Assessment of left ventricular structure and function in rats subjected to pressure-overload hypertrophy in time

Vili Stoyanova[1], Nikolai Zhelev[2], Evghenii Ghenev[1], Miroslava Bosheva[1]

[1] Department of Pediatrics and Medical Genetics, Medical University, Plovdiv, Bulgaria
[2] School of Contemporary Sciences, University of Abertay Dundee, Scotland, United Kingdom

Abstract

Background: Left ventricular hypertrophy (LVH) is an adaptive response to increased haemodynamic load and an independent risk factor for the development of heart failure. Although the pathophysiological features during the development of cardiac hypertrophy have been extensively studied, the time course of LVH is less clearly defined.

Aim: To define the time-dependency of the LVH process in vivo and compare the data by necropsy.

Methods: Using abdominal aortic banding (AAB) in male Wistar strain albino rats we assessed the changes of LV structure and function in short intervals of 5 days for a period of 45 days. We determined the changes by serial echocardiography and confirmed the results in a second echocardiographic experiment and by necropsy.

Results: In our model the magnitude of the pressure overload was sufficient to produce significant LVH within a 10-day time frame and further progression on the 15th day after AAB. Interestingly, on the 20th day after banding a short-lasting regression of LVH (and heart weight and LV wall thickness) was found. It was followed by an increase in the next 15 days (till the 35th day), after which LVH was roughly complete (as measured at the 45th day).

Conclusions: Following the development of LVH over a relatively long period of time and providing the changes in short intervals, a short lasting regression during ongoing pressure overload was noted. Understanding and targeting the associated signalling underlying this regression may have considerable clinical consequences.

Key words: cardiac hypertrophy, left ventricle, pressure overload, time-course, rats

Kardiol Pol 2009; 67: 27–34

Introduction

Left ventricular hypertrophy (LVH) is an adaptive response to increased haemodynamic load that works to normalise abnormal wall stress [1]. Findings from the Framingham studies have provided evidence that the presence of LVH is an independent risk factor for the development of heart failure [2]. Therefore, investigations concerning the time course for the progression of LVH contribute to finding the most suitable therapeutic strategy for this affliction for limiting heart failure.

Rats are suitable small animals for inducing and studying LVH. There are different experimental models of LVH in rats [3-6], but very few of them have been used to study the time-course of the progression of this hypertrophy [3, 5]. Even then the changes in LV function and structure have not been studied in short intervals of time.

In the present study we produced pressure overload-induced LVH in rats and followed the time-dependency of the hypertrophy process.

Methods

Animals

Experiments were performed with male Wistar strain albino rats, 200±10 g body weight at the beginning of the experiment. All procedures were performed in accordance with the Ethical Committee Note of the Medical University of Plovdiv (Bulgaria) for care and use of laboratory animals.

Address for correspondence:
Vili Stoyanova MD PhD, Department of Pediatrics and Medical Genetics, Medical University, Vasil Aprilov Blv 15A, Plovdiv, Bulgaria, tel.: +359 32 602 547, fax: +359 32 602 547, e-mail: vi1sto@abv.bg

Received: 29 August 2008. **Accepted:** 15 October 2008.

Animal model

Pressure overload was produced by abdominal aortic bending (AAB) subdiaphragmatically. Under anaesthesia with pentobarbital sodium (65 mg/kg) intraperitoneally (ip) and a midline abdominal laparotomy, a nearly 1 cm segment of abdominal aorta was separated free and a curved needle, having 0.95 mm external diameter, was placed on its anterior surface. A 2-0 surgical silk was tightly banded around the needle and aorta, providing a uniform degree of constriction. The needle was then carefully withdrawn from the ligature so that the diameter of the constriction approximated that of the needle. The muscle layer and then the skin were closed with a 3-0 silk suture. Sham operated (SO) animals underwent dissection of the abdominal aorta without banding. Postoperatively, all animals were fed commercial rat chow and had free access to water. On the final day of the study each rat with AAB was examined to verify the constriction.

Echocardiographic study

After sedation with pentobarbital sodium (65 mg/kg) injected intraperitoneally, rats were secured in a supine position, shaved at the precordium, and transthoracic echocardiography was performed by using Sono Site 180 (SonoSite Inc., USA) equipped with a 10 MHz linear transducer.

The heart was imaged in the 2D mode in the parasternal long-axis view. From this view, an M-mode cursor was positioned perpendicular to the interventricular septum and posterior wall of LV at the level of the papillary muscles. Left ventricular anterior wall thickness (AWT), posterior wall thickness (PWT), LV end-diastolic diameter (LVEDD), and LV end-systolic diameter (LVESD) were measured. All measurements were performed from leading edge to leading edge according to the American Society of Echocardiography guidelines [7]. Three representative cardiac cycles were analysed and averaged for each measurement.

Left ventricular mass (LVM) was calculated by use of the following formula, assuming a spherical left ventricular geometry and validated in rats [6]: LVM (g)=1.04 × [(LVEDD + PWT + AWT)^3 – LVEDD^3], where 1.04 is the specific gravity of myocardium. The percentage of LV fractional shortening (LV% FS) was calculated as [(LVEDDLVESD)/LVEDD] × 100.

Haemodynamic measurements

Mean arterial pressure (MAP) was measured in anaesthetised (pentobarbital sodium – 65 mg/kg ip) rats (n=5 from each group and at each time point). For this purpose the left carotid artery was cannulated with a fluid-filled polyethylene (PE-50) catheter connected to a pressure transducer (E xperimetria, Ltd, Hungary) in-line to Cardiostar CO-100 polygraph (Cardiostar CO-100, Experimetria MM, Ltd., Budapest, Hungary). Blood pressure below the ligation was not examined.

Experimental protocol

After the initial assessment by using echocardiography rats were randomly divided into 2 groups and subjected to AAB or sham operation.

First experiment: a group of 14 animals with AAB and 10 SO rats were followed 45 days after operation. They were studied echocardiographically on the 10th, 15th, 20th, 25th, 35th and 45th day and euthanized on the 45th day.

Second experiment: in 10-16 AAB rats and 10 SO rats transthoracic echocardiography was performed at the 10th day, 15th day, 20th day, 25th day and 35th day after surgery and on the appointed day they were euthanised.

Body weight (BW) was recorded for each rat, both on the day of operation and on the day of death. On the last day, each animal chest was opened through a left thoracotomy, the pericardium was exposed, and the heart was quickly removed, immersed in ice-cold PBS and weighed. The length of the tibia (TL) was measured. The right tibia was dissected, and its length from the condyles to the tip of the medial malleolus was measured with micrometer calipers by the method of Yin et al. [8].

Statistical analysis

Values are expressed as means ± SD. Serial echocardiographic studies were tested by repeated measures ANOVA followed by Fisher's protected least significant difference test. Results obtained in postmortem examination and in echocardiographic studies were analysed with analysis of variance of the multivariate profiles for dependent groups (MANOVA), followed by the Duncan post hoc test to assess differences between groups (AAB and SO), as well as the effect of aortic banding in time. A value of $p < 0.05$ was considered statistically significant. The relation between variables was examined by linear regression analysis.

Results

A total of 155 rats were initially enrolled in this study – 95 underwent AAB and 60 rats underwent sham operation. Upon surgery, the death rate for AAB was nearly 16%, mainly in the first 12 hours after the operation, and only one died on the third day. There were no fatalities in SO animals.

In vivo 2D guided M-mode echocardiograms were obtained on the 1st day before surgery and at each time point post-operation in both groups. The difference in PWT between SO and AAB rats was apparent (Figures 1B, 2A). In relation to SO, PWT in AAB rats increased significantly (e.g., by 19% at day 10, 39% at day 15, 18% at day 20, 31% at day 25, 43% at day 35 and 38% at day 45). Within the group of AAB rats PWT increased in time, such that at the 35th and 45th day it was significantly thicker than in AAB rats at earlier time points after operation. At day 20, PWT (0.18±0.02 cm) was significantly lower in respect to

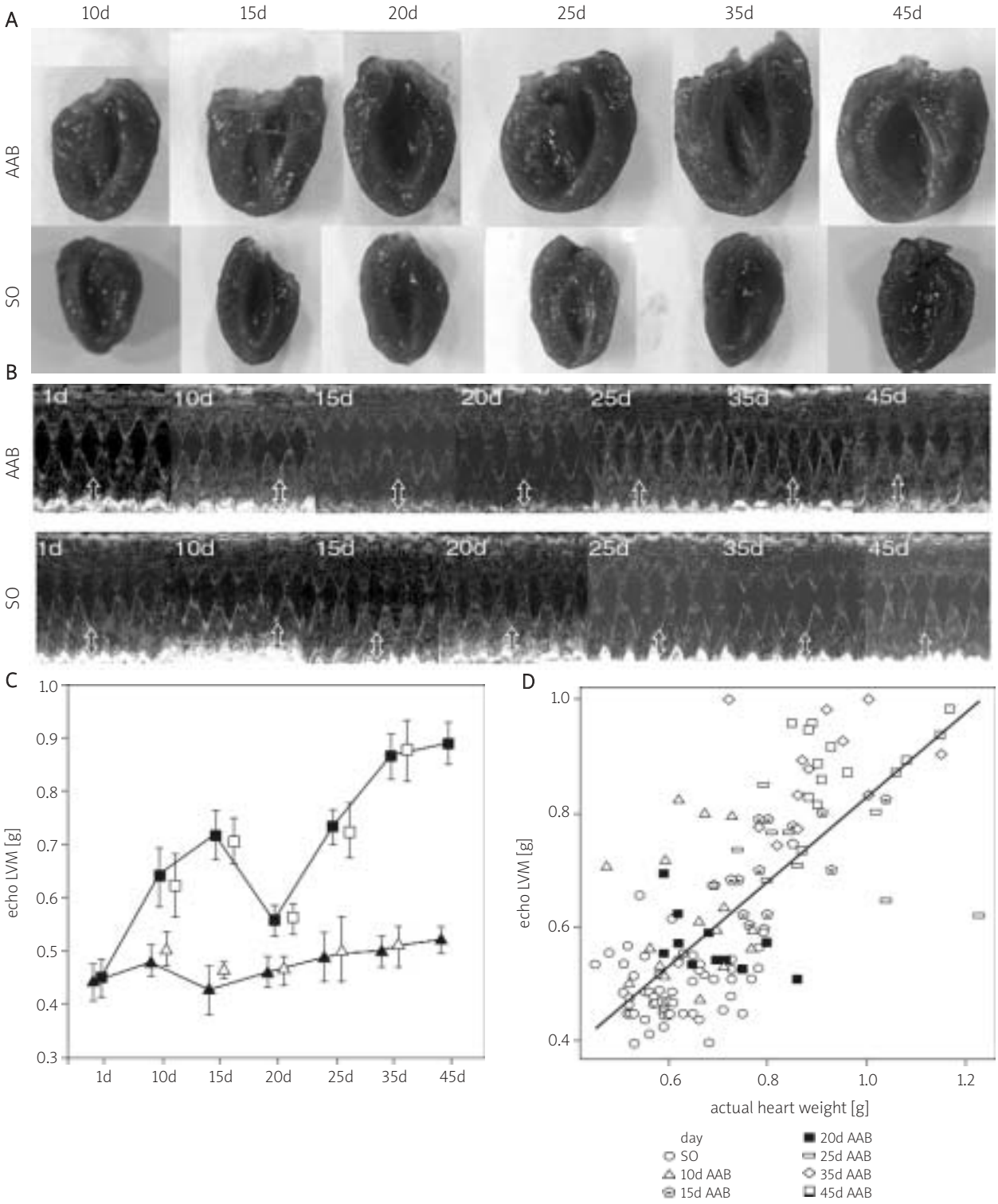

Figure 1. Time-dependent development of load-induced cardiac hypertrophy. **A** – Examples of heart sections on 10th day, 15th day, 20th day, 25th day, 35th and 45th day after operation. Scale: 1 mm. **B** – M-mode echocardiograms of AAB animal and SO control on the 1st day before surgery and at each time point after operation. Posterior wall thickness (PWT) is indicated with an arrow. **C** – left ventricular mass (LVM) of abdominal aortic banded (AAB) rats – ■ and sham-operated (SO) rats – ▲ from serial echocardiography and from second echocardiographic experiment (AAB – □ and SO – Δ) at 10th day, 15th day, 20th day, 25th day, 35th and on 45th day after operation. **D** – correlation between actual heart weight and echo-LV mass

r=0.741, y=0.09+0.741 x, p <0.0001, n=140, AAB – abdominal aortic banded rats, SO – sham operated rats, d – day

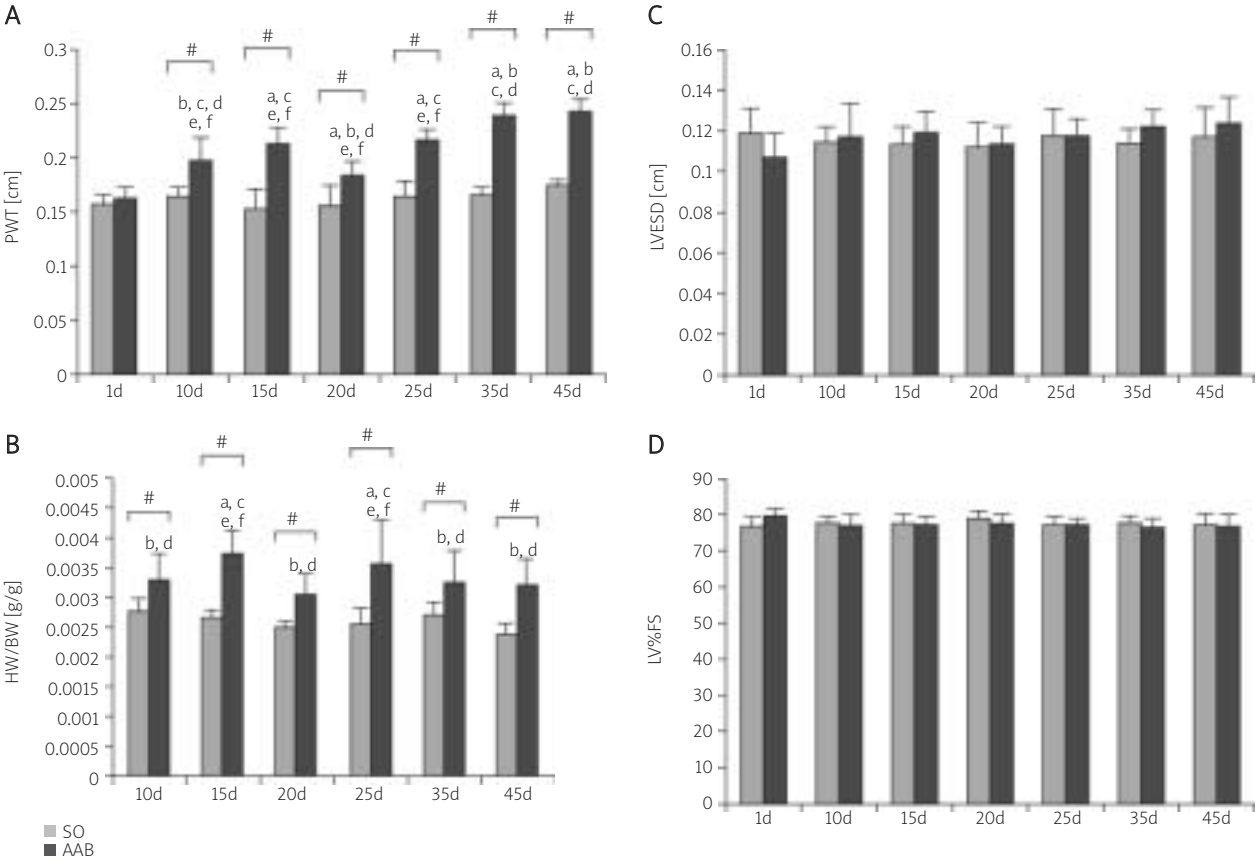

Figure 2. Time-course changes in **A** – posterior wall thickness (PWT), **B** – actual heart weight to body weight ratio (HW/BW), **C** – left ventricular end systolic diameter (LVESD), **D** – percentage of LV fractional shortening (LV%FS) assessed by serial echocardiography of 14 abdominal aortic banding rats (AAB) and 10 sham-operated (SO)
d – day; data are means ± SD, a – p <0.05 vs. 10 day AAB, b – p <0.05 vs. 15 day AAB, c – p <0.05 vs. 20 day AAB, d – p <0.05 vs. 25 day AAB, e – p <0.05 vs. 35 day AAB, f – p <0.05 vs. 45 day AAB, #p <0.05 vs. respective SO

the other time points in the AAB group (e.g. by 7% to 10th day, by 14% to 15th day, by 15% to 25th day, by 23% to 35th day, by 24% to 45th day).

The short-lasting regression of LVH on the 20th day, found in the first experiment in this model, was confirmed by the results of the second experiment. There was no significant difference between the values of LVM obtained on each day from serial echocardiographic study (first experiment) and the values of LVM assessed on the same day in rats from the second experiment (Figure 1C). What is more, the actual heart weight (HW) after necropsy corroborated the short-lasting regression of LVH (Figures 1A, 3A).

Actual HW was markedly greater in AAB than in SO rats at each time point. Heart hypertrophy was manifested as an increase in absolute HW and remained evident when normalised to BW (Figure 2B) and TL (Figure 3C). However, on the 20th day HW in the AAB group was significantly lower (0.69±0.08 g) compared to HW in the same group on day 15 (0.81±0.09 g); 25 (0.88±0.16 g), 35 (0.90±0.11 g) and 45 (0.96±0.1 g). Both PWT and echocardiographically derived LVM correlated significantly with actual heart mass at necropsy. The PWT had an r value of 0.730 (p <0.0001) compared with actual HW. For calculated echo LVM, r was 0.741 (p <0.0001) compared with actual heart mass (Figure 1D). In addition, the time dependent PWT and actual whole HW (Figure 2, 3), and echo LVM/TL and HW/TL (Figure 3) were well-coincided.

Arterial blood pressure measured through the left carotid artery was significantly higher in the AAB rats than in the SO rats at each time point, while there was no statistical difference within the groups. Relative to SO, MAP was increased by ≈53 mmHg in AAB rats at each time point after pressure overload.

However, no significant difference was observed in HR between AAB and SO rats and within each group across time (Table I).

Body weight and TL were analysed across groups and in time. There was a significant increase in BW and TL with time in rats from both groups, with no significant difference between the groups at each time point (Table I).

Table I. Measurements of body weight (BW), tibia length (TL), mean blood pressure (MBP), heart rate (HR) in sham-operated (SO) and abdominal aortic banded (AAB) rats at each time point after surgery

Day	BW [g]		TL [mm]		MBP [mmHg]		HR [beats/min]	
	SO	AAB	SO	AAB	SO	AAB	SO	AAB
10th	197±9	197±18	34.9±0.6	34.7±1.4	111±3	165±5#	403±15	372±16
15th	211±9	219±11	35.8±0.7	35.9±0.8	112±3	167±6#	410±11	399±17
20th	223±9	228±15	36.5±0.7	36.4±0.6	113±3	166±4#	409±8	409±13
25th	244±9	253±30	36.8±0.4	36.7±1.0	113±5	164±2#	410±7	405±17
35th	260±7	283±19	37.5±0.5	38.1±0.8	113±2	165±3#	410±7	406±17
45th	290±14	301±20	38.4±0.5	38.5±0.8	114±4	168±4	409±8	402±16

data are means ± SD;. #p <0.05 AAB vs. respective SO

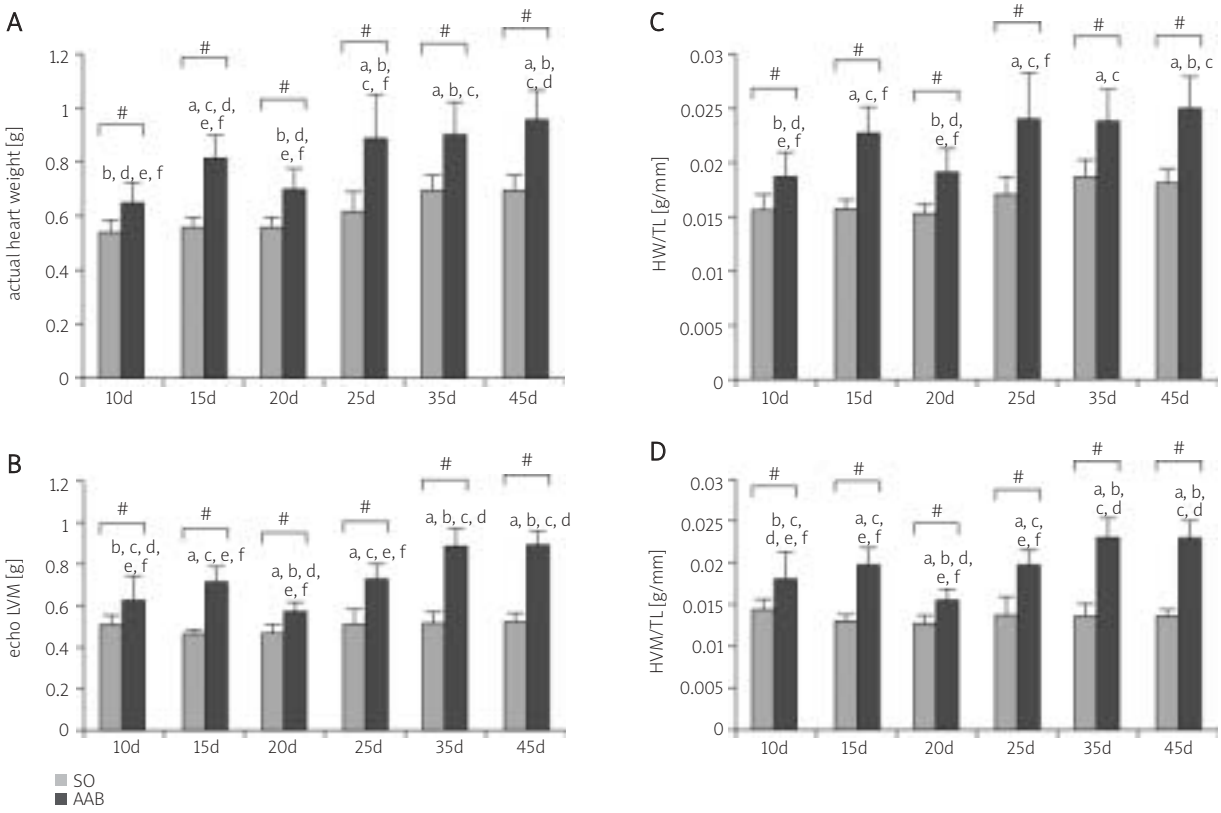

Figure 3. Changes in **A** – actual whole heart weight (HW), **B** – echo left ventricular mass (LVM), **C** – actual heart weight to tibia length ratio (HW/TL), **D** – echo left ventricular mass to tibia length ratio in abdominal aortic banded (AAB) rats n=16 at 10th day, n=15 at 15th day, n=13 at 20th day, n=10 at 25th day, n=12 at 35th and n=14 at 45th day after surgery and 10 sham operated (SO) rats at each time point

data are means ± SD; a – p <0.05 vs. 10 day AAB, b – p <0.05 vs. 15 day AAB, c – p <0.05 vs. 20 day AAB, d – p <0.05 vs. 25 day AAB, e – p <0.05 vs. 35 day AAB, f – p <0.05 vs. 45 day AAB, #p <0.05 vs. respective SO

Discussion

We report, for the first time, a short lasting regression on the 20th day during the development of compensated LVH in rats in this model. Changes in LV structure and function in rats subjected to pressure-overload hypertrophy were assessed in short intervals of time for a period of 45 days by serial echocardiography. The results were confirmed in a second echocardiographic experiment and corroborated by the actual HW after necropsy.

Recent studies examined the effects of pressure overload on cardiac structure and function via echocardiography in rabbits, dogs, cats and mice [9-12]. Numerous studies have also assessed cardiac structure and function in rats subjected to pressure overload

[5, 6, 13]. However, long-term analyses with assessment in short intervals were not carried out. Pawlush et al. [6] examined pressure overload hypertrophy at a single time point – four weeks after the operation. Turto [13] studied the development of LVH, using the same model as ours, but during the first 7 days. Ribeiro et al. [5] conducted a long-term study, but they evaluated the level of ventricular hypertrophy 2, 6, 12 and 21 weeks after surgery. Besides, Ribeiro et al. studied rats that underwent supravalvular aortic stenosis.

We assessed the changes of LV structure and function in short intervals of five days, with enough animals per group. Meanwhile, we observed in vivo by echocardiography and validated by postmortem examination that there is a short-lasting regression of LVH during its development.

Abdominal aorta banding just below the diaphragm in male Wistar rats was sufficient to produce significant hypertrophy and was confirmed by significantly increased blood pressure above the ligation. Blood pressure below the ligation was not examined. In this model LVH was induced by AAB just below the diaphragm. We did not expect any dramatic changes in the blood pressure below the ligation, which may influence the development of LVH in this model.

In our model, significant LVH developed within 10 days. However, we reported a further increase of wall thickness and echo LVM on the 15th day after AAB that coincided with actual HW and the ratio HW/BW and HW/TL on this day [3]. Interestingly, on the 20th day after operation there was a short lasting regression in LVM, followed by a new progression on the 25th and 35th day. Above all, one may ask whether our findings are simply an artefact. We consider this a possibility when we did the serial echo determination of LV mass overtime in the first experiment. That is why we did the second experiment which followed the time-dependency of the changes of LV structure by echocardiography once again and compared the alterations with actual HW at each time point. Furthermore, there was no significant difference between the values of echo LVM from the second experiment when compared with those at the respective day from the first experiment. The results found in vivo were confirmed by necropsy and the significant correlation between echocardiographically derived LVM and actual HW.

Therefore, we believe that during the progression of compensated LVH there is a short-lasting regression of LVH during ongoing pressure overload. It is found on the 20th day in this model.

Recent studies only mentioned a similar alteration in the progression of LVH. Comparable to our findings were those of Hill et al. [14], who followed the development of cardiac hypertrophy in mice 5 weeks after thoracic aortic banding. On the other hand, Akers et al. [3] assessed the changes in rats but only in long intervals of time – on day 3, 10, 30, 60 after banding. We suppose that the other investigators [3-5] have not encountered the short-lasting regression of LVH because of the long intervals of time at which they study the development of LVH.

Our finding can explain to some extent why in Kupari et al. [15] study, despite critical aortic stenosis, 19 of 85 patients did not have LVH. The mean age of their patients without LV hypertrophy was 71 years compared with 69 years in the 66 patients with LV hypertrophy. The authors did not discuss the duration of aortic stenosis formation in their patients. Providing that aortic stenosis developed at similar age in all these patients, they could have presented different stages of developing LVH.

In summary, our study extends previous findings by demonstrating an episode of short-lasting regression of LVM during development of LVH. A comprehensive explanation for this non-linear development of compensated LVH cannot be given at this time, though a number of factors could be responsible. However, the stage progression of LVH in the setting of sustained increased blood pressure suggests that pressure-overload may not be the sole stimulus for such a development. Further analysis of the concentrations of neurohormones in plasma would be reasonable [16]. In addition, relative changes in myocyte hypertrophy should be evaluated to better characterise the hypertrophic process.

A possible explanation of our finding is that adult ventricular myocytes, under certain circumstances, appear to re-enter the cell cycle and proliferate [17, 18]. In this aspect the short-lasting regression of LVH on the 20th day in the model we used may be a result of the small size of the new cardiomyocytes immediately after mitosis [18].

This study adds new important concepts for the time-dependency of the process of LVH, prompting a basis for further studies for prevention or regression of LVH as a possible therapeutic target for limiting heart failure.

References

1. Lorell B, Carabello B. Left ventricular hypertrophy pathogenesis, detection, and prognosis. *Circulation* 2000; 102: 470-9.
2. Kannel W. Vital epidemiologic clues in heart failure. *J Clin Epidemiol* 2000; 53: 229-35.
3. Akers W, Cross A, Speth R, et al. Renin-angiotensin system and sympathetic nervous system in cardiac pressure-overload hypertrophy. *Am J Heart Circ Physiol* 2000; 279: H2797-806.
4. Cantor E, Babick A, Vasanji Z, et al. A comparative serial echocardiographic analysis of cardiac structure and function in rats subjected to pressure or volume overload. *J Mol Cell Cardiol* 2005; 38: 777-86.
5. Ribeiro H, Okoshi K, Cicogna A, et al. Follow-up study of morphology and cardiac function in rats undergoing induction of supravalvular aortic stenosis. *Arq Bras Cardiol* 2003; 81: 569-75.

6. Pawlush DG, Moore RL, Musch TI, et al. Echocardiographic evaluation of size, function, and mass of normal and hypertrophied rat ventricles. *J Appl Physiol* 1993; 74: 2598-605.
7. Sahn DJ, DeMaria A, Kisslo J, et al. Recommendations regarding quantitation in M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation* 1978; 58: 1072-83.
8. Yin RC, Spurgeon HA, Rakusan K, et al. Use of tibial\length to quantify cardiac hypertrophy: application in the aging rat. *Am J Heart Circ Physiol* 1982; 243: H941-7.
9. Fard A, Wang CY, Takuma S, et al. Noninvasive assessment and necropsy validation of changes in left ventricular mass in ascending aortic banded mice. *J Am Soc Echocardiogr* 2000; 13: 582-7.
10. Moran AM, Friehs I, Takeuchi K, et al. Noninvasive serial evaluation of myocardial mechanics in pressure overload hypertrophy of rabbit myocardium. *Herz* 2003; 28: 52-62.
11. Pollack PS, Bailey BA, Budjak R, et al. Progressive feline pressure-overload: noninvasive assessment correlates with abnormalities in single cells. *Am J Heart Circ Physiol* 1993; 264: H1307-14.
12. Rouleau JR, Simard D, Blouin A, et al. Angiotensin inhibition and coronary autoregulation in a canine model of LV hypertrophy. *Basic Res Cardiol* 2002; 97: 384-91.
13. Turto H. Collagen metabolism in experimental cardiac hypertrophy in the rat and the effect of digitoxin treatment. *Cardiovascular Res* 1977; 11: 358-66.
14. Hill J, Karimi M, Kutschke W, et al. Cardiac hypertrophy is not a required compensatory response to short-term pressure overload. *Circulation* 2000; 101: 2863-9.
15. Kupari M, Turto J, Lommi A. Left ventricular hypertrophy in aortic valve stenosis: preventive or promotive of systolic dysfunction and heart failure? *Eur Heart J* 2005; 26: 1790-6.
16. Frey N, Katus HA, Olson EN, et al. Hypertrophy of the heart. A new therapeutic target? *Circulation* 2004; 109: 1580-9.
17. Beltrami A, Urbanek K, Kajstura J, et al. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med* 2001; 344: 1750-7.
18. Chaudhry H, Dashoush N, Tang H, et al. Cyclin A2 mediates cardiomyocyte mitosis in the postmitotic myocardium. *J Biol Chem* 2004; 279: 35858-66.

Ocena przebiegu zmian w budowie i czynności lewej komory poddanej przerostowi ciśnieniowemu u szczurów

Vili Stoyanova[1], Nikolai Zhelev[2], Evghenii Ghenev[1], Miroslava Bosheva[1]

[1] Klinika Pediatrii i Genetyki, Uniwersytet Medyczny, Plovdiv, Bułgaria

[2] Szkoła Nauk Współczesnych, Uniwersytet Abertaya Dundee, Szkocja, Wielka Brytania

Streszczenie

Wstęp: Przerost lewej komory (LVH) jest odpowiedzią na zwiększone obciążenie hemodynamiczne i stanowi niezależny czynnik ryzyka powstawania niewydolności serca. Mimo że patofizjologiczne zmiany zachodzące podczas tworzenia się przerostu mięśnia sercowego były przedmiotem wielu badań, ich przebieg w czasie nie został do końca ustalony.

Cel: Ocena zależności czasowych powstawania LVH *in vivo* i udokumentowanie tych zmian w badaniu anatomopatologicznym.

Metody: Oceniono zmiany w budowie i czynności LV u szczurów (samce rasy Wistar) w odstępach 5-dniowych podczas 45 dni obserwacji. Przerost lewej komory wywoływano przez podwiązanie aorty brzusznej. Oceny LVH dokonywano metodą echokardiografii oraz badań anatomopatologicznych po zakończeniu eksperymentu. W celu oceny powtarzalności wyników badanie przeprowadzono u dwóch grup zwierząt.

Wyniki: Przy zastosowaniu tego modelu przerostu istotny LVH obserwowano po 10 dniach obserwacji, z dalszym postępem LVH do 15. dnia. W 20. dniu obserwowano krótko trwającą regresję LVH, po czym do 35. dnia następowała ponownie progresja LVH, z utrzymującymi się zmianami na tym samym poziomie do 45. dnia. Zjawisko to wystąpiło w identycznej formie zarówno podczas pierwszego eksperymentu, jak i drugiego.

Wnioski: W procesie powstawania LVH jest krótki okres, kiedy przerost ulega przejściowej regresji. Zjawisko to może mieć istotne implikacje kliniczne.

Słowa kluczowe: przerost lewej komory, kardiologia eksperymentalna

Kardiol Pol 2009; 67: 27–34

Adres do korespondencji:

prof. dr hab. n. med. Vili Stoyanova, Klinika Pediatrii i Genetyki, Uniwersytet Medyczny, Vasil Aprilov Blv 15A, Plovdiv, Bułgaria,

tel.: +359 32 602 547, faks: +359 32 602 547, e-mail: vi1sto@abv.bg

Praca wpłynęła: 29.08.2008. **Zaakceptowana do druku:** 15.10.2008.