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 while paradoxically increasing cardiovascular morbidity and mortality. 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) 10−8 M, 10−7 M and 10−5 M stimulation. Left ventricular weight normalized by BW. Expression of thioredoxin 1 (Trx-1), peroxiredoxin 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)**. 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 < 0.05 vs. L, H, WKY, SHR). LVW/BW was higher in SHR and R (p < 0.05 vs. L, H, WKY, SHR). LVW/BW was higher in SHR and R (p < 0.05 vs. L, H, WKY, SHR).

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.

International Journal of Cardiology 238 (2017) 57-65

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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 10 (H), 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 (Cooperacidin–Argentina). All procedures were performed according to the Canadian Council on Animal Care (Guide to the care and use of experimental animals, 1988–1984, 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 PowerLab 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 (Buddell, 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 (WST) and left ventricular end diastolic posterior wall thickness (PW) were determined at the parasternal long axis at the midcaval 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 midcaval level. Shortening fraction (SF%) was calculated as: 100 x (LVDd – LVPW) / LVDd. Ejection Fraction (EF, %) was calculated as: (EF%) = (1 – LVPW/LVDd) x 100. Peroxiredoxin-2 (anti-Trx-1, anti-Grx-3, anti-Prx-2, Abcam Inc., Cambridge, Ma, USA) and antiredoxin antibodies were used: anti-thioredoxin-1, anti-glutaredoxin-3 and anti-peroxiredoxin-2 (anti-Trx-1, anti-Grx-3, anti-Px-2, Abcam Inc., Cambridge, MA, USA). Nuclear Trx-1 and Grx-3 expression and interstitial Px-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.

2.4. 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 (respectively: LWV 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 on 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, NaHCO3 24.8 mM, KH2PO4 1.2 mM, MgSO4 1.2 mM, CaCl2 1.5 mM and glucose 10 mM, bubbled with 95% O2–5% CO2 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 (Deltlam 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 / dtnmax) were calculated in order to evaluate contractility in response to isoproterenol (10−4 M, 10−7 M and 10−10 M).

2.6. Histological and immunohistochemical procedures

The resulting LV pieces were embedded in paraaffin 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 dehydration, 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. Immunohistochemical staining 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 thioredoxin antibodies were used: anti-thioredoxin-1, anti-glutaredoxin-3 and anti-peroxiredoxin-2 (anti-Trx-1, anti-Grx-3, anti-Px-2, Abcam Inc., Cambridge, MA, USA). Nuclear Trx-1 and Grx-3 expression and interstitial Px-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 54) 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.

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-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,
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