitium (Vv int) and Vv coronary microvessels (Vv cmv) was expressed as percentage. Individual myocyte mean cross-sectional area (MCSA, μm^2) was determined for 200 myocytes per animal, only in tissue areas with cross sectional arrangement of cardiomyocytes. In addition, the number of myocytes per mm^2 was estimated. Coronary microvessels' density (CMD) was expressed as microvessels/mm^2 cross-sectional area of myocardium. This data was used to calculate diffusion distance using the following equation: $R = 10^3 / \sqrt{N\pi}$, where N is number of capillaries/mm^2. This value represents mean half distance (μm) between two capillaries in cross-section and is used as an average index of capillary supply. Immunohistochemical expression for Trx-1, Prx-2, Grx-3, caspase-3 and BNP was estimated by a semiquantitative score [15].

2.8. Survival analysis

Kaplan-Meier and Cox proportional regression analyses were used to explore survival rate (censored survival time) across groups over follow-up time and relative risk of death, in untreated SHR rats compared with those receiving different antihypertensive treatments respectively (SPSS™ 15.0).

2.9. Statistical methods

Values were expressed as mean ± SD. Statistical analyses were based on absolute values. GraphPad Prism version 5.0 (GraphPad Software, Inc., San Diego, California, USA) was used for histology data. Gaussian distribution was assessed by the Kolmogorov and Smirnov method with an assumption test. Variables following a Gaussian distribution were examined using analysis of variance (ANOVA) and between-experimental group comparison was evaluated using the Tukey-Kramer multiple comparisons test. Histological values not following a Gaussian distribution, were analyzed by the Kruskal-Wallis test,