Rev Bras Cir Cardiovasc 2013;28(4):482-90

Reversible pulmonary trunk banding. IX. G6PD activity of adult goat myocardium submitted to ventricular retraining

Bandagem ajustável do tronco pulmonar. IX: atividade da G6PD do miocárdio de cabras adultas submetido ao treinamento ventricular

Renato Samy Assad[1], MD, PhD; Leonardo Augusto Miana[1,2], MD, PhD; Miriam Helena Fonseca-Alaniz[1], BPh, PhD; Maria Cristina Donadio Abduch[1], VMD, PhD; Gustavo José Justo da Silva[1,3], PE, PhD; Fernanda Santos de Oliveira[1]MD; Luiz Felipe Pinho Moreira[1], MD, PhD; José Eduardo Krieger[1], MD, PhD

DOI: 10.5935/1678-9741.20130079

RBCCV 44205-1501

Abstract

Objective: **Increased glucose 6-phosphate dehydrogenase activity has been demonstrated in heart failure. This study sought to assess myocardial glucose 6-phosphate dehydrogenase activity in retraining of the subpulmonary ventricle of adult goats.**

Methods: **Eighteen adult goats were divided into three groups: traditional (fixed banding), sham, and intermittent (adjustable banding, daily 12-hour systolic overload). Systolic overload (70% of systemic pressure) was maintained during a 4-week period. Right ventricle, pulmonary artery and aortic pressures were measured throughout the study. All animals were submitted to echocardiographic and hemodynamic evaluations throughout the protocol. After the study period, the animals were killed for morphological and glucose 6-phosphate dehydrogenase activity assessment.**

Results: **A 55.7% and 36.7% increase occurred in the intermittent and traditional right ventricle masses, respectively, when compared with the sham group ($P<0.05$), despite less exposure of intermittent group to systolic overload. No significant changes were observed in myocardial water content in the 3 groups ($P=0.27$). A 37.2% increase was found in right ventricle wall thickness of intermittent group, compared to sham and traditional groups ($P<0.05$). Right ventricle glucose 6-phosphate dehydrogenase activity was elevated in the traditional group, when compared to sham and intermittent groups ($P=0.05$).**

Conclusion: **Both study groups have developed similar right ventricle hypertrophy, regardless less systolic overload exposure of intermittent group. Traditional systolic overload for adult subpulmonary ventricle retraining causes upregulation of myocardial glucose 6-phosphate dehydrogenase activity. It may suggest that the undesirable "pathologic systolic overload" is influenced by activation of penthose pathway and cytosolic Nicotinamide adenine dinucleotide phosphate availability. This altered energy substrate metabolism can elevate levels of free**

[1] Heart Institute (InCor), University of São Paulo Medical School, São Paulo, SP, Brazil

[2] Medical School, Federal University of Juiz de Fora, Juiz de Fora, MG, Brazil

[3] University of Maastricht. Mastricht, Netherlands.

Work carried out at Heart Institute (InCor), University of São Paulo Medical School, São Paulo, SP, Brazil.

Work awarded at the 39th Congress of the Brazilian Society of Cardiovascular Surgery, Maceió, April 2012, and at the 67th Brazilian Congress of Cardiology, Recife, September 2012. Presented at the 46th Annual Meeting of the Association for European Paediatric and Congenital Cardiology, Istanbul, May 2012.

Funding: SILIMED, Rio de Janeiro, RJ (donation of adjustable banding devices and molecular biology kits), FAPESP (research grant nº 2006/50831)

Correspondence address:
Renato Samy Assad
Heart Institute (InCor), University of São Paulo Medical School,
Laboratory of Research in Cardiovascular Surgery (LIM-11) / Laboratory of Genetics and Molecular Cardiology
Av. Dr. Enéas de Carvalho Aguiar, 44 – Cerqueira César - São Paulo, SP
Brazil – Zip Code: 05403-000
E-mail: rsassad@cardiol.br

Article received on April 14th, 2013
Article approved on August 19th, 2013

Abbreviations, acronyms & symbols	
ANOVA	Analysis of variance
Ao	Aorta
CCTGA	Congenially corrected transposition of the great arteries
COBEA	Brazilian College of Animal Experimentation
DW	Dry weight
ECG	Electrocardiogram
G6PD	Glucose 6-phosphate dehydrogenase
IVS	Interventricular septum
IW	Initially weighed
LV	Left ventricle
NADPH	Nicotinamide adenine dinucleotide phosphate
PT	pulmonary trunk
PTB	Pulmonary trunk banding
RV	Right ventricle
TGA	Transposition of the great arteries

radicals by Nicotinamide adenine dinucleotide phosphate oxidase, an important mechanism in the pathophysiology of heart failure.

Descriptors: **Energy metabolism. Hypertrophy, right ventricular. Transposition of great vessels. Cardiac surgical procedures. Goats.**

Resumo

Objetivo: **O aumento da atividade miocárdica da Glicose 6-Fosfato Desidrogenase tem sido demonstrado na insuficiência cardíaca. Este estudo avalia a atividade miocárdica da Glicose 6-Fosfato Desidrogenase no treinamento do ventrículo subpulmonar de cabras adultas.**

Métodos: **Foram utilizadas 18 cabras adultas, divididas em três grupos: convencional (bandagem fixa), sham e intermitente (bandagem ajustável; 12 horas diárias de sobrecarga). A sobrecarga sistólica (70% da pressão sistêmica) foi mantida durante quatro semanas. As avaliações hemodinâmica e ecocardiográfica foram realizadas durante todo o estudo. Depois de cumprido o protocolo, os animais foram mortos para avaliação morfológica e da atividade da Glicose 6-Fosfato Desidrogenase dos ventrículos.**

Resultados: **Apesar de haver sobrecarga sistólica proporcionalmente menor no ventrículo subpulmonar do grupo intermitente (*P*=0,001), ambos os grupos de estudo apresentaram aumento da massa muscular de magnitude similar. Os grupos intermitente e convencional apresentaram aumento da massa de 55,7% e 36,7% (*P*<0,05), respectivamente, em comparação ao grupo sham. O conteúdo de água do miocárdio não variou entre os grupos estudados (*P*=0,27). O ecocardiograma demonstrou maior aumento (37,2%) na espessura do ventrículo subpulmonar do grupo intermitente, em relação aos grupos sham e convencional (*P*<0,05). Foi observada maior atividade da Glicose 6-Fosfato Desidrogenase na hipertrofia miocárdica do ventrículo subpulmonar do grupo convencional, comparada aos grupos sham e intermitente (*P*=0,05).**

Conclusão: **Ambos os grupos de treinamento ventricular desenvolveram hipertrofia ventricular, a despeito do menor tempo de sobrecarga sistólica no grupo intermitente. A maior atividade de Glicose 6-Fosfato Desidrogenase observada no grupo convencional pode refletir um desequilíbrio redox, com maior produção de fosfato de dinucleotídeo de nicotinamida e adenina e glutationa reduzida, um mecanismo importante da fisiopatologia da insuficiência cardíaca.**

Descritores: **Metabolismo energético. Hipertrofia ventricular direita. Transposição dos grandes vasos. Procedimentos cirúrgicos cardíacos. Cabras.**

INTRODUCTION

Both adult and adolescent patients diagnosed with congenially corrected transposition of the great arteries (CCTGA) as well as those with transposition of the great arteries (TGA) who underwent either Senning or Mustard surgery can develop right ventricle dysfunction (systemic) [1,2]. Subpulmonary ventricular retraining for anatomical correction, in those patients, still presents disappointing results. Hypertrophy induced by acute pressure overload can lead to foci of cellular necrosis in the hypertrophied myocardium and, consequently, to late ventricular dysfunction [3,4]. At present, it is imperative to understand the molecular mechanisms involved in hypertrophy of the mature myocardium induced by pulmonary trunk banding (PTB) in order to establish retraining protocols for a more "desirable" physiological hypertrophy.

In physiological conditions, fatty acids, especially the long chain ones, act as the main myocardial energy substrate [5]. Fatty acid oxidation in mitochondria represents approximately 70% of all ATP production in a normal heart [6,7]. On the other hand, several studies have shown that there is greater preference for glucose oxidation by cardiac substrates in heart failure and in a hypertrophic heart [8,9]. However, the change in energy substrate varies according to the etiology and severity of the ventricular dysfunction, where the degree of metabolic modulation plays an important role in determining adaptive or maladaptive function in terms

of states leading to pathological hypertrophy.

G6PD is the first and rate-limiting enzyme in the pentose phosphate pathway, which is an alternate and independent pathway from glycolysis, generating nicotinamide adenine dinucleotide phosphate (NADPH) and pentose (ribose-5-phosphate). It catalyzes the conversion of Glucose 6-Phosphate into 6-Phosphogluconolactone. The process is divided into two phases: generation of NADPH and non-oxidative synthesis of pentose. It maintains the level of the NADPH coenzyme, which in turn maintains the level of reduced glutathione, protecting the cells, in normal conditions, from oxidative lesions. Therefore, the more NADPH is needed, the greater the activity level of the G6PD enzyme.

There is evidence that greater G6PD activity leads to increased production of superoxide radicals as well as oxidative stress in diabetes, heart failure, and hypertension. Previous studies of the energy metabolism of acute myocardial hypertrophy in young animals have shown an increase in G6PD activity associated with ventricular dysfunction in continuous systolic overload, compared to intermittent overload [10].

In the subset of the subpulmonary ventricle retraining of adult patients for the double switch operation , it would be also interesting to analyze changes in energy metabolism. This study sets out to evaluate qualitative differences in the process of myocardial hypertrophy induced by continuous and intermittent pressure overload, through biological markers such as G6PD, which show occasional phenotypic changes to the energy metabolism.

METHODS

This study was approved by the Ethics Committee for the Analysis of Research Projects at – InCor University of Sao Paulo Medical School (process: SDC 2660/05/080) and carried out in accordance with the regulations on the use of animals in teaching and research of the Brazilian College of Animal Experimentation (COBEA). Eighteen adult goats with comparable weight (*P*=0.63) were used, divided into three groups: (1) Traditional group (n=6, weight=26.33 kg ± 2.32 kg, PTB with continuous systolic overload of the RV); (2) Sham or Positive Control group (n=6, weight=26.42 kg±2.63 kg, loose banding, no overload of the RV); and Intermittent group (n=6, weight=25.17 kg ± 2.48 kg, PTB with adjustable device and 12 hours/day of intermittent systolic overload of the RV).

Anesthesia

The animals were fasted for 24 hours before the surgery and received Xylazine 2%, 0.1 mg/kg IM, as preanesthetic medication. Anesthesia was induced with propofol 1% (7 mg/kg), intravenously (IV) for orotracheal intubation. The animals were maintained under mechanical ventilation (Takaoka Fuji Maximus, São Paulo, SP), with inspired oxygen fraction between 60 and 100% and Isoflurane inhalation (1 to 2%). The goats were placed in the right lateral decubitus and monitored with continuous electrocardiogram (ECG) and invasive arterial pressure (auricular artery). Postoperative pain relief was obtained in the first three days by administering Tramadol chlorhydrate (2 mg/kg, intramuscular).

Surgical Procedure

The goats were prepared for the sterile procedure, as described in previous studies [11,12]. For animals in the Traditional group, pulmonary trunk (PT) banding was performed with cardiac tape (Polysuture, São Sebastião do Paraíso, MG), positioned about 10 mm above the valve. Animals in the Sham and Intermittent groups had an adjustable banding device implanted immediately above the pulmonary valve and fixed to the PT adventitia. The insufflation button was implanted and fixed subcutaneously in the thorax. Hemodynamic monitoring catheters were exteriorized and maintained filled with heparin. The following antibiotics were administered prophylactically to all animals: cefazolin (30 mg/Kg), gentamicin (4 mg/Kg), and Benzathine Penicillin (500,000 IU), intramuscular. The Benzathine Penicillin dose was repeated after two weeks. In addition, Heparin Sodium 5,000 IU was administered daily by subcutaneous injection until the end of the protocol.

Description of the adjustable banding device

The adult model device was developed in collaboration with SILIMED (*Indústria de silicone e instrumental médico-cirúrgico e hospitalar Ltda.*, Rio de Janeiro, RJ), as previously published [11,12]. It has an outer diameter of 24 mm and is 6 mm wide. The inner surface has a distensible silicon layer, 0.6 to 0.8 mm thick, whose expansion compresses the lumen of the PT according to the amount of liquid injected in the insufflation button. This button is implanted subcutaneously so that fine adjustments to the diameter of the banding ring can be made percutaneously.

RV systolic overload protocol and pressure readings

Traditional Group

RV training started during the banding implant surgery. The animals remained under continuous systolic overload of the RV for a period of four weeks, with conventional fixed banding adjusted on the day of the surgery in order to reach an RV systolic pressure of about 70% of systemic systolic pressure, provided that there was no more than a 10% drop in systemic systolic pressure. Pressures of RV, PT, and aorta (Ao) were recorded twice a week with the animal conscious and immobilized on a special stretcher.

Sham Group

As in the Traditional group, hemodynamic measurements

were taken twice a week during the four weeks of the study and the banding device was kept deflated throughout the protocol.

Intermittent Group

The RV training started after approximately 60 hours of convalescence. As in the Traditional group, baseline pressures of RV, PT, and Ao were recorded with the animal conscious and the device completely deflated. Subsequently, the adjustable banding device was insufflated with 0.9% saline in order to reach the same RV systolic overload as the Traditional group. The pressures were then measured once again. The RV systolic overload was maintained for a period of 12 hours, after which arterial pressures were read one more time. After that, the device was deflated and pressures were measured again. The process of insufflating and deflating the device was performed daily for four weeks, with pressures being measured three times a week. On alternate days, the injected volume was the same as the volume calculated on the last day of hemodynamic measurements.

Echocardiographic study

All animals underwent echocardiographic evaluation by the same observer prior to the protocol and weekly after surgery. During the exams, the goats were sedated with Ketamine (10 mg/kg, intramuscular) and kept in the left lateral decubitus. The ACUSON Cypress (Siemens Healthcare, Mountain View, USA) echocardiography machine was used as well as the multi-frequency sector transducer (1.8-3.6 MHz) to capture images and analyze flow. The thicknesses of the interventricular septum (IVS) and left ventricle (LV) posterior wall were measured by two-dimensional echocardiogram at the end of diastole through longitudinal parasternal section at the mitral valve cusps [13]. The thickness of the RV free wall was obtained through transverse parasternal section (at the great vessels and at the papillary muscles level) and longitudinal four-chamber section in the region where its boundaries were more easily seen. Finally, the arithmetic mean of those values was obtained.

Weighing cardiac masses

At the end of the protocol, the animals were euthanized for resection of the heart. Anesthesia was deepened and heparin (5 mg/kg) was administered endovenously. Then, potassium chloride was administered until cardiac arrest. At that time, samples of the RV, LV, and interventricular septum were collected, weighed, and immediately placed in tagged plastic containers. The samples were stored in liquid nitrogen (-80ºC) until they could be transferred to the Genetics and Molecular Cardiology Laboratory, where they were kept in a special freezer (Forma Scientific -80º C). The heart was then removed from the thorax. The great vessels, atria, as well as the cardiac valves and epicardial fat were carefully resected. Ventricular and septal walls were separated so that cardiac masses could be weighed individually in a METTLER AE-200 (Mettler-Toledo AG, Greifensee, Switzerland) digital scale.

Tissue water content

After weighing the cardiac masses, samples were collected from each one for water content assessment. Every sample was initially weighed (IW), then wrapped in aluminum foil and properly identified before being placed in an incubator (temperature: 55-60º C). After about 70 hours of dehydration, every sample was weighed once again to determine dry weight (DW). The percentage of water content was then determined by the following formula, assuming the water distribution was homogenous in the septum and ventricles:

$WC\ (\%) = 100 - (DW \times IW^{-1} \times 100)$

Analysis of glucose 6-phosphate dehydrogenase (G6PD) maximum activity

G6PD maximum activity was determined in the septum and ventricle samples obtained. Samples were stored in liquid nitrogen and homogenized in extraction buffer (proportion 1:5 weight/volume). The material kept in ice was homogenized using Polytron (PT 3100, Kinematica AG, Littau-Lucerne, Switzerland), at maximum speed for 30 seconds. Cell remnants were separated by centrifugation (15,000 g, 15 minutes, 4ºC) using a Centrifuge 5417 C/R-Eppendorf (Hamburg, Germany). Enzymatic activity was analyzed using the supernatant of the last centrifugation.

Proteins were quantified using the BCATM protein assay kit (PIERCE Biotechnology, Rockford, IL, USA). Results were expressed as $nmol.min^{-1}.mg^{-1}$ of protein present in the extract. The extraction buffer for G6PD contained Tris-HCl (50 mM) and EDTA (1 mM), with a pH of 8.0. The assay buffer (270 mL/sample) was comprised of Tris-HCl (8.6 mM), $MgCl_2$ (6.9 mM), $NADP^+$ (0.4 mM), and Triton X-100 0.05% (volume/volume), with a pH of 7.6. The reaction was initiated by adding 15 mL of Glucose-6-phosphate (1.2 mM) to the enzymatic extract (15 mL sample) and it was monitored for 10 minutes at 25º C. Absorbance was monitored at 340 nm, the extinction coefficient being 6.22 for that particular wavelength.

The biochemical reaction is based on glucose phosphorylation and subsequent oxidation of glucose-6-phosphate to 6-phosphogluconate catalyzed by G6PD. G6PD activity levels were determined indirectly as the total NADPH produced in the pentose phosphate pathway.

Statistical Analysis

Means of the hemodynamic and echocardiographic variables were compared between groups and throughout the protocol by two-way analysis of variance (ANOVA) with repeated measures. Values for mass, water content, and G6PD maximum activity in the RV, LV, and IVS were compared

by one-way ANOVA. Both analyses were followed by the Bonferroni post hoc test. Systolic overload imposed on the RV, in the Traditional and Intermittent groups, was assessed by calculating the areas under the curve (trapezoidal method). The comparison between those areas was made through unpaired Student's t-test. Values were expressed as mean ± standard deviation (SD), unless otherwise indicated. The significance level was 5% for all cases. Statistical analyses were done using GraphPad Prism v.4 (San Diego, CA, USA) and SigmaStat v.3.11.0 (Systat Software, Inc., San Jose, CA, USA).

RESULTS

Hemodynamics

RV/PT pressure gradient

The Traditional group showed a decrease in gradient after the second postoperative day (from 45.00 mmHg ± 4.90 mmHg to 39.25 mmHg ± 13.05 mmHg), but remained stable until the end of the protocol (limits: 36.33 mmHg ± 4.04 mmHg to 40.00 mmHg ± 7.72 mmHg). During euthanasia, there was a significant decrease in the RV/PT gradient in the Traditional group ($P<0.05$), compared to the values of the fourth week. In the Sham group, as opposed to the others, pressure gradient (limits: 4.67 mmHg ± 2.08 mmHg to 9.40 mmHg ± 4.51 mmHg) was maintained throughout the protocol ($P<0.05$).

Peak pressure gradients were significantly higher in the Intermittent group (limits: 46.67 mmHg ± 6.80 mmHg to 59.00 mmHg ± 8.29 mmHg) ($P<0.05$), alternating with periods of "rest" of the RV. The RV/PT gradient remained elevated in both study groups compared to the Sham group ($P<0.001$). The Traditional group was subjected to a significantly larger area of systolic overload (23,764 mmHg.h ± 2,192 mmHg.h) than the Intermittent group (17,414 mmHg.h ± 1,144 mmHg.h; $P<0.0001$). Both Traditional and Intermittent groups had larger areas of systolic overload than the Sham group (3,841 mmHg.h ± 1,298 mmHg.h; $P<0.05$).

RV/Ao pressure ratio

Baseline RV/Ao pressure ratio of approximately 0.25 was similar in all groups. During surgery, RV/Ao ratio of 0.70 was reached in the stimulated groups. However, after the first week, the ratio decreased significantly in the Traditional group ($P<0.05$) and remained stable for the remainder of the study. In the Sham group, the baseline RV/Ao ratio remained stable throughout the protocol ($P<0.05$). In the Intermittent group, the maximum RV/Ao ratio was kept at around 0.7 throughout the study, as opposed to the Traditional group (Figure 1; $P<0.05$).

Echocardiographic findings

Thickness of the cardiac walls. There was significant percentage variation in the thickness of the RV free wall in the Intermittent group, starting in the first week of the study when compared to the Sham group ($P<0.001$), and in the second and fourth weeks of the study when compared to the Traditional group ($P<0.01$; Figure 2). There was no variation among the groups in the thicknesses of the interventricular septum and the LV posterior wall.

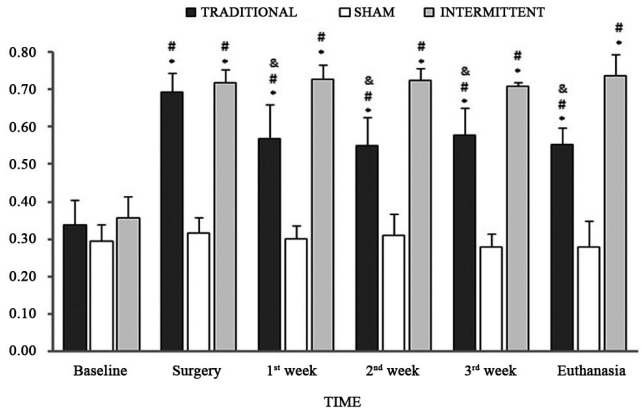


Fig. 1 – Temporal comparison of the RV/Ao maximum ratio in the Traditional, Sham, and Intermittent groups
**P<0.05 compared to Baseline instant in its respective group; # P<0.05 difference between the Traditional and Intermittent groups compared to the Sham group; & P<0.05 difference between the Traditional and Intermittent groups.*

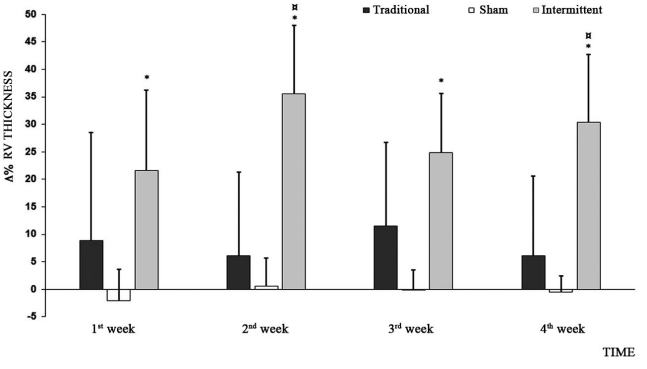


*Fig. 2 – Percentage variation of the thickness (delta) of the free wall of the right ventricle (RV), measured by echocardiography, in the Traditional, Sham, and Intermittent groups throughout the four weeks of study. *P<0.001 compared to Baseline in the Intermittent group and between the Intermittent and Sham groups.*

Morphological findings

Measurement of cardiac mass

Table 1 shows the weight of the ventricular chamber masses. The Intermittent and Traditional groups showed an increase in RV mass of 57.0% and 36%, respectively, compared to the Sham group ($P<0.05$). There was no significant variation in the weight of the IVS ($P=0.09$) and LV ($P=0.30$) masses among the groups.

Water content

There was no significant variation in water content of the RV myocardium in the Traditional (79.67% ± 1.25%), Sham (79.16 ± 1.28), and Intermittent (80.61 ± 1.87) groups ($P=0.27$).

Glucose 6-phosphate dehydrogenase (G6PD) maximum activity

Table 2 shows the mean of absolute values for G6PD maximum activity in the Traditional, Sham, and Intermittent groups. In the Traditional group, maximum activity levels of this enzyme in the RV were 55.2% higher than in the Sham group and 40.7% higher than in the Intermittent group ($P=0.05$). These data are graphically represented in Figure 3. No significant differences were found between the groups in G6PD maximum activity in the LV ($P=0.39$) and Septum ($P=0.31$).

DISCUSSION

This experimental study set out to compare right ventricular hypertrophy in adult goats subjected to intermittent versus traditional systolic overload, highlighting the energy metabolism in the ventricular retraining of mature myocardium. From a morphological standpoint, differences in hypertrophy in favor of the Traditional group were expected due to the higher exposure of the myocardium to hypertrophic stimuli, quantified by the larger area of systolic overload of the right ventricle.

Table 1. Weight of cardiac masses of the right ventricle (RV), interventricular septum (IVS), and left ventricle (LV), normalized by the weight of the animals in the Traditional, Sham, and Intermittent groups.

	Traditional N = 6	Sham N = 6	Intermittent N = 6	*P* Value
RV	1.08 ± 0.17 *	0.79 ± 0.15	1.24 ± 0.16 #	<0.05
IVS	0.96 ± 0.19	0.84 ± 0.20	1.09 ± 0.13	0.09
LV	1.52 ± 0.21	1.35 ± 0.22	1.47 ± 0.10	0.30

*Values= average in g/Kg ± standard deviation; * P<0.05 compared to the Sham group; # P<0.001 compared to the Sham group.*

Table 2. Maximum activity of the glucose-6-phosphate dehydrogenase (G6PD) enzyme in the Traditional, Sham, and Intermittent groups.

	Traditional	Sham	Intermittent	*P* Value
RV	2.11 ± 0.88	1.36 ± 0.14	1.50 ± 0.24*	0.05
LV	1.85 ± 0.22	1.80 ± 0.17	1.71 ± 0.16	0.39
Septum	0.96 ± 0.28*	0.86 ± 0.25	1.13 ± 0.32*	0.31

*Values = average ± standard deviation; Measures: nmol/min/mg of protein; * n = 5*

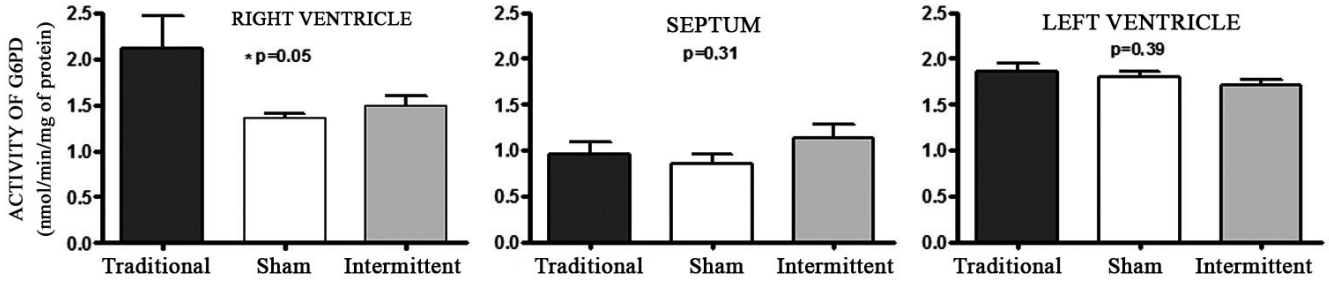


Fig. 3. – *Maximum activity of the Glucose-6-phosphate Dehydrogenase (G6PD) enzyme in the myocardium of the Traditional, Sham, and Intermittent groups. Measures: nmol/min/mg of protein ± standard error. n= 6*

Even though both ventricular retraining groups were able to promote ventricular hypertrophy of similar magnitude compared to the Sham group, the echocardiographic findings of the Intermittent group showed increased RV free wall thickness. However, the length of time of the hypertrophic process was greater than that found in in young animals submitted to just 96-hour intermittent systolic overload protocol.

Likewise, morphological and/or echocardiographic analyses revealed no changes in septal thickness, which also diverges from previous studies in young animals that showed significant increase in the septal mass [10]. Perhaps this deviation can be explained by a more efficient protein production in young as opposed to mature myocardium. All three groups showed no variation in myocardium water content, a sign of cellular edema, suggesting the gain in mass was mainly due to enhanced protein synthesis.

Previous studies from our laboratory have demonstrated impaired functional and morphological RV performance of adult goats submitted to Traditional systolic overload under the same protocol. [11,12]. Likewise, there was a concordance of impaired RV function and increased RV G6PD activity of Traditional group after 4-week study period, corroborating the findings of the 96-hour systolic overload in young animals [10]. Increased G6PD activity indicates an exacerbation of the pentose phosphate pathway and it can mean loss of redox balance, with higher production of NADPH and reduced glutathione, as well as development of oxidative stress derived from superoxide anions associated with NADPH oxidase.

Under pathological conditions, NADPH is produced by activation of G6PD after stimuli from a range of factors, such as angiotensin II, thrombin, and alpha tumor necrosis factor [14,15]. Cardiomyopathy related to protein aggregation and myocardial lesion would be the final consequence. There is growing evidence that increased G6PD activity is associated with oxidative and reductive stress, with new drugs being developed in order to inhibit its activity [16]. For instance, patients with diabetes mellitus show increased G6PD activity and NADPH levels. This metabolic disorder is associated with endothelial dysfunction due to the inhibition of nitric oxide synthesis [17].

Although the mechanisms through which most of the free radicals are produced in the heart are not completely known, it has been suggested that higher glucose oxidation increases the potential of the mitochondrial membrane thereby augmenting NADPH oxidase activity in the vascular system and, consequently, increasing the production of superoxide anions [18,19]. The latter would act as mediators of diabetic vasculopathy and precursors of myocardial dysfunction related to the disease [20,21]. There is a 10-fold increase in G6PD activity in pacing-induced heart failure compared to normal hearts [22].

This study did not directly assess the production of free radicals associated with NADPH oxidase; however, it can be speculated that the overexpression of G6PD observed in the RV of the Traditional group indicates that the Pentose pathway increases the availability of NADPH, providing the release of free radicals via NADPH oxidase and nitric oxide synthase. Thus, this could lead to myocardial lesion caused by accumulation of superoxide anions and protein aggregation and, subsequently, to ventricular dysfunction should the overload continue. This is corroborated by the fact that functional recovery of hearts with compensated hypertrophy is significantly higher than that of non-hypertrophic hearts when myocardial perfusion is intermittently reestablished, a maneuver which prevents or minimizes the accumulation of glycolytic products and H^+ ions. Indeed, reestablishment of this subendocardial coronary flow during the resting periods of the Intermittent group would avoid the accumulation of the products of glycolysis and H^+ ions.

Besides, fatty acid oxidation is directly stimulated during the reestablishment of subendocardial reperfusion in the resting periods from systolic overload as a result of changes in the enzymes and metabolites responsible for the regulation of fatty acid oxidation. Predominance of fatty acid oxidation during periods of rest of the RV could lead to decreased myocardial glucose uptake. On the other hand, increased efficiency of intermittent systolic overload could be related to the release of hypertrophic stimuli and the cascade of protein synthesis, just as in the Traditional group, yet with decreased myocardial energy expenditure. The mechanisms of the hypertrophic process triggered by molecular cascade are likely to happen under good conditions during the 12-hour resting period and ideal oxygen delivery. Therefore, the type of retraining, in terms of level and duration of the systolic overload as well as its impact on the myocardium, must be considered. Even though the increase in G6PD is an unspecified pathway for production of free radicals, this study has found an agreement between previously shown right ventricular dysfunction in the Traditional group and the increase in G6PD activity, a situation where there is inadequate oxygen delivery due to continuous systolic overload, likely high consumption of ATP, and, hence, larger production of free radicals.

Limitations of the study

There are limitations to implementing the results of this study in human beings. Firstly, metabolism and morphological aspects can differ among species. Secondly, the great arteries were normally related in the animals; hence, the ventricle studied (anatomically right) is not the same as the target population in humans (anatomically left).

Finally, it is difficult to make meaningful inferences about a hypertrophic process involving several factors based solely on the activity of a particular enzyme. To this

end, it would be ideal to also analyze the production of free radicals and/or markers of myocardial lesion since there are several influences at play from other metabolic pathways. This rational suggests that future studies of the production of oxidized glutathione, free radicals, and apoptosis may provide important information to this research line.

CONCLUSION

This study has shown that ventricular retraining in both groups of adult goats led to right ventricular hypertrophy without myocardial edema. Continuous systolic overload promoted increased G6PD activity in the RV myocardium. This enzymatic hyperactivity may be related to increased production of free radicals caused by greater demand for constant myocardial overload stimulus. Conversely, intermittent systolic overload enabled a more efficient RV hypertrophy, considering the smaller area of systolic overload of the RV and decreased G6PD activity.

ACKNOWLEDGEMENTS

The authors are grateful to SILIMED, Rio de Janeiro, RJ, for the donation of the adjustable banding devices and to FAPESP, for the research grant nº 2006/50831.

Authors' roles & responsibilities	
RSA	Design, supervision, and execution of the project; statistical analysis; writing and review of the draft
LAM	Execution of the project; surgical procedures; data collection; and writing of the draft
MHFA	Processing of samples and energy metabolism analysis
MCDA	Echocardiographic exams; writing and review of the draft
GJJS	Statistical analysis and writing of the draft
FSO	Assistance in surgical procedures
LFPM	Supervision; statistical analysis; and review of the draft
JEK	Design; energy metabolism analysis; supervision; and review of the draft

REFERENCES

1. Cochrane AD, Karl TR, Mee RB. Staged conversion to arterial switch for late failure of the systemic right ventricle. Ann Thorac Surg. 1993;56(4):854-61.

2. Mee RB. Severe right ventricular failure after Mustard or Senning operation. Two stage repair: pulmonary artery banding and switch. J Thorac Cardiovasc Surg. 1986;92(3 Pt 1):385-90.

3. Siehl DL, Gordon EE, Kira Y, Chua BHL, Morgan HE. Protein degradation in the hypertrophic heart. In: Glaumann H, Ballard FJ, eds. Lysosomes: their role in protein breakdown. London: Academic; 1987.

4. Takahashi Y, Nakano S, Shimazaki Y, Kadoba K, Taniguchi K, Sano T, et al. Echocardiographic comparison of postoperative left ventricular contractile state between one- and two-stage arterial switch operation for simple transposition of the great arteries. Circulation. 1991;84(5 Suppl):III180-6.

5. Van der Vusse GJ, Glatz JF, Stam HC, Reneman RS. Fatty acid homeostasis in the normoxic and ischemic heart. Physiol Rev. 1992;72(4):881-940.

6. Taegtmeyer H. Energy metabolism of the heart: from basic concepts to clinical applications. Curr Probl Cardiol. 1994;19(2):59-113.

7. Grynberg A, Demaison L. Fatty acid oxidation in the heart. J Cardiovasc Pharmacol. 1996;28(Suppl 1):S11-7.

8. Huss JM, Kelly DP. Mitochondrial energy metabolism in heart failure: a question of balance. J Clin Invest. 2005;115(3):547-55.

9. Sambandam N, Lopaschuk GD. Brownsey RW, Allard MF. Energy metabolism in the hypertrophied heart. Heart Fail Rev. 2002;7(2):161-73.

10. Assad RA, Atik FA, Oliveira FS, Fonseca-Alaniz MH, Abduch MC, Silva GJ, et al. Reversible pulmonary trunk banding. VI: Glucose-6-phosphate dehydrogenase activity in rapid ventricular hypertrophy in young goats. J Thorac Cardiovasc Surg. 2011;142(5):1108-13.

11. Miana LA, Assad RS, Abduch MC, Gomes GS, Nogueira AR, Oliveira FS, et al. Sobrecarga sistólica intermitente promove melhor desempenho miocárdico em animais adultos. Arq Bras Cardiol. 2010;95(3):364-72.

12. Miana LA, Assad RS, Abduch MC, Silva GJ, Nogueira AR, Aiello VD, et al. Reversible pulmonary trunk banding VIII: Intermittent overload causes harmless hypertrophy in adult goat. Ann Thorac Surg. 2013;95(4):1422-8.

13. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, et al; Chamber Quantification Writing Group; American Society of Echocardiography's Guidelines and Standards Committee; European Association of Echocardiography. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr. 2005;18(12):1440-63.

14. Matsui R, Xu S, Maitland KA, Hayes A, Leopold JA, Handy DE, et al. Glucose-6 phosphate dehydrogenase deficiency decreases the vascular response to angiotensin II. Circulation. 2005;112(2):257-63.

15. Li JM, Mullen AM, Yun S, Wientjes F, Brouns GY, Thrasher AJ, et al. Essential role of the NADPH oxidase subunit p47(phox) in endothelial cell superoxide production in response to phorbol ester and tumor necrosis factor-alpha. Circ Res. 2002;90(2):143-50.

16. Gupte SA. Glucose-6-phosphate dehydrogenase: a novel therapeutic target in cardiovascular diseases. Curr Opin Investig Drugs. 2008;9(9):993-1000.

17. Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, et al. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxidase synthase. Circulation. 2002; 105(14):1656-62.

18. An D, Rodrigues B. Role of changes in cardiac metabolism in development of diabetic cardiomyopathy. Am J Physiol Heart Circ Physiol. 2006;291(4):H1489-506.

19. Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, et al. Mechanisms underlying endothelial dysfunction in diabetes mellitus. Circ Res. 2001;88(2):E14-22.

20. Park J, Rho HK, Kim KH, Choe SS, Lee YS, Kim JB. Overexpression of glucose-6-phosphate dehydrogenase is associated with lipid dysregulation and insulin resistance in obesity. Moll Cell Biol. 2005;25(12):5146-57.

21. Serpillon S, Floyd BC, Gupte RS, George S, Kozicky M, Neito V, et al. Superoxide production by NAD(P)H oxidase and mitochondria is increased in genetically obese and hyperglycemic rat heart and aorta before the development of cardiac dysfunction. The role of glucose-6-phosphate dehydrogenase-derived NADPH. Am J Physiol Heart Circ Physiol. 2009;297(1):H153-62.

22. Recchia FA, McConnell PI, Bernstein RD, Vogel TR, Xu X, Hintze TH. Reduced nitric oxide production and altered myocardial metabolism during the decompensation of pacing-induced heart failure in the conscious dog. Circ Res. 1998;83(10):969-79.

Rev Bras Cir Cardiovasc 2008; 23(1): 60-69

IV Pulmonary trunk reversible banding: Analysis of right ventricle acute hypertrophy in an intermittent loading experimental model

Bandagem reversível do tronco pulmonar IV: análise da hipertrofia aguda do ventrículo direito em modelo experimental de sobrecarga intermitente

Acrisio Sales VALENTE[1], Renato Samy ASSAD[2], Maria Cristina D. ABDUCH[3], Gustavo J. J. SILVA[4], Petronio G. THOMAZ[5], Leonardo A. MIANA[6], José E. KRIEGER[7], Miguel BARBERO-MARCIAL[8]

RBCCV 44205-951

Abstract

Objectives: **Adjustable pulmonary trunk (PT) banding device may induce a more physiological ventricle retraining for the two-stage Jatene procedure. This experimental study evaluates theacute hypertrophy (96 hours) of the right ventricle (RV) submitted to intermittent systolic overload.**

Methods: **Five groups of seven young goats were distributed according to RV intermittent systolic overload duration (0, 24, 48, 72 and 96 hours). The zero-hour group served as a control group. Echocardiographic and hemodynamic evaluations were performed daily. After completing the training program of each group, the animals were sacrificed for water content and cardiac mass evaluation.**

Results: **There was a significant increase in RV free wall thickness starting with the 48-hour group ($p<0.05$). However, a decreased RV ejection fraction, associated with an important RV dilation and a significant increase in the RV volume-to-mass ratio was observed at 24-hour training period, when compared to 96-hour period ($p=0.003$), with subsequent recovery throughout the protocol. A 104.7 % increase in RV mass was observed in the 96-hour group, as compared to the control group, with no differences in water content between these two groups. The daily mean increase in RV mass during the study period was 21.6% ± 26.8 %. The rate of RV mass acquisition for the overall study period of intermittent systolic overload was 0.084 g/h ± 0.035 g/h.**

Conclusion: **Intermittent PT banding has allowed a significant RV mass acquisition in the 96-hour trained group. No myocardial water content changes were observed in this group, suggesting an increased myocardial protein synthesis.**

Descriptors: **Heart ventricles/physiopathology. Hypertrophy/physiopathology. Right ventricular hypertrophy. Transposition of great vessels/surgery. Cardiac surgical procedures/methods. Goats.**

1. PhD, cardiovascular surgeon
2. Full professor, cardiovascular surgeon
3. PhD, veterinary doctor
4. PhD, post-doctorate student
5. PhD, cardiovascular surgeon
6. PhD Candidate, cardiovascular surgeon
7. Director of Genetics and Mollecular Cardiology Laboratory – InCor – HCFMUSP
8. Titular Professor of Cardiovascular Surgery of University of São Paulo Medical School

This study was carried out at the Instituto do Coração HCFMUSP, São Paulo, SP

Address for correspondence:
Acrisio Sales Valente. Rua Alberto Feitosa Lima, 180/602 - Fortaleza, CE – CEP 60810-018 Phone: (85) 32410990/(85) 99880444.
E-mail address: acrisiovalente@yahoo.com

Support: SILIMED Silicone e Instrumental Médico-Cirúrgico e Hospitalar Ltda., Rio de Janeiro, RJ.

Article received on September 5th, 2007
Article accepted on October 10th, 2007

Resumo

Objetivo: **A bandagem ajustável do tronco pulmonar (TP) pode proporcionar treinamento ventricular mais fisiológico para cirurgia de Jatene em dois estágios. Este estudo experimental analisa a hipertrofia aguda (96 horas) do ventrículo direito (VD) submetido à sobrecarga sistólica intermitente.**

Método: **Cinco grupos de sete cabritos jovens foram dispostos conforme o tempo de sobrecarga sistólica do VD (0, 24, 48, 72 e 96 horas). O grupo zero hora funcionou como grupo controle. Avaliações ecocardiográficas e hemodinâmicas foram feitas diariamente. Os animais foram sacrificados para avaliação do conteúdo de água e pesagem das massas cardíacas.**

Resultados: **Houve aumento da espessura do VD a partir de 48 horas de treinamento (p<0,05) e rebaixamento da fração de ejeção do VD, com dilatação importante desta câmara nas primeiras 24 horas do protocolo, recuperando-se posteriormente. Houve aumento da relação volume/massa nas primeiras 24 horas do protocolo, em relação ao momento 96 horas (p=0,003). A massa do VD apresentou aumento de 104,7% no grupo 96 horas em relação ao controle. Não houve diferença quanto ao conteúdo de água do VD. A média diária de aumento da massa do VD foi de 21,6% ± 26,8%. A taxa de ganho de massa muscular do VD para todo o período de estudo foi de 0,084 g/h ± 0,035 g/h.**

Conclusão: **O protocolo de bandagem intermitente do TP permitiu ganho de massa muscular do VD, significativa no grupo de 96 horas de estudo. Esta hipertrofia não foi acompanhada de aumento no conteúdo de água, o que sugere maior síntese protéica nos tecidos cardíacos.**

Descritores: **Ventrículos cardíacos/fisiopatologia. Hipertrofia/fisiopatologia. Hipertrofia ventricular direita. Transposição dos grandes vasos/cirurgia. Procedimentos cirúrgicos cardíacos/métodos. Cabras.**

INTRODUCTION

The establishment of the Jatene procedure as the best option for treatment of the transposition of large arteries (TGA) [1], as well as the well-studied concept about the right ventricle (RV)'s inability to maintain appropriate performance as a long-term systemic ventricle in atrial inversion operations or in congenitally corrected transposition of large arteries (CTTGA) [2,3], lead us to the following alternative: training of the hypotrophied left ventricle (LV), aiming to surgically recruit it for systemic circulation.

In 1977, before the Jatene procedure, Yacoub et. al. [4] described, for the first time, the idea of performing a ventricular preparation by banding the pulmonary trunk (PT) to a pulmonary-systemic shunt, aiming for LV hypertrophy as the first stage of the surgery, with a subsequent Jatene procedure months later. However, high rates of mortality connected to the procedure were described [5].

In 1989, Jonas et. al. [6] introduced a concept of rapid LV preparation by banding it to the pulmonary trunk for TGA correction in two stages, showing appropriate hypertrophy of this chamber in a mean period of nine days. However, the long-term evolution also showed ventricular dysfunction and dilatation in approximately 25% of the evaluated patients (aspects that were not observed in patients who were operated early without need for previous ventricular preparation) [7]. Other studies also showed that acute pressure overload can lead to cellular necrosis focus in the hypertrophied myocardium and consequently late ventricular dysfunction [8-11].

The best option for subpulmonary ventricle training for congenital cardiopathies with ventriculoarterial discordance still remains undefined. Intermittent overload periods with adjustable banding devices seem to be related to healthier hypertrophic processes [12,13]. However, a detailed analysis of the increase in subpulmonary ventricle mass that has undergone intermittent systolic overload has not yet been established. Thus, the aim of this study is to analyze the RV acute hyperthophy process in young goats that underwent intermittent systolic overload, in terms of hemodynamic, echocardiographic and morphological aspects at 24, 48, 72 and 96 hours.

METHODS

This study was carried out in the Experimental Division of the Heart Institute of Hospital das Clínicas, University of São Paulo Medical School, meeting the norms of Animal Experimentation in Teaching and Research of Animal Research Inspection Commission – COFIPA.

Four study groups with seven young goats each (between 30 and 60 days of age) were used for induction of RV systolic overload using an experimental model of reversible PT banding [14-16]. Each group underwent twelve hours of systematic stimuli of RV systolic overload, followed by 12 hours of resting divided in training groups of 24 hours (Group 24, weight: 9.64 Kg ± 0.95 Kg), 48 hours (Group 48,weight: 9.64 Kg ± 0.95 Kg), 72 hours (Group 72, weight: 8.76 Kg ± 1.17 Kg) and 96 (Group 96, weight: 8.13 Kg ± 0.75 Kg). A control group (group C – zero hours, weight: 7.65 Kg ± 1.88 Kg), also with seven animals with similar weight and age, was used to compare the cardiac mass and water volume of these animals to those from the study groups.

Preoperative evaluation

All animals underwent echocardiography exams and were previously examined by veterinarians to eliminate any animals with preexisting diseases. Animals with preoperative RV-PT gradients higher than or equal to 10mmHg were not selected.

Surgical Procedure

The animals were operated under general anesthesia and mechanical ventilation. They also were prepared for aseptic surgery through left lateral thoracothomy in 4th intercostal space After the opening of pericardial flap and exposition of outlet right ventricle, PT and descending thoracic aorta, previously heparinized catheters were implanted in these structures. The catheters were fixed with 5-0 polypropylene purse-string sutures and exteriorized and fixed to the thoracic skin wall. The pressures were measured using ACQ Knowledge software v.3.01 (Biopac Systems, Inc., Goleta, CA, USA).

Pulmonary Trunk Banding device

After dissection between the PT and the ascending aorta, the banding device is positioned in the PT immediately above the pulmonary valve, and is fixed to the PT adventitia in order to avoid its migration. The banding device used in this study represents the development and improvement of 15 years of this research line, working together with SILIMED (Rio de Janeiro, RJ). The proposed improvements resulted in the development of a completely hermetic adjustable banding device, which is thinner and more delicate, to be used for the treatment of several cardiopathies (Figure 1). After device implantation, thoracic water-sealed drainage was performed. The ribs were approximated and a layered suture of the soft parts was performed.

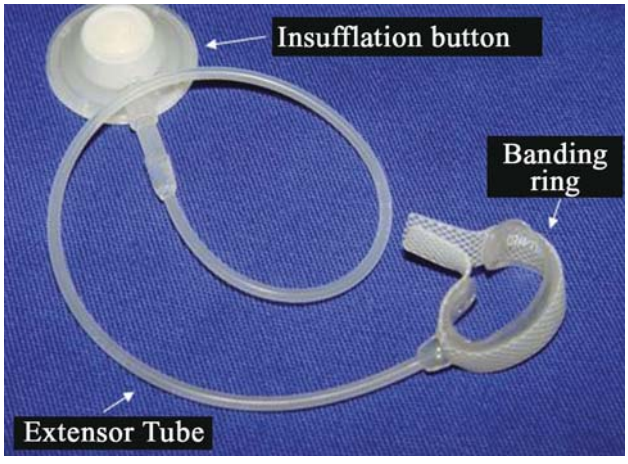


Fig. 1 – PT adjustable banding device used in the protocol, manufactured by SILIMED (Rio de Janeiro)

A preoperative antibiotic prophilaxy was performed in the animals and was spread out over the time of their stay in an animal care facility until the end of the study protocol of each group.

Device insufflation protocol

After 48 hours of total operation recovery, we initiated the ring intermittent inflation protocol (inflated in 12-hour blocks and then alternated with 12 hours of resting) for induction of RV progressive systolic stress, for 24 hours in the Group 24, for 48 hours in Group 48, and so on for all groups. The animals in Group 24 received the inflation only once in 12 hours. Before and after each stimulus, and at 12-hour intervals, and with the conscious animal immobilized on a special stretcher, the RV, PT and aortic basal pressures were measured. The ring inflation was performed with saline solution in an attempt to understand the pressure relationship of approximately 70% between the RV and the aorta, inasmuch as this did not drop the systemic systolic pressure by more than 10%, according to what was previously described in other studies [17-19].

In the case of any signs of instability of the animal, the banding volume would have been reduced to a tolerable level. The animals remained with RV systolic overload during the determined periods for each group, with progressive inflations at the maximum tolerated limit.

Echocardiographic study

All animals were submitted to echocardiographic evaluation by the same specialist (MCDA), awake and in esternal decubitus position in order to decrease interobserver variability. The examination was performed before the start of the protocol, as also after each inflation period in order to evaluate the RV hypertrophy process of all stimulated groups, as well as to record heart function in terms of RV ejection fraction (RVEF) and right ventricular diastolic volume [20]. The animals of the control group were also evaluated once before being euthanized.

Weighing of heart mass

After the closure of the protocol of each group, the animals were euthanized under general anesthesia, heparinization and induction of hyperpotassemia for resection of the heart. The epicardial fat was carefully resected and the ventricular and septal walls were separated following the Fulton et. al. technique [21]. Next, the RV, LV and interventricular septum were weighed in a digital balance (METTLER AE-200, Mettler-Toledo AG, Greifensee, Switzerland). Due to the variation in the weight of the animals, the measurements were standardized by indexing the weight of the heart muscle mass to the respective body weights of the animals, following the suggestions of Bishop & Cole [22]. The indexed weights were expressed in g/kg.

The animals of the control group also underwent the same protocol of ressection and weighing of the hearts.

Water volume of the tissues

Samples of each of the cardiac walls were harvested for water volume evaluation. Each sample was correctly identified and weighed. They were stored in appropriate containers and placed in an incubator (temperature of 55º-60ºC) for about 70 hours for dehydration. After this period, the dry weight of each sample was obtained. The percentage of water volume was then obtained and compared to that of the control group in order to clarify whether the increase of RV weight of the study groups should be associated with myocardial edema or with the gain of muscle mass.

Statistical analysis

The normal distribution of each variable was evaluated using the Kolmogorov-Smirnov test. Comparisons of hemodynamic, echocardiographic, morphological and morphofunctional variables were made through the analysis of variance (ANOVA) of one factor, with the respective times of 0,12, 24, 36, 48, 60, 72, 84 e 96 hours). Analysis of variance reaching the established significance level was performed by Bonferroni's test for multiple comparisons. Values are presented as means ± standard deviation. For all cases, the level of significance used was 5%. Statistical analyses were carried out by the softwares Prism v.4 (San Diego, CA, USA) and ESTATISTICA v.6 (Tulsa, OK , USA).

RESULTS

All animals completed the protocols of their specific group. Migration or rupture of the adjustable banding device was not observed in any of them. There were no observed statistical differences between the animals' body weights in the different groups (p=0,65).

Hemodynamic measurements

The animals showed progressively larger RV-PT gradients along the protocol parallel during the process of hypertrophy/ventricular adaptation (Figure 2). From an initial value of RV/PT pressure gradient of 36.6 mmHg ± 9.3 mmHg, the ventricles reached values of 80.0 mmHg ± 13.0 mmHg at the end of 96 hours of intermittent overload.

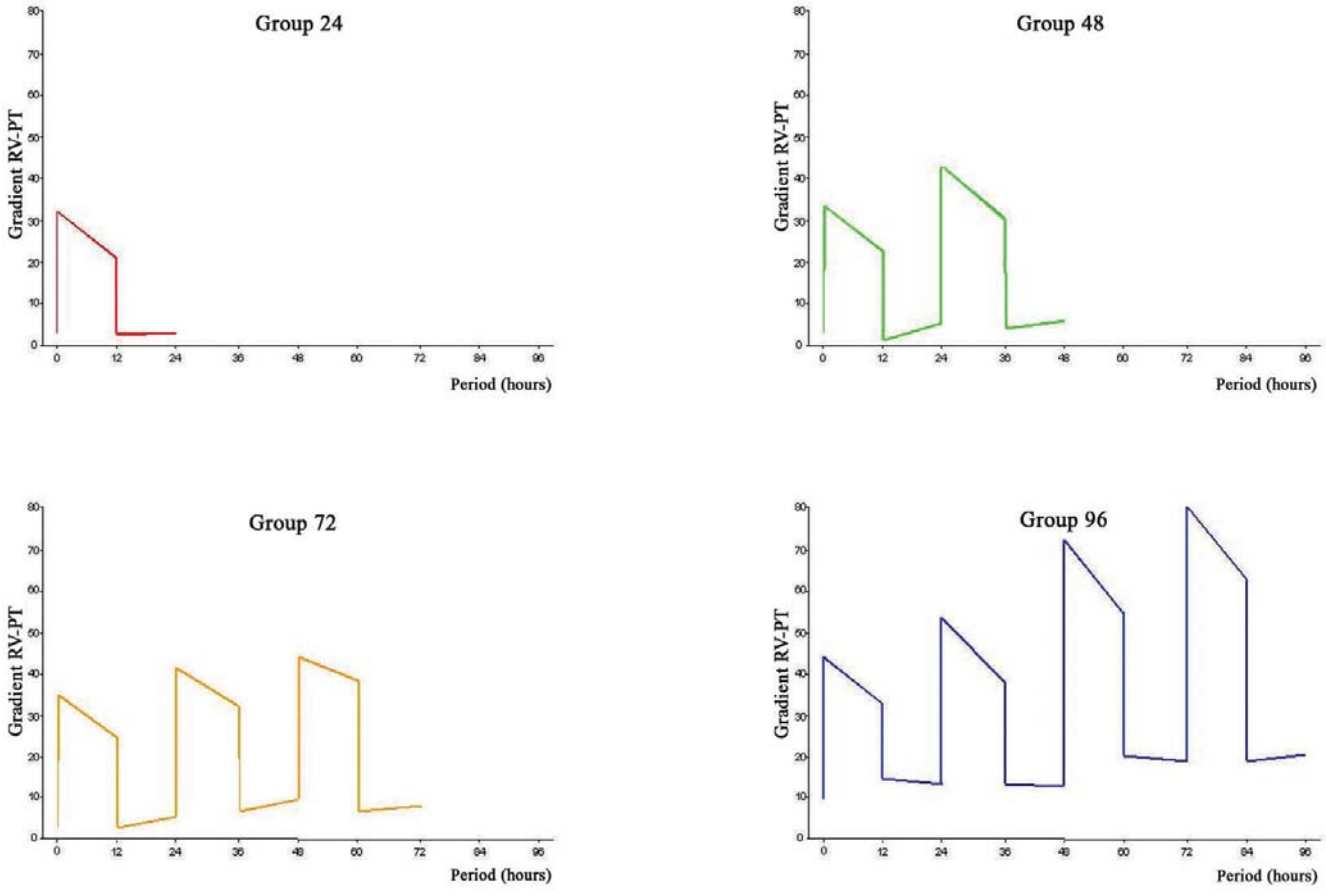


Fig. 2 – Demonstrative diagram of the intermittence of RV systolic overload in the four study groups

The pressure ratio of the animals RV/LV are shown in Figure 3. The ratios were significantly increased during all periods of RV systolic overload compared to the values found in base levels (p=0.0001). Paradoxically, the means of the periods of 60, 84 and 96 hours – periods in which the banding devices were deflated – were also significantly increased compared to the base values (p=0.05).

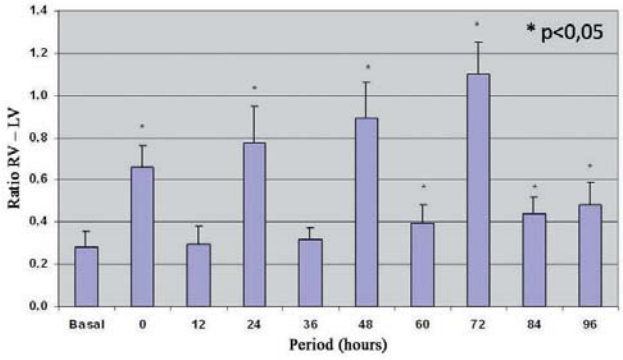


*Fig. 3 – RV/LV pressure ratio in the four study groups. Note the ratio intermittence in each period of 12 hours. Values = mean ± standard deviation. *p<0.05 vs. base values*

Group 24 in relation to the base period (p=0.0001). After 48 hours of overload, there was recovery of this parameter, reaching, at the end of the protocol, the value of 81.9% ± 30.4% of the RV end-diastolic base volume.

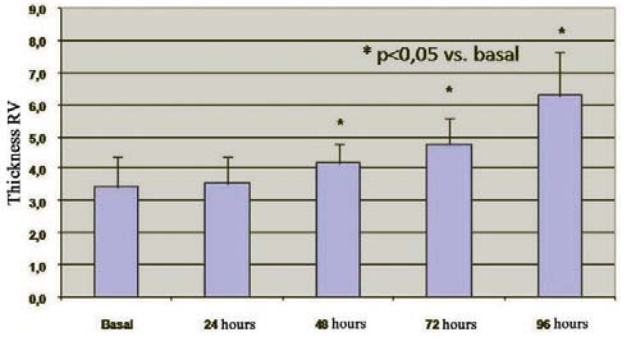


*Fig. 4 – The thickness of the RV wall presented significant increase from the period of 48 hours through the intermittent systolic overload protocol imposed upon the RV in the four study groups. n = 28 (base value and 24 hours); n = 21 (48 hours); n = 14 (72 hours); n = 7 (96 hours). Values (mm) = mean ± standard deviation. * = p<0.5 vs. base values*

Echocardiographic measurements

Neither the LV wall nor the ventricular septum presented alterations in thickness that were visible by echocardiogram during the protocol of RV intermitent systolic overload in four study groups. Figure 4 shows combined analysis of the animals' RV wall thickness during the protocol of intermittent systolic overload. Significant increase of this parameter was observed (48 hours after the initiation of intermittent overload) exceeding the values of septal and of LV thickness in the 96 hours of training (p<0.05).

The acute overload induction, despite of established resting periods, resulted in significative decrease of the animals RVEF, 24 hours after initiation of the intermittent systolic overload in relation to the base period (p=0.0001; from 0.68 ± 0.08 to 0.52 ± 0.21) with its significant recovery in the following periods (48, 72 and 96 hours).

Concomitantly to RVEF decrease, an increase in the RV final diastolic volume of 229.5% ± 133.4% was observed in

Morphological measurements

The ventricular septum and the RV presented progressive increase of the weighed mass, as shown in Figure 5. The RV mass presented significant increase in Group 96 (1.76 g/kg ± 0.52 g/kg) in relation to the following groups: the control group, Group 24, Group 48, and Group 72 (p=0.0001). The ventricular septum mass also presented significant increase in Group 96 (1.44 g/kg ± 0.25 g/kg), when compared to the other groups (p.0.01). The LV mass did not present a significant increase during the protocol (p=0.14).

Daily mean of RV mass increase was 21.6% ± 26.8%, with a 104.7% mass gain in Group 96 compared to the control group. The rate of RV muscle mass increase in all periods of intermittent systolic overload was 0.084 g/h ± 0.035 g/h. Figure 6 shows the daily gain of RV muscle mass, indexed by body weight. The highest gain of ventricular mass was seen in Group 96.

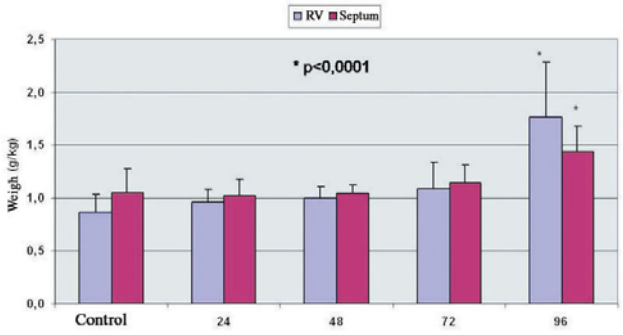


Fig. 5 – Septum and RV masses (indexed to body weight) evaluated during the intermittent systolic overload protocol, showing significant increase of both masses at the group 96 (p<0.0001). n=7

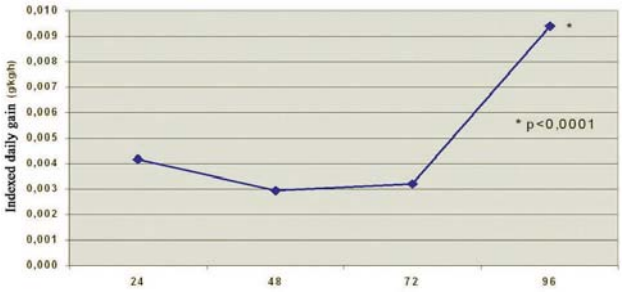


*Fig. 6 – Evolution of the daily muscle mass gain of the RV (indexed to body weight) during the intermittent systolic overload protocol, showing significant increase at the group 96. (p<0.0001) n=7. Values (g/kg/h) = mean ± Standard deviation.*p<0.0001*

Concerning the water volume of the RV, LF and septum muscle masses, increases in the RV (p=0.014) and septum (p=0.003) in Group 72 were found; though slight, the increases were significant when compared to the control group and Group 24. The RV and ventricular septum masses of Group 96 – which presented significative weight gain – did not present significative variation in water volume in relation to the control group.

Morphofunctional measurements

Figure 7 shows the RV's volume/mass relationship during the intermittent systolic overload protocol. Significant increase in the first 48 hours of protocol was observed in relation to Group 96 (p=0.006); Group 96 tended to have a lower volume/mass ratio when compared to the control group.

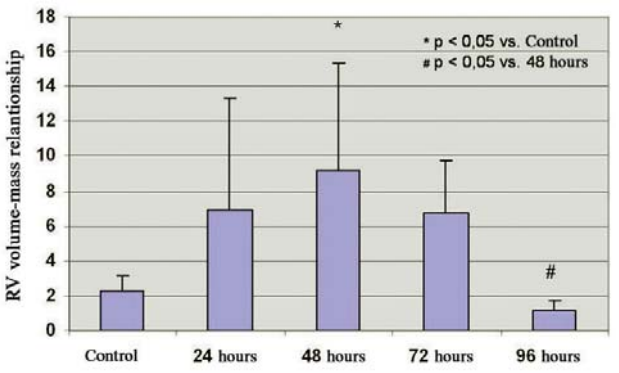


*Fig. 7 – Evolution of the volume/mass relationship of the RV during the intermittent systolic overload protocol, showing significant increase at the 48-hour mark. N = 7. Values (ml/g/kg) = mean ± Standard deviation * p < 0.05 vs. control group; # p < 0.05 vs. 48 hours*

The pressure generated on the myocardial wall is directly proportional to the result of the RV intracavitary pressure multiplied by volume/mass ratio of the RV [23]. Figure 8 shows the graphic elucidation of the RV's wall pressure during the systolic overload period (inflated cuff); it was significantly increased in Group 48 compared to Group 24 (p=0.003). From then on, keeping consistent with ventricular adaptation, this pressure presented a progressive decrease, reaching final values that were less than base values.

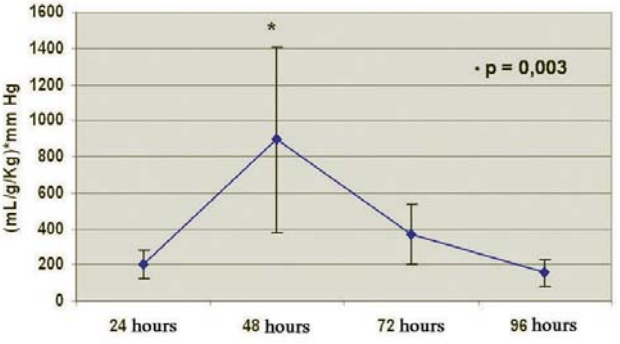


*Fig. 8 – Image of the RV pressure wall during the protocol of intermittent systolic overload. Values (mmHg.ml.g^{-1}. Kg^{-1}) = mean ± Standard deviation; * p=0.003*

DISCUSSION

This study was performed to establish, with the highest accracy possible, the daily gain patterns of a ventricular

mass submitted to intermittent systolic overload and the degree of its hypertrophy by direct weighing of the myocardial muscle mass in groups with different periods of RV intermittent overload. During 96 hours of RV intermittent systolic overload, a significant increase in mass was observed at the end of protocol, reaching more than two times the gain of the control group (an increase of 104.7%). This fact indicates that the RV has rapid hypertrophic capacity in only four periods of 12 hours of systolic overload stimulus per day. The weighing of cardiac masses consistently shows the development of the hypertrophic process when compared to the echocardiographic estimation used in studies, such as that of Boutin et. al. [23].

Boutin et. al.'s study was a clinical study in Boston. With respect to the rapid preparation of the LV for the Jatene procedure in two stages, the LV mass reached its peak of hypertrophy in seven days, with a mean increase of 100% with an initial significant gain within 36 hours of continuous systolic overload. In some studies, the highest total overload in the continuous protocols (in relation to the intermittent protocols) results in a early and more accentuated hypertrophy. However, according to observations by Perrino et. al. [24], the higher and highest early mass gain seems to be associated with a higher myocardial dysfunction. The rate of increase in muscle mass of 0.08 g/h ± 0.04 g/h of this study was slightly larger than the values found by the echocardiographic evaluation of the Boston group in the rapid preparation for the Jatene procedure (0.06 g/h ± 0.04 g/h), despite less systolic overload time and consequently lesser energetic consumption by the myocardium [23]. It is likely that, after the early activation of hypertrophy genes, the mechanism of this hypertrophic process may lead to more favorable conditions during the resting periods and more transportation of oxygen.

Concerning the interventricular septum mass, a significant gain of 37% observed after 96 hours of training is corroborated by the findings of previous studies in our laboratory [13], which also failed to show echocardiographic alterations parallel to the hypertrophic process of this ventricular mass area.

Morphofunctional parameters

The capacity of the subpulmonary ventricle - prepared to support the systemic vascular resistance during the second stage of the Jatene procedure – primarily depends on the afterload to which it will be postoperatively submitted. Generally, the simple ventricular mass measurement is not enough to predict if the ventricle would be capable of anatomical correction of the TGA. Although the ventricular mass may be proportional to the body surface of the patient, the trained ventricle may still be poorly prepared for a connection to systemic circulation. The best situation would be to anticipate the volume/mass relationship to which the ventricle would be submitted in the postoperative of Jantene procedure, in order to use it as successfully prognostic index of the trained ventricle.

In this study, the volume/mass relationship of the RV presented maximum increase in the first 48 hours of intermittent overload with total recovery at the end of the protocol. This moment indicated a condition that is favorable for supporting the systemic circulation afterload. The improvement of this RV ratio contrasts with the findings about rapid preparation in the Boston clinical study, which indicated progressive increase of this parameter, reaching its maximum in a period of seven to ten days, characterizing the progressive ventricular dilatation observed until the moment of the Jatene procedure [23]. Normalization of RV dilatation in this study, associated with its significant mass gain may be attributed to the intermittent pattern of hypertrophic stimulus.

RV pressure was also analyzed in this study, as it is considered the best parameter of afterload submitted to the ventricle by banding and also the best paramater of energy use dispensed by the ventricle in a given moment. Although we have been observing that the induced RV-PT gradients and the RV/LV pressure relation have been progressively larger over time, the values of wall pressure did not comply with this tendency. We observed a peak of this parameter at the 48-hour mark, when the RV presented high intracavitary pressure with severe dilatation but without significant mass gain.

Later, a progressive decrease was observed in the subsequent periods due to the significant mass gain of the RV. This study clearly showed that the RV wall pressure is at its maximum in the initial periods of overload, with progressive decrease during **ventricular adaptation** through muscle hypertrophy and a decrease in final diastolic volume of the RV. The more hypertrophied heart— that is, the more conditioned muscle— would "suffer" less with the induced afterload.

In relation to myocardial water volume, the four periods of intermittent systolic overload caused significant RV and septum hypertrophy of Group 96, and was not followed by myocardial edema. It is likely that this increase of the aforementioned cardiac mass weight was due to increased protein synthesis, due the fact that there was no significant difference in the RV and septum myocardial water volume between Group 96 and and the control group.

Echocardiographic parameters

The intermittent systolic overload (imposed by the PT

banding) allowed for a rapid RV hypertrophy; as a result, the thickness of its free wall superceded the thickness of the septum and LV walls, beginning at the 72-hour mark of the protocol. Paradoxically, the septum thickness did not show detectable hypertrophy in the echocardiogram during the protocol, which differs from the significant increase of the septa weighing in Group 96. Perhaps, this difference may be explained by the larger protein content and muscle density of the wall, even without the proportional macroscopic increase visible in the echocardiogram.

The decrease in the ejection fraction and the parallel increase of final RV diastolic volume occurring 24 hours after the RV intermittent systolic overload, especially in the Group 48 and Group 72, were not followed by a significant drop in systemic arterial pressure. In the rapid preparation protocol of the Boston group with fixed and continuous TP banding, the ventricular dysfunction was earlier and the recovery time was longer (12 hours and 84 hours, respectively) [23]. This fact may be explained by RV diastolic dysfunction with its contractile function largely unaffected, according as suggested by Gaynor et. al. [25]. These authors suggest compensatory mechanisms in atrial compliance in order to avoid systemic hemodynamic alterations.

In this study, the recovery from the ejection fraction and the RV final diastolic volume in the subsequent periods are probably related to the four intervals (12 hours) of rest between the periods of systolic overload, which induces remodeling and "healthier" myocardial hypertrophy of the RV. The periods of intermittent resting during the protocol may optimize the subendocardial coronary flow and, consequently, enlarge the quantity of substrates in the myocardial hypertrophic process, which limits the seriousness of a continuous systolic overload imposed upon the RV. This improvement in prerformance of the RV contrasts with the findings of Leeuwenburgh et al. [26], which showed a drop in the cardiac debt in protocol of PT chronic banding in young sheep, despite the increase of RV contractility. The authors concluded that the chronic banding of the RV causes an increase in RV relaxation and decrease of the diastolic compliance, both indicative of diastolic dysfunction of the RV.

Clinical implications

In this study, it was clear that the intermittent systolic overload of the RV caused a significant gain in muscle mass without the loss of contractile function. However, this kind of protocol was not still clinically applied in the Jatene procedure in two stages. It is still not known if this means of ventricular preparation - although it allows for significant hypertrophy of the RV - would be effective in preparing the ventricle for the anatomical correction with the proposed overload and time rate.

Evaluating some aspects that are commonly considered as effective ventricular preparation criteria, the systolic overload of this protocol would be enough and appropriate for ventricular preparation, due to the following facts:

- Within 24 hours of intermittent systolic overload, the RV already tolerated a pressure ratio higher than 0.75 in relation to the systemic ventricle;
- Within 72 hours, the RV thickness already had reached the values of the systemic ventricle;
- The ventricular function and the RV dilatation was also perfectly recovered within 72 hours;
- However, the gain of ventricular mass showed significance only within 96 hours; and,
- Within 96 hours, the volume/mass relationship tended to have values that were less than those of the control group.

Limitations of the study

Considering the limitations of clinical inferences from observations of an experimental group, we can not affirm that the behavior of the human heart with TGA would be exactly the same as what was observed in our experiment. In this study, the evaluated subpulmonary ventricle was the RV and not the LV, as in the TGA. On the other hand, the experiments with aortic banding in animals occur with mortality rates of 30%-50%. Furthermore, the aortic banding would submit the coronary system to high pressures, which may cause several repercussions. This experimental approach also would not correspond with reality because, in the TGA, the LV is connected to the pulmonary artery. In the case of adult patients with CTTGA, we can not affirm that the adaptive behavior of the heart will be the same, which could limit our conclusions about the subpulmonary ventricle preparation of this class of patients for the Jatene procedure in two stages. Studies with adult animals and the use of molecular biology to analyze the markers of the hypertrophic process certainly may be enlightening and may generate knowledge that would allow for a better understanding of the ventricular hypertrophic process that would offer the best ventrical training.

CONCLUSION

This study showed that the four periods of intermittent systolic overload caused significant septum and RV hypertrophy in the 96-hour group without myocardial edema. Therefore, this intermittent pattern of hypertrophy stimulus may decrease the time needed for ventricular

preparation of 96 hours for patients with TGA beyond the neonatal period.

ACKNOWLEDGMENT

Authors acknowledge the support of SILIMED Silicone e Instrumental Médico-Cirúrgico e Hospitalar Ltda., specially to the managers Margaret Figueiredo and Antoine Robert and to Ana Soares and André Esteves of the Research and Development Department-.

REFERENCES

1. Jatene AD, Fontes VF, Paulista PP, Souza LC, Neger F, Galantier M, et al. Successful anatomic correction of transposition of the great vessels. A preliminary report. Arq Bras Cardiol. 1975; 28(4):461-4.

2. Devaney EJ, Charpie JR, Ohye RG, Bove EL. Combined arterial switch and Senning operation for congenitally corrected transposition of the great arteries: patient selection and intermediate results. J Thorac Cardiovasc Surg. 2003;125(3):500-7.

3. Graham Jr TP, Markham L, Parra DA, Bichell D. Congenitally corrected transposition of the great arteries: an update. Curr Treat Options Cardiovasc Med. 2007;9(5):407-13.

4. Yacoub MH, Radley-Smith R, Maclaurin R. Two-stage operation for anatomical correction of transposition of the great arteries with intact interventricular septum. Lancet. 1977;1(8025):1275-8.

5. Robertson MA, Penkoske PA, Duncan NF. Right pulmonary artery obstruction after pulmonary artery banding. Ann Thorac Surg. 1991;51(1):73-5.

6. Jonas RA, Giglia TM, Sanders SP, Wernovsky G, Nadal-Ginard B, Mayer JE Jr, et al. Rapid, two-stage arterial switch for transposition of the great arteries and intact ventricular septum beyond the neonatal period. Circulation. 1989;80(3 Pt 1):I203-8.

7. Boutin C, Wernovsky G, Sanders SP, Jonas RA, Castaneda AR, Colan SD. Rapid two-stage arterial switch operation. Evaluation of left ventricular systolic mechanics late after in acute pressure overload stimulus in infancy. Circulation. 1994;90(3):1294-303.

8. Bishop SP, Melsen LR. Myocardial necrosis, fibrosis, and DNA synthesis in experimental cardiac hypertrophy induced by sudden pressure overload. Circ Res. 1976;39(2):238-45.

9. Zimmer HG, Ibel H, Gerlach E. Significance of the hexose monophosphate shunt in experimentally induced cardiac hypertrophy. Basic Res Cardiol. 1980;75(1):207-13.

10. Weber KT, Janicki JS, Pick R, Abrahams C, Shroff SG, Bashey RI, et al. Collagen in the hypertrophied, pressure-overloaded myocardium. Circulation. 1987;75(1 Pt 2):I40-7.

11. Takahashi Y, Nakano S, Shimazaki Y, Kadoba K, Taniguchi K, Sano T, et al. Echocardiographic comparison of postoperative left ventricular contractile state between one- and two-stage arterial switch operation for simple transposition of the great arteries. Circulation. 1991;84(5 Suppl):III180-6.

12. Le Bret E, Lupoglazoff JM, Borenstein N, Fromont G, Laborde F, Bachet J, et al. Cardiac "fitness" training: an experimental comparative study of three methods of pulmonary artery banding for ventricular training. Ann Thorac Surg. 2005;79(1):198-203.

13. Assad RS, Rodriguez MQ, Abduch MC, Valente AS, Andrade JL, Krieger JE, et al. Bandagem ajustável do tronco pulmonar: comparação de dois métodos de hipertrofia aguda do ventrículo subpulmonar. Rev Bras Cir Cardiovasc. 2006;21(4):418-28.

14. Dias CA, Assad RS, Caneo LF, Abduch MCD, Aiello VD, Dias AR, et al. Modelo experimental de bandagem ajustável do tronco pulmonar para preparo rápido do ventrículo. Rev Bras Cir Cardiovasc. 2000;15(4):328-37.

15. Dias CA, Assad RS, Caneo LF, Abduch MC, Aiello VD, Dias AR, et al. Reversible pulmonary trunk banding. II: An experimental model for rapid pulmonary ventricular hypertrophy. J Thorac Cardiovasc Surg. 2002;124(5):999-1006.

16. Câneo LF, Dias CA, Assad RS, Abduch MCD, Aiello VD, Moreira LFP, et al. Preparo do ventrículo subpulmonar através de dois diferentes modelos ajustáveis de bandagem do tronco pulmonar: estudo experimental. Rev Bras Cir Cardiovasc. 2001;16(1):35-48.

17. Muraoka R, Yokota M, Aoshima M, Nomoto S, Kyoku I, Kobayashi A, et al. Extrathoracically adjustable pulmonary artery banding. J Thorac Cardiovasc Surg. 1983;86(4):582-6.

18. Higashidate M, Beppu T, Imai Y, Kurosawa H. Percutaneously adjustable pulmonary artery band. An experimental study. J Thorac Cardiovasc Surg. 1989;97(6):864-9.

19. Ahmadi A, Rein J, Hellberg K, Bastanier C. Percutaneously adjustable pulmonary artery band. Ann Thorac Surg. 1995;60(6 Suppl):S520-2.

20. Pontes SC Jr, Assef JE, Barretto RB, Chaccur P, Moreira DA, S Nina VJ, et al. Estimation of right ventricular mass by two-dimensional echocardiography. J Am Soc Echocardiogr. 2005;18(5):427-34.

21. Fulton RM, Hutchinson EC, Jones AM. Ventricular weight in cardiac hypertrophy. Br Heart J. 1952;14(3):413-20.

22. Bishop SP, Cole CR. Production of externally controlled progressive pulmonis stenosis in the dog. J Appl Physiol. 1969;26(5):659-63.

23. Boutin C, Jonas RA, Sanders SP, Wernovsky G, Mone SM, Colan SD. Rapid two-stage arterial switch operation. Acquisition of left ventricular mass after pulmonary artery banding in infants with transposition of the great arteries. Circulation. 1994;90(3):1304-09.

24. Perrino C, Naga Prasad SV, Mao L, Noma T, Yan Z, Kim HS, et al. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. J Clin Invest. 2006;116(6):1547-60.

25. Gaynor SL, Maniar HS, Bloch JB, Steendijk P, Moon MR. Right atrial and ventricular adaptation to chronic right ventricular pressure overload. Circulation 2005;112(9 suppl):I212- 8.

26. Leeuwenburgh BP, Steendijk P, Helbing WA, Baan J. Indexes of diastolic RV function: load dependence and changes after chronic RV pressure overload in lambs. Am J Physiol Heart Circ Physiol. 2002;282(4):H1350-8.

ORIGINAL ARTICLE

Braz J Cardiovasc Surg 2006; 21(4): 418-428

Adjustable pulmonary trunk banding: comparison of two methods of acute subpulmonary ventricle hypertrophy

Bandagem ajustável do tronco pulmonar: comparação de dois métodos de hipertrofia aguda do ventrículo subpulmonar

Renato Samy ASSAD[1], Miguel Quintana RODRIGUEZ[2], Maria Cristina ABDUCH[3], Acrísio Sales VALENTE[4], José L. ANDRADE[5], José Eduardo KRIEGER[6], Miguel BARBERO-MARCIAL[7]

RBCCV 44205-850

Abstract

Objective: **This study compares ventricular hypertrophy induced by continuous versus intermittent systolic overload of the pulmonary ventricle (RV) of young goats.**

Methods: **Three groups of seven goats were used (control, continuous, and intermittent). Systolic overload was maintained for 96 hours in the continuous group, while the intermittent group suffered four 12-hour periods of systolic overload, alternating with 12-hour resting periods. Echocardiographic and hemodynamic evaluations were performed every day. The animals were then killed for myocardial water content and weight evaluation.**

Results: **Both study groups achieved significant increases in RV mass ($p<0.05$). However, significant increases of the septum mass were observed only in the Intermittent Group ($p<0.05$). A greater increase in the RV wall thickness was observed in the Intermittent Group ($p<0.05$). There was a significant difference in RV diastolic volume between the two groups ($p= 0.01$), with a greater RV dilation in the Continuous Group after 24 hours of continuous overload (p<**

1 - Professor Livre Docente of the Medical School of the University of Sao Paulo; Heart surgeon of the Infant Surgery Unit, Heart Institute HCFMUSP

2 - Cardiovascular surgeon; PhD in Sciences from the Medical School of the University of Sao Paulo

3 - Veterinary Doctor; PhD in Sciences from the Medical School of the University of Sao Paulo; Assistant veterinary of the Veterinary Medical School and Zoology of the University of Sao Paulo.

4 - Cardiovascular Surgeon of the Hospital Universitário in Fortaleza

5 – Professor Livre Docente da UNIFESP- Paulista Medical School; Head of the Echocardiography Laboratory of the Sírio Libanês Hospital, São Paulo

6 - Professor Livre Docente of the Clinical Medicine Department Medical School of the University of Sao Paulo; Directory of the Genetics and Molecular Cardiology Laboratory of the Heart Institute of Hospital das Clínicas of the Medical School of the University of Sao Paulo

7 - Associate professor of the Medical School of the University of Sao Paulo; Director of the Child Surgery Unit f the Heart Institute of Hospital das Clínicas of the Medical School of the University of Sao Paulo

Work carried out in the Heart Institute of Hospital das Clínicas of the Medical School of the University of Sao Paulo

Correspondence address

Renato S. Assad. Instituto do Coração HC – FMUSP. Unidade Cirúrgica Infantil Av. Dr. Enéas de Carvalho Aguiar, 44 - São Paulo, SP – CEP: 05403-000 Phone/fax: (011) 3069-5089.

E-mail: rsassad@cardiol.br

Article received in June, 2006

Article accepted in November, 2006

0.03). In both groups, the RV ejection fraction was maintained within the normal range throughout the protocol. A smaller RV perimeter was observed in the Intermittent Group after 96 hours of systolic overload (p<0.05). There was no significant difference in RV myocardial water content between the study groups and the Control Group.

Conclusions: **Adjustable pulmonary artery bandages permit rapid RV hypertrophy in both groups. Nevertheless, it is more efficient in the Intermittent Group. This study suggests that preparation of the pulmonary ventricle with intermittent systolic overload might provide better results for the 2-stage arterial switch operation.**

Descriptors: **Heart ventricles, physiopathology. Hypertrophy, physiopathology. Right ventricular hypertrophy. Transposition of great vessels, surgery. Cardiac surgical procedures, methods. Goats.**

Resumo

Objetivo: **Este estudo compara a sobrecarga contínua versus intermitente do ventrículo direito (VD) de cabritos, para induzir a hipertrofia ventricular.**

Método: **Foram utilizados três grupos de sete cabritos jovens (controle, contínuo, intermitente). A sobrecarga sistólica foi imposta por 96 horas, no contínuo e por quatro períodos de 12 horas, alternados com 12 horas de descanso, no intermitente. Avaliações ecocardiográficas e hemodinâmicas foram feitas diariamente. Os animais foram, então, mortos para avaliar o conteúdo de água e peso das massas cardíacas.**

Resultados: **O Intermitente mostrou aumento dos pesos de VD e de septo, em relação ao controle (p<0,05), enquanto o contínuo aumentou apenas a massa do VD (p<0,05). Houve maior aumento da espessura do VD no Intermitente (p<0,05). O volume diastólico final do VD mostrou diferença significativa entre os grupos (p=0,01), com maior dilatação do VD do grupo contínuo, no momento 24 horas de sobrecarga sistólica (p<0,03). A fração de ejeção do VD se manteve dentro da normalidade nos dois grupos ao longo do protocolo. Foi observado menor perímetro do VD no intermitente, após 96 horas de treinamento (p<0,05). Não houve diferença significante entre os grupos de estudo e o controle quanto ao conteúdo de água do miocárdio do VD.**

Conclusões: **A bandagem ajustável do tronco pulmonar permitiu um rápido processo hipertrófico do VD em ambos os grupos, sendo, porém, mais eficiente no intermitente. Nosso estudo sugere que a preparação do ventrículo subpulmonar de forma intermitente poderá proporcionar melhor resultado para a operação de Jatene em dois estágios.**

Descritores: **Ventrículos cardíacos, fisiopatologia. Hipertrofia, fisiopatologia. Hipertrofia ventricular direita. Transposição dos grandes vasos, cirurgia. Procedimentos cirúrgicos cardíacos, métodos. Cabras.**

INTRODUCTION

The concept of rapid preparation of the left ventricular (LV) was introduced by the group from Boston in 1989 to treat transposition of the great arteries (TGA) after the neonatal period. Retraining of the subpulmonary ventricle was obtained in a mean time of nine days and after this Jatene's operation was performed [1]. These authors achieved good preliminary results, however, they were not reproduced in other centers due to high morbimortality. One of the greatest limitations of this technical approach is related to the lack of adjustability of the pulmonary trunk (PT) banding. The degree of PT banding may be inadequate or imprecise and can cause an important acute systolic overload of the LV [2, 3].

Heart hypertrophy is the main adaptive response of a heart submitted to physiological or pathological overload. It is interesting to note that the acquisition of LV mass during physical conditioning of athletes who practice swimming for example, reaches a peak in about only one week of training. Then the LV mass remains relatively constant [4]

In an attempt to improve the rapid hypertrophic process without causing injury to the myocardium of the subpulmonary ventricle, we tried to find an analogy between the physiological hypertrophic processes in static-type exercises and intermittent PT banding, where periods of systolic overload are alternated with periods of subpulmonary ventricle relaxation.

We formed a hypothesis that the subpulmonary ventricle, submitted to the gradual and progressive systolic overload alternated with periods of relaxation, could cause a more beneficial hypertrophic process, similar to the process observed in the myocardium of athletes who perform exercises causing systolic overload. Faced with the possibility of intermittently adjusting the systolic overload of the subpulmonary ventricle, a better understanding of the changes occurring during this acute hypertrophy process is necessary with the objective of evaluating its impact on myocardial function. Thus, induction of a more physiological hypertrophy may be achieved, in order to preserve the future ventricular function.

The objective of this study was to compare acute

myocardial hypertrophy of the subpulmonary ventricle (RV) of two groups of young goats, with the first one being submitted to continuous and progressive RV systolic overload and the second undergoing intermittent RV systolic overload using an adjustable PT banding device. We also analysed and compared rapid hypertrophy, from hemodynamic and echocardiographic points of view, in the two processes of systolic overload of the subpulmonary ventricle (intermittent versus continuous). Moreover the weight and myocardial water content were compared between the study groups and a control group.

METHOD

This study was approved by the Ethics Research Commissions of the Hospital das Clínicas and the Medical School of the University of São Paulo in accordance with the norms on animal use in teaching and research established in the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy of Sciences, Washington, D.C., 1996) and the ethical principles for animal experimentation of the Brazilian College of Animal Experimentation (COBEA).

Twenty-one goats with ages between 30 and 60 days were included in the study and divided into three groups: Control (n = 7; weight = 7.5 ± l.9 kg, no surgical procedure), Continuous (n = 7; weight = 9.3 ± 1.4 kg, continuous systolic overload of the RV), Intermittent (n = 7; weight 8.1 ± 0.8 kg, 12 hours/day of intermittent systolic overload of the RV), all studied for a period of 96 hours.

Preoperative assessment

All the animals were submitted to echocardiography (Apogee CX, Advanced Technology Laboratories, Bothell, WA, USA), in the preoperative period to confirm the thickness of the RV free wall in relation to the left ventricle (LV).

Anesthesia

The animals remained 24 hours fasting before the surgery. Anesthetic induction was achieved with ketamine (30 mg/kg, intramuscular). The animals were weighted and subsequently their jugular veins were punctured with a Jelco nº18 catheter for the administration of drugs and infusion of saline solution. The animals were then sedated with Nembutal (from 5 to 10 mg/kg, endovenous) and intubated. Mechanical ventilation (Harvard 708, South Natick, MA, USA) was established with an inspired oxygen fraction of 100% and tidal volume of 15 mL/kg. The animals were positioned in the right lateral decubitus position, monitored by ECG and prepared for the sterile procedure. Anesthesia was maintained with sodium pentobarbital (Nembutal, 5 -10 mg/kg, intravenously) and ketamine (1 mg/kg endovenously). All animals received antibiotic therapy initiated before the surgery and maintained until the end of the protocol (cephazolin 500 mg and gentamicin 10 mg, intramuscular, every 12 hours). Digoxin (0.005 mg/kg endovenously every 12 hours) and heparin (2500 IU every 12 hours subcutaneously) were also administrated until the end of the protocol.

Surgical procedure

Left lateral thoracotomy was performed at the 4th intercostal space. The lung was dislocated and the pericardial sac was opened. An adequate view of the RV outflow tract, the PT and descending thoracic aorta were obtained. At this moment, three previously-heparinized catheters (intracath 17G) were implanted in the RV, PT and descending thoracic aorta. The catheters were fixed with prolene 5-0 purse-string sutures and exteriorized through the thoracic wall, near to the spinal column, where they were also anchored to the skin using 3-0 cotton thread. Subsequently, these catheters were tested (permeability and pressure curves) and maintained heparinized. Pressures, proximal and distal to the adjustable PT banding device, were measured as was the systemic arterial pressure using the ACQ Knowledge 3.01 software system (Biopac Systems, Inc., Goleta, CA, USA).

PT banding device

The PT was dissected to implant the PT banding device as described and utilized in previous publications [5, 6]. The cuff occluder was immediately positioned over the pulmonary valve and fixed to the adventitial layer of the PT to prevent its migration. The insufflation button of the device was implanted subcutaneously thereby allowing fine percutaneous adjustment of the diameter of the banding ring.

After device implantation, the thorax was drained. The ribs and thoracic wall tissues were closed by layers. After approximately six postoperative hours, the thorax drain was removed after verifying minimal drainage, the absence of broncopleural fistulae and good pulmonary expansion.

Protocol of RV systolic overload

RV training was started after total recovery from the surgery (72 hours of convalescence). With the animal conscious and immobilized on a special stretcher, the RV, PT and aorta basal pressures were measured with the PT banding device completely deflated. After measurement of the basal pressures, percutaneous insufflation of the device with saline solution was initiated using an insulin syringe, observing the pressures curves of the RV and aorta with an aim of reaching a RV pressure of approximately 70% of the systemic systolic pressure, as long as this did not drop by 10% or more.

If the animal presented clinical signs of significant

hypoxia, such as agitation, breathing difficulties or arrhythmias, the volume of the device was reduced to a tolerable level. Insufflation of the device and measurement of the aortic, RV and PT pressures were performed every day for the two study groups. The volume of fluid in the device was measured and compared to the previous day to assess possible losses. The device was insufflated again, trying to achieve the desired parameters; in general it was possible to increase the volume every day. The pressure gradient between the RV and the pulmonary arterial trunk was calculated by subtraction of their systolic pressures.

Training of the continuous group

The animals remained under continuous systolic RV overload for a period of 96 hours with progressive insufflations at 24-hour intervals to the maximum tolerated limit.

Training of the intermittent group

The animals were submitted to four 12-hour periods of RV systolic overload (during the day), alternated with 12 hours of relaxation (at night), over the same period of 96 hours as the continuous group. The pressures were measured both during the day and at night.

Echocardiographic study

All animals were submitted to echocardiographic assessment by the same specialist, before the start of the insufflation protocol and on each day after the initial insufflation of the device, to assess the RV hypertrophy process of both study groups over the 96 hours of RV systolic overload. The animals of the Control Group were evaluated just once before being sacrificed.

Transducers of 7.5 MHz were utilized to obtain the images and 2.5-MHz transducers to assess blood flows. The thickness of interventricular septum and LV posterior wall were measured at M-mode at the end of diastole by a transversal parasternal plane at the papillary muscles. The RV/PT pressure gradient caused by the device was obtained by continuous Doppler. The RV free wall perimeter and its thickness were measured by 2-dimensional echocardiogram always at the end of diastole using the same parasternal plane near to the great vessels and the papillary muscles. The measurements of the RV perimeter were indexed to the RV wall thickness at the site of the perimeter measurement. These measurements were also taken for the four-chamber longitudinal plane. The RV free wall thickness is the mean of three measurements taken in each examination. The right ventricle final systolic (FSV) and final diastolic volumes (FDV) were obtained by the formula area x length in the longitudinal four-chamber slice. The hemodynamic efficiency was evaluated from the RV ejection fraction (RVEF), which was attained from the difference of the volumes using the formula proposed by Pontes et al. [7].

$$RVEF = (FDV - FSV) \times FDV^{-1}$$

Weighing the heart masses

After the end of the protocol of each animal, sacrifice was achieved by resection of the heart. Before anesthetic induction, the RV, PT and aorta pressures were measured. After general anesthesia using ketamine (30 mg/kg, IM) with Nembutal (15mg/kg, EV) and orotracheal intubation, left thoracotomy was performed at the site of the previous incision to expose of heart. Dissection of the superior and inferior vena cavas and great arteries were performed. After increasing the anesthesia, heparin (50 mg EV) and potassium chloride were administrated to cause cardiac arrest.

The heart was then resected. The epicardial fat was carefully removed and the ventricular and septal walls separated according to the technique published by Fulton et al. [8]. The aorta and PT were resected near the semilunar valves. Atria were resected together with the atrioventricular valves and identified. The RV free wall was separated from the interventricular septum, incising it in the same direction as the anterior and posterior interventricular arteries. The same procedure was performed to separate the LV free wall from the septal wall.

Subsequently, the RV, LV, interventricular septum and atria were weighed using a digital balance (Mettler AE-200, Mettler-Toledo AG, Greifensee, Switzerland). Due to the variation in the weight of animals, the measurements were standardized by indexing the weight of the heart muscle mass to respective body weights of the animals. The indexed weights are expressed in g/kg. The animals of the Control Group were also submitted to the same scarification, resection and weighing procedures.

Tissue water content

The next step was to select samples of each of the cardiac walls to assess water content (WC). The initial weight (IW) of each sample was obtained. The samples were then placed in appropriate flasks, closed with filter paper and duly identified, before being placed in an incubator (temperature: 55-60º C). After about 70 hours of dehydration, each sample was again weighed to obtained the dry weight (DW). The percentage water content was then obtained by the following formula, assuming that the distribution of water was homogenous in the septum and in the ventricles:

$$WC\ (\%) = 100 - (DW \times IW^{-1} \times 100)$$

The water content of the heart muscles in the Continuous and Intermittent Groups were compared to the Control Group to check if the increase in weight of the RV in the study groups was associated to myocardial edema.

Statistical analysis

The normality of the distribution of each variable was assessed by means of the Kolmogorov-Smirnov test. Comparisons of the medians of variables such as the RV/LV ratio and RV heart muscle thickness were evaluated using the Kruskal-Wallis non-parametrical test followed by the Student-Newman-Keuls multiple comparison test. The comparisons of the aortic systolic pressures, RV ejection fraction (measured by echocardiography), RV/PT pressure gradient, perimeter and RV final diastolic volume in the Continuous and Intermittent Groups were made by analysis of 2-way variance (ANOVA), followed by Fisher's exact test for multiple comparisons. However, the masses of the RV, LV and septum and the water content of the RV, LV and septum were compared using the one-way ANOVA test followed by Bonferroni's test for multiple comparisons. The systolic overload applied to the RV of the Continuous and Intermittent Groups was evaluated by calculating the areas under the curves (trapezoidal method), which describe the behavior of the pressure gradient between the RV and the PT (RV/PT) in these groups. A comparison of the areas under the curves was made by means of Student's t-test for unmatched data. Values are presented as means ± standard deviation (SD) for analyses using parametrical tests and as medians with respective interquartiles (25%-75%) using non-parametric tests. In all cases, the level of significance employed was 5%. Statistical analyses were made with the GraphPad Prism v.4 (San Diego, CA –USA) and Statistics v.6 software programs (Tulsa, OK – USA).

RESULTS

All animals completed the 96-hour protocol of RV systolic overload. The weight of the animals of the three groups were similar to each other (p=0.11). The Intermittent Group supported a higher filling volume of the inflatable occluder than the Continuous Group (0.63 mL ± 0.19 mL versus 0.47 mL ± 0.16 mL, p= 0.001). There was a minimal loss of volume injected into the device during the study period.

Hemodynamical measurements

Systemic arterial pressure

The systemic systolic pressure of the two study groups of animals was analysed by the two-way ANOVA parametric test, demonstrating that there was no significant difference using the different overload stimuli (p=0.21 and p=0.12 for group and time, respectively), independent of the method (continuous or intermittent). It is interesting to observe that there was a trend of lower systolic pressures in the Continuous Group when compared to the Intermittent Group.

RV/PT pressure gradient

The two-way ANOVA test demonstrated a statistical significance (p=0.02) for the RV/PT gradient ratios. In respect to RV training, Figure 1 demonstrates a progressive increase in pressure gradient for RV/PT only for the Intermittent Group at 48 hours (72.00 mmHg ± 15.17 mmHg) and 72 hours (80.00 mmHg ± 12.99 mmHg) when compared to the basal measurements (9.57 mmHg ± 9.45 mmHg; p< 0.05). However, there was no significant difference in the values of gradient caused by the RV between the Continuous and Intermittent Groups at all time intervals (0, 24, 48, 72 and 96 hours, p> 0.05).

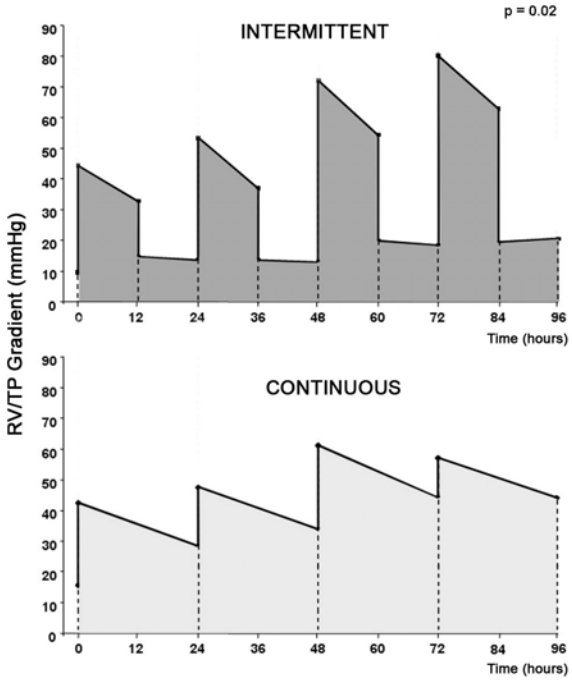


Fig. 1 – Diagram of RV systolic overload. Upper graph: RV/PT gradient (mmHg) of the group submitted to 12 hours of RV systolic overload alternated with 12 hours of relaxation. Lower graph: RV/PT gradient (mmHg) of group submitted to continuous RV systolic overload. (p=0.02)*

The drop in the RV/PT gradient at the last measurement (96 hours) of the Intermittent Group is due to the fact that the animals of this group were relaxing. In spite of this, higher gradients caused by the RV at this moment were observed compared with the basal gradient, even with the adjustable banding device deflated. However, these differences were not statistically significant. Figure 2 shows the systolic overload area applied to the RV in the two groups. The data were obtained by calculating the product

of the pressure gradient RV/PT over time of systolic overload. The systolic overload area was lower in the Intermittent Group when compared to the Continuous Group (p=0.002).

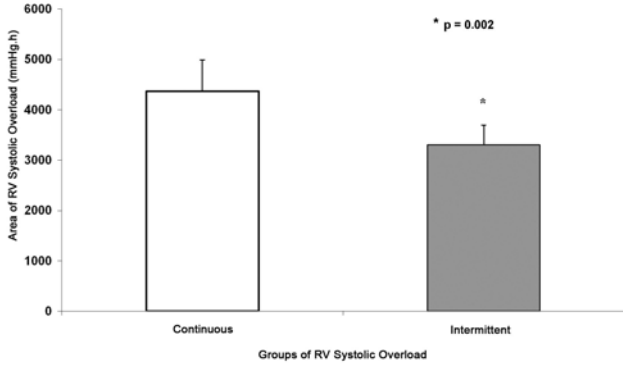


*Fig. 2 – Diagram of RV systolic overload area. Overload area of groups submitted to continuous banding versus intermittent PT banding. (*P=0.002)*

RV/LV pressure ratio

The RV/LV pressure ratios of both groups of RV systolic overload are demonstrated in Table 1. Together with the RV/PT gradient, we observed a progressive increase of the RV/LV ratio in the two groups when compared to the basal values (p<0.05). Nevertheless, higher values were observed in the Intermittent Group when compared to the Continuous Group, at 24, 48 and 72 hours. The significant drop in the RV/LV ratio of the Continuous Group at 96 hours of protocol (p<0.05) may be related to the gradual loss of volume from the adjustable PT banding device. In regards to the Intermittent Group, as the device was deflated during the 96-hour hemodynamics measurements, due to the prior relaxing period a lower RV/LV ratio was expected, however it was still higher than the basal value (p<0.05).

Echocardiographic findings

Heart wall thicknesses

All animals included in the protocol presented with thinner RV thicknesses compared to the septum and the LV at the start of the study. The Kruskal-Wallis' non-parametrical test demonstrated that there was no alteration in the LV thickness or of the septum with the continuous or intermittent overloads (p=1.00).

Figure 3 shows a progressive increase in the RV wall thickness during the protocol. Both groups presented with significant increases in the RV free wall thickness. Nevertheless, this increase was significantly greater in the Intermittent Group with an increase of 132.1% at the end of the protocol compared to an increase of 63.7% in the Continuous Group (p<0.05). The LV contractility was preserved during the protocol.

Table 1. Comparison of mean of RV/LV pressure ratios in groups of continuous versus intermittent systolic overload, calculated by means obtained from pressure measurements by vascular catheters

Time (hours)	Ratio RV/LV		p-value
	Continuous group	Intermittent group	
Basal	0.34 (0.29-0.39)	0.33 (0.30-0.44)	>0.05
0	0.68 (0.62-0.84)	0.70 (0.65-0.81)	>0.05
24	0.79 (0.61-0.81)	0.79 (0.73-0.96)	<0.05
48	0.84 (0.76-0.95)	1.08 (0.85-1.20)	<0.05
72	0.85 (0.74-0.97)	1.10 (0.94-1.25)	<0.05
96	0.69 (0.54-0.74)	0.49 (0.37-0.54)	<0.05

N=7; Values = mean (interquartile 25% - 75%); continuous group = continuous RV systolic overload; Intermittent group = intermittent RV systolic overload.

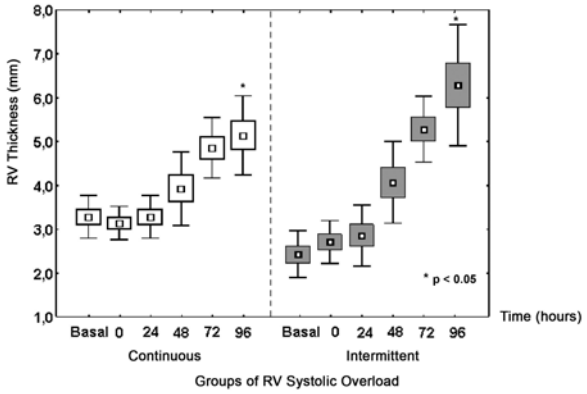


*Fig. 3 – Echocardiographic measurements of RV. RV free wall thickness of groups submitted to continuous and intermittent systolic overload (*p<0.05)*

RV/PT pressure gradient

The two-way ANOVA test gave a p-value < 0.001 for the RV/PT pressure gradients. However, the multiple-comparison test did not show any statistical difference between the groups at any time interval (0, 24, 48, 72 and 96 hours).

Similar to the measurements attained using catheters, a significant increase in the RV/PT gradient of the Intermittent Group was noted 72 hours after the start of the systolic overload (30.29 mmHg ± 6.16 mmHg) in relation to the basal measurement (2.14 mmHg ± 1.46 mmHg; $p<0.05$) by echocardiography. Different to the measurements observed using catheters, the Continuous Group demonstrated increments in the RV/PT gradient greater than the basal value after 48, 72 and 96 hours of systolic overload as identified by Doppler ($p<0.05$).

It is important to highlight that according to what we observed by hemodynamic measurements verified by catheter, at 96 hours of RV overload in the Intermittent Group, with the RV already trained, higher RV/PT pressure gradients than the basal measurements were also observed despite of the animals being in a period of relaxation. However, these differences were not statistically significant.

RV end diastolic volume

Figure 4 demonstrates the percentage variation of the RV diastolic volume during the systolic overload protocol compared to the preoperative echocardiographic evaluation. ANOVA of the RV end diastolic volume showed a significative difference between the groups (p=0.01). An analysis of multiple comparisons (Fisher's test) revealed a greater dilatation of the RV in the Continuous Group after 24 hours of systolic overload when compared to the basal reading and greater dilations at the 48-, 72- and 96-hour time intervals of the Intermittent Group ($p<0.03$). Temporal differences in variations of the RV end diastolic volume within the groups were not observed during the study (p=0.24).

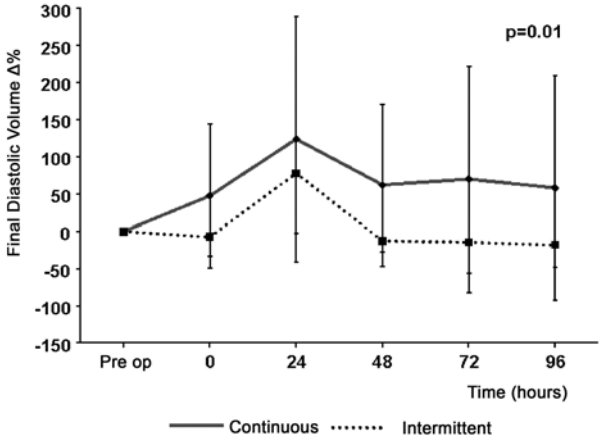


Fig. 4 – RV diastolic volume variation. Percentage variation of RV end diastolic volume of groups submitted to continuous systolic overload versus intermittent RV systolic overload. (p=0.01)

RV functional evaluation

The RV ejection fractions of the two groups were normal during the RV systolic overload protocol, thus, no harm was caused by adjusting the PT band. ANOVA did not identify significant changes of this parameter between the groups (p=0.07), although lower increments of the RV ejection fraction were seen in the Continuous Group during the protocol compared to the Intermittent Group.

RV perimeter

Table 2 illustrates the RV perimeters measured near to the great vessels and indexed to the RV wall thickness for the two study groups during the protocol. ANOVA demonstrated a significative difference in relation to time (p=0.006), but no difference between the groups (p=0.18). In the multiple comparisons (Fisher's test), a smaller RV perimeter was observed in the Intermittent Group at 96 hours of training compared to the first day of training of the Continuous Group ($p<0.05$). In respect to the percentage variation of the RV perimeter in comparison to the preoperative measurements, although the Continuous Group always had positive values and values similar to the Intermittent Group, no statistically significant differences were evidenced (p=0.18).

Table 2. RV perimeter measured by echocardiography near the great vessels (continuous versus intermittent systolic overload groups)

Time (hours)	RV Perimeter (cm) Continuous group	Intermittent group	p-value
0	5.77±1.48	6.00±0.92	0.14
24	6.86±1.07	6.46±0.83	0.79
48	6.41±1.15	5.46±0.81	0.93
72	5.91±1.32	5.67±0.62*	0.20
96	5.87±1.25*	5.29±0.95*	0.92

Intermittent Group = intermittent RV systolic overload; Continuous Group = continuous RV systolic overload; Values = means in cm ± standard deviation; n = 7; overload time: hours= p<0.05 when compared with the basal measurements of the Continuous Group and with both groups after 24 hours*

Weighing the heart tissue

Table 3 shows the heart tissue weights indexed to the animals' body weight. One-way ANOVA demonstrated that there were only significant differences in the RV (p=0.001) and septum masses (p=0.026). The RV hypertrophic process

did not influence the LV muscle mass (p=0.53). The systolic overload determined similar increases in the RV masses of both groups compared to the Control Group, with 55.6% increase in the Continuous Group and 88.9% in the Intermittent Group. In respect to the increase in septal muscle mass, a significant increase of 40% was observed in the Intermittent Group when compared to the Control Group (p=0.03).

Table 3. Weight of muscle tissues in the Control, Continuous and Intermittent systolic overload Groups

Cardiac Mass	Weight (g/Kg of body weight) of RV (cm) Control	Continuous	Intermittent	p-value
RV	0.9±0.2	1.4±0.3*	1.7±0.5*	0.001
LV	1.6±0.2	1.7±0.3	1.7±0.3	0.529
Septum	1.0±0.2	1.2±0.2	1.4±0.3*	0.026

*Significant difference between groups (*p<0.05): RV Control versus RV Intermittent; RV Control x RV Continuous; Control septum x Intermittent septum; Values = mean ± standard deviation; Measurements: g/kg of body weight; n=7; RV intermittent systolic overload group; Continuous = RV continuous systolic overload group; Control = Control Group*

When the RV mass was indexed to the systolic overload area (Figure 5) for the Continuous and Intermittent Groups, a more significant increase was observed in the Intermittent Group. This data demonstrated that, for similar variations of systolic overload applied to the RV, the increase in mass was greater in Intermittent Group (p=0.02).

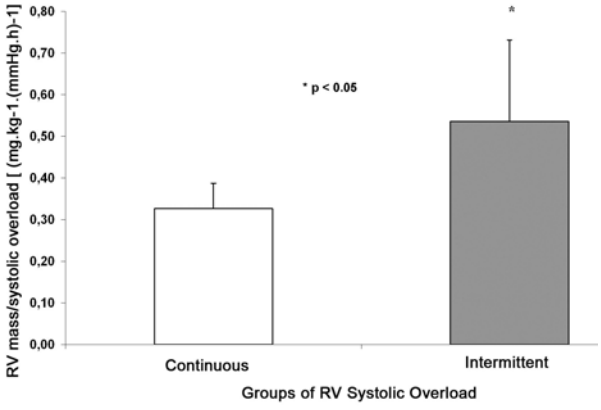


*Fig. 5 – RV mass Index. RV mass indexed by area of systolic overload of continuous and intermittent groups (*p<0.05)*

Water content of the tissues

In spite of RV and septum weight gains in the study groups, there were no significant differences in the RV myocardial water content between these groups and the Control Group according to one-way ANOVA (RV: p=0.10; septum: p=0.45 and LV: p=0.88).

Discussion

Percutaneous adjustment of PT banding is an extraordinary instrument to manage the type and quality of systolic overload applied to the subpulmonary ventricular myocardium. A definition of the ideal frequency and amount of overload required to cause physiological cardiac re-hypertrophy has not yet been established.

This study tries to assess and improve the re-hypertrophy process of the subpulmonary ventricle, attempting to find alternatives to improve the quality of the myocardial hypertrophy by studying concepts from physical training programs of the athlete's skeletal striated musculature.

Training protocol

It is very clear in this study that, in spite of there being no harm nor differences in RV performance between the two study groups resulting from the systolic overload imposed by adjustable PT banding, the RV training program to promote the myocardial hypertrophic process was more efficient in the Intermittent Group than in the Continuous Group. A proportionally smaller overload in the Intermittent Group caused significantly greater hypertrophy, as was seen by the RV free wall thickness at echocardiography and by greater increases in septal mass.

The group of Perrino [9] from Duke University, USA, analysed the development of LV hypertrophy in rats submitted to continuous and intermittent aortic arch banding, comparing them to two groups of rats submitted to running and swimming exercises during a period of four weeks. Both physical exercises and intermittent overload were performed twice per day, during a 90-minute period. The authors observed an increase in the LV weight of the intermittent group similar to the Running and Swimming Groups, in contrast to the group submitted to the continuous LV overload, which presented a more significant hypertrophic response. In spite of the greater hypertrophy in the Continuous Group, there was significative deterioration in the heart function during the study protocol, as demonstrated by echocardiography.

Undoubtedly, the current study would better show the trend of worse hemodynamic performance of the Continuous Group, if it were over a period of more than four weeks as in the study of Perrino et al. [9].

Echocardiographic parameters

The echocardiographic findings indicate a significative increase in the free wall thickness of both study group subpulmonary ventricles, corroborating with the increase in the RV tissue weight. However, the septal thicknesses did not change according to echocardiographic analysis, different to the significant increase of the septal mass in the Intermittent Group. Perhaps, this divergence may be explained by a higher protein content and muscle density of this wall, but without a proportional macroscopic increase visible by echocardiography. Additionally, a greater RV dilatation and a trend of greater increase in the RV perimeter observed by echocardiography in the animals of the Continuous Group may represent a greater physiopathological effect on the myocardium submitted to continuous systolic stress without alternating relaxation.

Carrol et al. [10] found intense inflammatory infiltration in the myocardium of swine submitted to pulmonary banding after 7 hours of systolic overload. Areas of variable degrees of cellular necrosis in the hypertrophied myocardium [11-13] and a consequent late ventricular dysfunction [14] have also been demonstrated in hearts submitted to acute systolic stress, probably due to imbalance between the oxygen supply and demand ratio in the hypertrophic myocardium.

LV hypertrophy induced by systemic arterial hypertension results in a reduction of the coronary flow reserve (number of arterial vessels/unit volume) [15]. In studies on RV hypertrophy, a coronary arterial tree remodeling has already been seen with an increased number of vessels and a reduction in the resistance to intracoronary flow [16]. However, it is still not clear if this difference occurs due to hypertension imposed on the coronary arteries in LV hypertrophic models. However, it is interesting to observe that, in hearts with TGA, the LV is connected to the pulmonary artery, and thus, pulmonary banding does not cause hypertension of the coronary system, a fact that is not assessed in experimental studies, due to the difficulty of creating a similar model. It may be that this detail is important in the understanding of the phenomena that occurs with the induced hypertrophy of these hearts.

Apart from possible cellular necrosis, there is a change in the genetical profile of cardiomyocytes with proteic alterations that are related to worsening of myocardial contractility.

Maybe intermittent periods of relaxation during the protocol may optimize subendocardial coronary flow and, consequently, provide more substrates to the myocardial hypertrophic process, thereby limiting the degree of continuous systolic stress imposed on the RV of the Continuous Group.

Weighing the heart tissues

The greater efficiency of intermittent systolic overload may be related to the triggering of the hypertrophic stimulus and the proteic synthesis cascade in the same manner as in the Continuous Group, but with less energy loss of the myocardium. Probably, the mechanism of this hypertrophic process triggered by the molecular cascade may be developed in good conditions during periods of relaxation and with ideal oxygen transport and, hence, without the development of fibrosis resulting from relative ischemia.

This hypothesis is corroborated by the studies of Le Bret et al. [17] who caused RV hypertrophy of sheep in only two hours daily of RV systolic overload during a period of five weeks. Fibrosis was then observed in animals submitted to a conventional banding regime and in those submitted to only two readjustments of RV systolic overload during the five-week protocol.

Recently, several studies have recommended stem cell implantation in the myocardium, as a therapeutic alternative in the treatment of heart failure, with the goal of improving the ventricular function performance. The French group of Borenstein [18] reported in 2005, the first experimental study on cell cardiomyoplasty in a training protocol with PT banding, for possible clinical application in Jatene's two-stage operation. However, the authors did not observe additional hemodynamic benefits of this strategy in a group submitted to banding associated to cell transplantation in the subpulmonary ventricle.

Water content

This study demonstrated similar increases in RV mass in both study groups when compared to the Control Group. A greater increase of septal muscle mass was observed in the Intermittent Group in comparison to the Control Group. Probably, this increase in heart tissue weight was a result of increased proteic synthesis, as there was no significant difference in the RV myocardial and septal water contents between the study groups and the Control Group.

Implications

This 96-hour protocol analyzes the performance of acute RV hypertrophy of young goats submitted to two programs of systolic overload, demonstrating the greater efficiency of the Intermittent Group. In patients with TGA after the neonatal period or in those already submitted to the atrial repair, such as the Senning or Mustard procedures, and even in patients with repaired TGA, who evolve with right ventricular dysfunction (systemic), the left ventricle morphologically (subpulmonary) requires retraining to support the systemic circulation after Jatene's operation. To achieve this objective with conventional PT banding is problematic, as some patients need to be reoperated to

relieve the banding due to the excessive constriction of the banding leading to ventricular dysfunction, or to tighten the banding in cases of inadequate left ventricle training, because of a loose band. These reoperations are associated to high morbimortality rates. Clinical application of an intermittent program of subpulmonary ventricle retraining in this setting could reduce the postoperative complications between the two-stages of surgical treatment. It is not know if the response to intermittent systolic overload stimulus in adult animals will be similar, considering the clinical application of Jatene's operation in older children and teenagers.

In the clinical experience of Mavroudis & Backer [19], the mean time between conventional banding and conversion of the atrial repair to the Jatene operation was 15.6 months in patients with ages between 2 to 23 years. These authors demonstrated that left ventricular dysfunction occurred after the banding, as was confirmed by transesophageal echocardiography performed in the operating room. Indeed, the physiopathology involved in this procedure is the same as what occurs in LV rapid training, as pulmonary banding or systemic pressure will cause the same LV hypertrophic stimulus. LV dysfunction can occur over the long-term, probably related to the degree of abrupt systolic stress imposed on the ventricle during its retraining.

In the clinical experience of the Boston group, Boutin et al. [20] associated late left ventricular dysfunction to an extreme acute overload in patients submitted to Jatene's operation after LV rapid training. This dysfunction was inversely proportional to a quicker hypertrophy after PT banding.

Maybe our intermittent systolic overload model promotes a more physiological subpulmonary ventricular hypertrophy when compared to continuous banding, where the systolic stress is constant. With the proposal of a more efficient process, with intermittent overload in this setting, for the two-stage surgical treatment, it is possible to reduce the interval between the two operations and so reduce postoperative morbidity in preparing the left ventricle morphologically.

Future studies utilizing molecular biology as an instrument to analyze pathologic hypertrophic markers might compare the different training methods for the subpulmonary ventricle. As the pathologic overload stimulus is generally chronic while physiological overload is intermittent by nature, it is possible that qualitatively different overloads stimulate distinct hypertrophies in the heart, even if applied during similar periods of time, producing different phenotypic responses.

As the physiologic hypertrophy is characterized by normal or increased capillary density associated with little or no myocardial fibrosis, the morphological evaluation of suitable mechanisms of the contractile and non-contractile elements of the myocardium submitted to the acute hypertrophy process can also provide important contributions to the understanding of the fine adjustment in the preparation for Jatene's operation. The end point would be to minimize the cell lesions and maximize the efficiency of the pulmonary banding, thereby elucidating the best program of subpulmonary ventricular training.

CONCLUSIONS

Adjustable PT banding allows rapid RV hypertrophy in young goats, in groups submitted to a short period of continuous or intermittent RV systolic overload. The intermittent systolic overload allowed a more efficient hypertrophic process of the RV compared to the group submitted to continuous systolic overload, considering the greater septal hypertrophy. In spite of less systolic overload imposed on the RV of the Intermittent Group, the muscle mass acquired in this group was greater per unit of overload. There was no difference in the performance of the heart function between the two groups submitted to intermittent and continuous systolic overloads. The increase of mass identified in both study groups was probably due to an increased proteic synthesis and not to the accumulation of water in the heart tissues.

ACKNOWLEDGMENTS

Our thanks go to Dr. Gustavo José Justo da Silva, for his guidance with the statistical analysis and for his important suggestions which were included in this study.

REFERENCES

1. Jonas RA, Giglia TM, Sanders SP, Wernovsky G, Nadal-Ginard B, Mayer JE Jr, et al. Rapid two-stage arterial switch for transposition of the great arteries and intact ventricular septum beyond the neonatal period. Circulation. 1989;80(3 pt 1):I203-8.

2. Wernovsky G, Giglia TM, Jonas RA, Mone SM, Colan SD, Wessel DL. Course in the intensive care unit after 'preparatory' pulmonary artery banding and aortopulmonary shunt placement for transposition of the great arteries with low left ventricular pressure. Circulation. 1992;86(5 Suppl.):II133-9.

3. Takahashi Y, Nakano S, Shimazaki Y, Kadoba K, Taniguchi K, Sano T, et al. Echocardiographic comparison of postoperative left ventricular contractile state between one and two-stage arterial switch operation for simple transposition of the great arteries. Circulation. 1991;84(5 Suppl.):III180-6.

4. Ehsani AA, Hagberg JM, Hickson RC. Rapid changes in left ventricular dimensions and mass in response to physical conditioning and deconditioning. Am J Cardiol. 1978;42(1):52-6.

5. Dias CA, Assad RS, Caneo LF, Abduch MCD, Aiello VD, Dias AR, et al. Modelo experimental de bandagem ajustável do tronco pulmonar para preparo rápido do ventrículo. Rev Bras Cir Cardiovasc. 2000;15(4):328-37.

6. Caneo LF, Dias CA, Assad RS, Abduch MCD, Aiello VD, Moreira LFP, et al. Preparo do ventrículo subpulmonar através de dois diferentes modelos ajustáveis de bandagem do tronco pulmonar: estudo experimental. Rev Bras Cir Cardiovasc. 2001;16(1):35-48.

7. Pontes SC Jr., Assef JE, Barretto RB, Chaccur P, Moreira DA, Nina VJS, et al. Estimation of right ventricular mass by two-dimensional echocardiography. J Am Soc Echocardiogr. 2005;18(5):427-34.

8. Fulton RM, Hutchinson EC, Jones AM. Ventricular weight in cardiac hypertrophy. Br Heart J. 1952;14(3):413-20.

9. Perrino C, Prasad SV, Mao L, Noma T, Yan Z, Kim HS, et al. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. J Clin Invest. 2006;116(6):1547-60.

10. Carroll SM, Nimmo LE, Knoepfler PS, White FC, Bloor CM. Gene expression in a swine model of right ventricular hypertrophy: Intracellular adhesion molecule, vascular endothelial growth factor and plasminogen activators are upregulated during pressure overload. J Mol Cell Cardiol. 1995;27(7):1427-41.

11. Bishop SP, Melsen LR. Myocardial necrosis, fibrosis, and DNA synthesis in experimental cardiac hypertrophy induced by sudden pressure overload. Circ Res. 1976;39(2):238-45.

12. Siehl DL, Gordon EE, Kira Y, Chua BHL, Morgan HE. Protein degradation in the hypertrophic heart. In: Glaumann H, Ballard FJ, eds. Lysosomes: their role in protein breakdown. London:Academic;1987.

13. Zimmer HG, Ibel H, Gerlach E. Significance of the hexose monophosphate shunt in experimentally induced cardiac hypertrophy. Basic Res Cardiol. 1980;75(1):207-13.

14. Takahashi Y, Nakano S, Shimazaki Y, Kadoba K, Taniguchi K, Sano T, et al. Echocardiographic comparison of postoperative left ventricular contractile state between one and two-stage arterial switch operation for simple transposition of the great arteries. Circulation. 1991;84(5 Suppl.):III180-6.

15. White FC, Nakatani Y, Nimmo L, Bloor CM. Compensatory angiogenesis during progressive right ventricular hypertrophy. Am J Cardiovasc Pathol. 1992;4(1):51-68.

16. Kassab GS, Imoto K, White FC, Rider CA, Fung YC, Bloor CM. Coronary arterial tree remodeling in right ventricular hypertrophy. Am J Physiol. 1993;265(1 Pt 2):H366-75.

17. Le Bret E, Lupoglazoff JM, Borenstein N, Fromont G, Laborde F, Bachet J, et al. Cardiac "fitness" training: an experimental comparative study of three methods of pulmonary artery banding for ventricular training. Ann Thorac Surg. 2005;79(1):198-203.

18. Borenstein N, Jian Z, Fromont G, Bruneval P, Hekmati M, Behr L, et al. Noncultured cell transplantation in an ovine model of right ventricular preparation. J Thorac Cardiovasc Surg. 2005;129(5):1119-27.

19. Mavroudis C, Backer CL. Arterial switch after failed atrial baffle procedures for transposition of the great arteries. Ann Thorac Surg. 2000;69(3):851-7.

20. Boutin C, Wernovsky G, Sanders SP, Jonas RA, Castaneda AR, Colan SD. Rapid two-stage arterial switch operation. Evaluation of left ventricular systolic mechanics late after an acute pressure overload stimulus in infancy. Circulation. 1994;90(3):1294-303.

Rev Bras Cir Cardiovasc 2001;
16(1): 35-48.

Preparo do ventrículo subpulmonar através de dois diferentes modelos ajustáveis de bandagem do tronco pulmonar: estudo experimental

Luiz Fernando CANÊO*, Carlos A. DIAS*, Renato Sammy ASSAD*, Maria Cristina Donadio ABDUCH*, Vera Demarchi AIELLO*, Luiz Felipe Pinho MOREIRA*, Domingos D. LOURENÇO FILHO*, Noedir A. G. STOLF*

RBCCV 44205-528

Canêo L F, Dias C A, Assad R S, Abduch M C D, Aiello V D, Moreira L F P, Lourenço Filho D D, Stolf N A G - Preparo do ventrículo subpulmonar através de dois diferentes modelos ajustáveis de bandagem do tronco pulmonar: estudo experimental. *Rev Bras Cir Cardiovasc* 2001; **16**(1): 35-48.

RESUMO: Dois modelos de dispositivos ajustáveis foram implantados em cabritos jovens, com o objetivo de avaliar suas capacidades de bandagem do tronco pulmonar (TP) e eficiência no treinamento do ventrículo subpulmonar. Foram utilizados 3 grupos de 7 animais, submetidos ao implante de cateter balão (grupo I), implante de dispositivo de bandagem externa (grupo II) e para obtenção dos pesos dos ventrículos direito (VD), esquerdo (VE) e septo interventricular em condições normais (grupo controle). Os animais foram submetidos a ajustes progressivos dos dispositivos, a cada 24 horas, durante um período de 96 horas, avaliados através de monitorização hemodinâmica e ecocardiograma seriado. A hipertrofia ventricular foi avaliada através de parâmetros morfológicos e de microscopia óptica. Todos os animais concluíram o período de treinamento proposto. O gradiente VD-TP, a razão VD/VE e a pressão sistólica do VD foram significativamente maiores no grupo II ($p<0,05$). Observou-se aumento significativo da espessura da parede do VD, ao ecocardiograma, nos grupos I e II ao longo dos momentos avaliados, não havendo diferença significativa entre eles. O peso indexado e o peso seco do VD foi significativamente maior nos grupos I e II que no grupo controle ($p<0,05$), não havendo diferença significativa entre os grupos I e II ($p>0,05$). O perímetro e a área dos miócitos mostraram um aumento significativo no final do treinamento, comparados à amostra retirada no momento do implante dos dispositivos. Observamos maior grau de estenose do TP com dispositivo de bandagem externa, porém ambos tiveram a capacidade e a eficiência de preparar o ventrículo subpulmonar.

DESCRITORES:Ventículo cardíaco, cirurgia. Artéria pulmonar, cirurgia. Procedimentos cirurgicos cardiovasculares, métodos. Hipertrofia ventricular esquerda,etiologia. Ventrículo cardíaco, fisiologia. Hipertrofia ventricular esquerda, cirurgia. Obstrução do flluxo ventricular externo.Transposição das grandes artérias, cirurgia.

Trabalho realizado no Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. São Paulo, SP, Brasil.
Recebido para publicação em novembro de 2000.
* Do Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo.
Endereço para correspondência: Luiz Fernando Canêo. Av. Dr. Enéas de Carvalho Aguiar, 44 – Divisão Cirúrgica. São Paulo, SP, Brasil. CEP: 05403-000. Tel. (11)3069-5432. e-mail: dcicaneo@incor.usp.br

INTRODUÇÃO

Nos pacientes portadores de transposição das grandes artérias (TGA), o ventrículo esquerdo (VE) está conectado à circulação pulmonar. Nas primeiras semanas após o nascimento, há uma redução relativa da massa do VE quando comparada à massa do ventrículo direito (VD), em decorrência da diminuição progressiva da resistência vascular pulmonar [1,2]. Conseqüentemente, dentro das primeiras semanas, o VE perde a sua capacidade de manter um débito cardíaco adequado quando trabalhando com a pós-carga sistêmica normal. A operação de Jatene, atualmente aceita como a primeira escolha para o tratamento da TGA [3-5], deve ser realizada numa fase onde o VE ainda está adaptado, com sua massa ventricular preservada, com capacidade de suportar a resistência vascular sistêmica [1].

Sempre que essa capacidade está comprometida, a bandagem do tronco pulmonar (TP) poderá induzir a hipertrofia do VE, ou seja do ventrículo subpulmonar na TGA, podendo então prepará-lo para a operação de Jatene [6,7]. O preparo do ventrículo subpulmonar através da bandagem, seguido da operação de Jatene, pode ser aplicado ainda nos pacientes já submetidos à correção no plano atrial (operação de Senning ou Mustard), que evoluíram com disfunção do ventrículo sistêmico, ou seja do VD [8].

Na primeira fase do preparo ventricular observa-se grande morbidade decorrente da bandagem do TP [9-12], que pode impor uma sobrecarga de pressão não tolerada pelo ventrículo, causando disfunção no mesmo. Isto se deve à dificuldade em ajustar o diâmetro da bandagem do TP, realizada em condições não fisiológicas, com o paciente anestesiado, em decúbito dorsal e com o tórax aberto.

O ajuste da bandagem do TP durante o período pós-operatório, realizado externamente, sem a necessidade de reabertura do tórax, foi descrito por vários autores [13] e devemos destacar 2 modelos: os dispositivos de bandagem externa [13-21] e o balão de oclusão do tronco pulmonar (dispositivo de bandagem interna) [22-27].

Estes dispositivos foram aplicados em 2 situações: controle do hiperfluxo pulmonar e no preparo do ventrículo subpulmonar para a operação de Jatene. Quando aplicados com o objetivo de induzir a hipertrofia ventricular, observamos que os regimes de preparo do ventrículo subpulmonar variam entre os diversos estudos, assim como os critérios de avaliação dessa hipertrofia.

Considerando as vantagens teóricas dos 2 tipos de dispositivos e a variação de tempo necessário para o preparo obtido com eles, resolvemos estudá-los, comparativamente, com o intuito de avaliar a capacidade em promover a estenose do tronco pulmonar e de realizar o preparo do ventrículo subpulmonar dos mesmos.

MATERIAL E MÉTODOS

Foram estudados 21 cabritos machos, com idades entre 4 e 8 semanas de acordo com as normas de uso de animais em ensino e pesquisa da Divisão de Experimentação do Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, onde foi realizado.

O experimento consistiu no preparo do ventrículo subpulmonar, representado pelo VD nesse estudo experimental, através de 2 tipos de bandagens do TP, em 14 animais, divididos em 2 grupos:

- ✔Grupo I – bandagem interna: 7 animais submetidos ao implante de um cateter balão.
- ✔Grupo II – bandagem externa: 7 animais submetidos ao implante de um dispositivo de bandagem externa.

Como grupo controle para obtenção dos pesos do ventrículos direito, esquerdo e septo, em condições normais, foram utilizados os outros 7 cabritos.

No grupo I o dispositivo de bandagem interna utilizado foi o cateter balão descrito por ASSAD et al. [27] e no grupo II o dispositivo de bandagem externa descrito por DIAS [13].

O animais foram anestesiados com pentobarbital sódico (5 a 10 mg/kg) e midazolan (0,5 mg/kg) e intubados com tubo traqueal de calibre adequado. Instituiu-se a ventilação mecânica controlada (ventilador Harvard 708, South Natik, MA, EUA), mantida com fração inspirada de oxigênio em 100% e volume corrente de 15ml/kg. A manutenção da anestesia foi realizada através da administração de doses suplementares de pentobarbital sódico e midazolan conforme necessário. A monitorização padronizada consistiu de eletrocardiografia e registro de pressão arterial, obtida após a colocação do cateter na aorta, e registrada continuamente através de sistema computadorizado (Biopac Systems, Inc., Goleta, CA, EUA) e software de interpretação ACQknowledge 3.01.

A antibioticoterapia profilática foi feita com cefalotina (100 mg/kg/dia) e gentamicina (2mg/kg/dia) por via endovenosa. Antes da colocação dos cateteres foi administrada heparina por via endovenosa (500 U/kg).

A operação foi realizada por toracotomia lateral E, no quarto espaço intercostal.

Nos 2 grupos foram colocados cateteres para a monitorização das pressões da aorta, VD e TP. Devemos salientar que, embora o cateter balão apresentasse 2 vias que permitiam a monitorização das pressões do VD e do TP, preferimos excluir essas vias e usar os mesmos cateteres necessários para essas medidas, no grupo II (dispositivo de bandagem externa). O objetivo foi uniformizar as pressões medidas em ambos os grupos, diminuindo a chance de erros devido ao mau posicionamento do cateter balão ou obstrução das vias de medida de pressão desse dispositivo, mais finas que os cateteres de PVC.

Os cateteres para a monitorização das pressões da aorta, VD e TP foram colocados por punção direta desses vasos e exteriorizados por contrabertura no dorso do animal e mantidos heparinizados e ocluídos.

Após a abertura do pericárdio o VD foi tracionado gentilmente para realização de biópsia de sua parede anterior (3 amostras), que foram enviadas para estudo histológico.

No grupo I, foi realizada uma sutura em bolsa com prolene 5-0 na via de saída do VD, cerca de 3cm abaixo do anel pulmonar, por onde foi introduzido o cateter balão. Uma vez posicionado com sua extremidade distal no interior do TP, o mesmo foi exteriorizado por contrabertura na região dorsal do animal próximo aos demais cateteres de monitorização já citados.

No grupo II, o TP foi amplamente dissecado e o anel de bandagem colocado externamente a ele, abraçando-o acima da valva pulmonar. O tubo extensor do anel foi exteriorizado por contrabertura, no espaço intercostal inferior à incisão e levado até o dorso do animal através de um túnel no subcutâneo, onde o botão de insuflação do dispositivo foi implantado.

Antes do fechamento da toracotomia, foi realizada a insuflação do cateter balão através da injeção de água destilada no cateter ou insuflação do manguito do dispositivo de bandagem externa, observando o seu correto funcionamento pelas curvas de pressão no sistema de monitorização acoplado aos cateteres.

A cavidade torácica foi então drenada sob selo de água e a incisão fechada por planos. Foi realizado o curativo e proteção da ferida com coletes apropriados.

Após o implante dos dispositivos de bandagem ajustáveis os animais foram mantidos no biotério em gaiolas individuais, recebendo heparina por via subcutânea (2500 U) e antibioticoterapia por via intramuscular (cefazolina 500 mg/kg e gentamicina 10 mg/kg de 12 em 12h), durante as 96h subseqüentes.

Quando os animais já se encontravam em condições clínicas estáveis, isto é, 1 a 3 dias após o implante, dava-se início à insuflação dos dispositivos.

Após a primeira insuflação dos dispositivos implantados nos animais do grupo I, ou do primeiro ajuste do dispositivo de bandagem externa, foi administrado lanatosídeo-C 0,5 μg/kg/dia por via intramuscular, diariamente, até o sacrifício do animal.

O Ajuste da Bandagem

Após um período de convalescença que variou de 1 a 3 dias, os animais recuperados da cirurgia do implante dos dispositivos e cateteres, estáveis clinicamente, alimentando-se e ativos, foram levados para a sala de experimentação em macas especiais para quadrúpedes (Divisão de Bioengenharia, InCor-HC-FMUSP). Nessas macas, foram mantidos acordados em decúbito ventral, sem nenhum tipo de sedação, com as patas suspensas.

Na sala de experimentação os cateteres implantados foram então conectados ao sistema de pressão (ACQknowledge 3.01, Biopac Systems, Inc., Goleta, CA, EUA), monitorando simultaneamente as pressões do VD, Ao e TP. Observando-se as curvas de pressões, iniciavam-se os ajustes dos dispositivos de bandagem. A insuflação dos 2 dispositivos foi realizada através da introdução de água destilada na via de insuflação do balão do cateter do grupo I ou pela punção percutânea, com agulha de insulina, do botão de insuflação autoselante do dispositivo de bandagem externa. Os critérios iniciais de ajuste foram: manter a pressão sistólica do VD entre 70 a 80% do VE, desde que a queda da pressão sistólica da aorta não diminuísse mais que 10 a 15% ou o animal apresentasse algum sinal de baixo débito (irritabilidade, taquidispnéia ou síncope). Uma vez terminado o ajuste, o animal retornava ao biotério, onde ficava acomodado em gaiola individual. A cada 24h do primeiro ajuste, o animal retornava à sala de experimentação, registravam-se as pressões do VD, Ao e TP, simultaneamente e novo ajuste era feito, observando os mesmos critérios iniciais. Essa rotina se repetiu durante um período de 96h em cada um dos 14 animais.

Durante todo o treinamento dos ventrículos foram registradas as pressões sistólica, diastólica e média do VD, aorta e TP, antes e após o ajuste dos dispositivos.

O gradiente sistólico entre o VD e o TP (gradiente VD/TP) foi calculado subtraindo-se a pressão sistólica do TP da pressão sistólica do VD. A razão

entre a pressão sistólica do VD e a pressão sistólica do VE (razão VD/VE) foi calculada dividindo-se a pressão sistólica do VD pela pressão sistólica da aorta e expressa em porcentagem.

Avaliação Ecocardiográfica

Os exames ecocardiográficos foram realizados com aparelho Ultramark 4 (Advanced Technology Laboratories, Bothell, WA, EUA), utilizando-se transdutores de 2,5 e 5 mHz, apropriados para o tamanho do animal.

Os exames foram realizados em modo M, para a medida das espessuras das paredes dos ventrículos e septo; para a medida dos gradientes sistólicos entre o VD e o TP foi utilizado o Doppler contínuo. Esse exame foi feito com o animal acordado, sem nenhum tipo de sedação, através da via para-esternal direita, sempre pelo mesmo observador, um veterinário especializado em ecocardiografia. A espessura da parede e do septo foram avaliadas em corte transverso, ao nível dos músculos papilares. O gradiente entre o VD e o TP foi também obtido em corte transverso, ao nível dos vasos da base, alinhando-se a via de saída do VD com o TP.

Após a chegada dos animais ao biotério foi realizado o primeiro ecocardiograma para avaliação das espessuras das paredes dos ventrículos e septo. Os animais que apresentavam espessura do VD semelhante à do VE eram devolvidos ao criador. Imediatamente após o procedimento cirúrgico foi realizado novo exame ecocardiográfico, para determinar a posição do cateter ou do dispositivo de bandagem externa, verificando a presença de gradiente transvalvar pulmonar e o aumento na espessura da parede do VD. Os animais que apresentavam gradiente VD/VE significativamente maior que a média obtida com o estudo piloto eram excluídos do experimento.

No primeiro dia de início do treinamento do ventrículo subpulmonar, foi realizado um ecocardiograma com o animal acordado, em repouso, com o cateter balão ou o dispositivo de bandagem externa desinsuflado. Após a primeira insuflação e diariamente após cada ajuste, realizava-se novo exame ecocardiográfico. Isso se repetiu a cada 24h durante um período de 96h, em cada um dos 14 animais.

Em todos os exames foram observados os seguintes parâmetros:

- as espessuras das paredes ventriculares direita, esquerda e septo
- o gradiente transvalvar pulmonar

Avaliação Morfológica

Após 96 horas de treinamento do ventrículo subpulmonar os animais foram sacrificados seguindo as normas de uso de animais em ensino e pesquisa da Divisão de Experimentação do Instituto do Coração do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo.

Ainda no biotério, foi administrada quetamina via intramuscular (5mg/kg) como medicação pré-anestésico e na sala de cirurgia, após punção da veia jugular com cateter venoso percutâneo, foram administrados nembutal (15 mg/kg), heparina 500 U/kg e cloreto de potássio (20ml), por via endovenosa.

O coração foi então retirado através da secção dos vasos da base, veias cavas e pulmonares. A separação das paredes ventriculares e septo foi realizada conforme a técnica descrita por FULTON et al. [28], em 1952. As estruturas atriais foram ressecadas no plano das valvas atrioventriculares. A seguir, foi realizada a secção da via de saída do VD rente ao septo na porção basal. Em seguida, realizou-se a separação da parede livre do VD, com tesoura, rente ao septo indo da porção basal até o ápice na face anterior. O mesmo movimento foi realizado na face diafragmática seccionando totalmente a parede livre do VD. A separação do septo da parede livre do VE foi feita com tesoura, rente ao septo e acompanhando a sua espessura.

Após o sacrifício do animal e retirada do coração em bloco, a valva pulmonar e o TP foram observados para a presença de lesão endotelial no local do balão ou do manguito, presença de trombos em seu interior ou vegetações.

Para comparar os pesos do septo e dos ventrículos foram sacrificados os 7 animais do grupo controle, seguindo a mesma rotina dos 2 grupos de animais treinados.

As paredes livres do VD, VE e o septo interventricular dos animais dos grupos treinados (grupo I e grupo II) e do grupo controle foram pesados em balança de precisão (METTLER AE 200, Mettler-Toledo AG, Greinfense, Suiça), considerando até centésimos de grama. Após serem pesados, foram retirados fragmentos com cerca de 4x4cm de cada uma das paredes e enviados para obtenção do peso seco, como descreveremos adiante. O restante do material foi fixado em formalina tamponada a 10%, durante um período de 24 h e encaminhados para a microscopia óptica.

No intuito de diminuir as variações de peso dos animais, permitindo uma melhor comparação entre eles, calculamos o respectivo peso indexado, conforme sugerido por BISHOP et al. [29]. Esse valor resul-

tou da divisão dos pesos dos ventrículos e septo (em gramas) obtidos, como descrito anteriormente, e divididos pelo peso do animal (em kg). Foram denominados: peso do VD indexado, peso do VE indexado e peso do septo indexado, e expressos em g/kg.

Com a finalidade de esclarecer quanto da variação da massa observada nos corações treinados, comparada aos corações do grupo controle, se deu por aumento de água, ou seja, por um possível edema, o peso seco foi calculado para cada um dos ventrículos e septo interventricular dos animais dos grupos I, II e controle. Fragmentos medindo 4x4cm foram retirados dos ventrículos e septo dos corações treinados e do grupo controle, logo após a pesagem para obtenção dos respectivos pesos indexados. Foram então pesados, acondicionados em frascos apropriados, fechados com papel filtro, devidamente identificados e mantidos em estufa seca a 55-60 graus centígrados por um período de 6 dias. Após 6 dias, os fragmentos foram retirados da estufa e novamente pesados. Assumindo que a distribuição de água foi homogênea no septo e em cada um dos ventrículos, pode-se calcular o peso seco dos mesmos, utilizando a fórmula: $Ps = Ps\ frag \times P0 \times (P0\ frag)^{-1}$

Onde:

- ✔ Ps = peso seco do VD, VE ou septo
- ✔ Ps frag = peso seco do fragmento do VD, VE ou septo
- ✔ P0 = peso inicial das paredes livres do VD, VE ou septo
- ✔ P0 frag = peso inicial do fragmento do VD, VE ou septo

Os fragmentos do VD retirados no intra-operatório e o restante dos fragmentos dos ventrículos e septo dos corações obtidos após o sacrifício do animal foram fixados em formalina tamponada a 10% por um período de 24h e encaminhados para microscopia óptica.

Após processamento histológico de rotina, secções de 5μm foram coradas com hematoxilina e eosina. Na microscopia óptica (Leica DLMS), com aumento de 400x, foram selecionados os campos em que os miócitos estavam em corte transversal ao nível do núcleo [30] para a medida de seus perímetros e áreas. O sistema de análise de imagem utilizado foi o Quantimet500 (Quantimet-Leica, Leica Cambridge Ltd., Cambridge, Reino Unido).

Nas lâminas dos fragmentos obtidos por biópsia intra-operatória (antes do treinamento ventricular) foram escolhidos quatro campos por animal e após o sacrifício (após o treinamento) foram escolhidos três campos por animal, onde analisamos 50 miócitos por lâmina.

Análise Estatística

A hipótese de igualdade de médias foi verificada fazendo uso da Análise de Variância a um fator [31], com comparações múltiplas feitas através do teste de Newman-Keuls. Para averiguar o comportamento dos grupos considerando as condições estudadas fez-se uso da técnica Análise de Perfil [32]. O nível de significância utilizado para os testes foi de 5%.

RESULTADOS

Os animais mantiveram-se ativos durante todo o experimento e não manifestaram sinais de disfunção do VD. Não houve óbito nos grupos I e II e todos completaram as 96 horas. Não observamos lesões na valva pulmonar e no TP, assim como a presença de trombos ou vegetações. Em 2 casos do grupo I, observou-se pequena quantidade de fibrina na ponta do cateter.

No grupo de bandagem externa (grupo II), os critérios objetivos de ajuste determinados previamente (queda de no máximo 10% da pressão sistólica da aorta e ou a razão VD/VE ser de 80%) foram seguidos na maior parte das vezes, enquanto no grupo I nem sempre isso foi possível.

O dispositivo de bandagem interna apresentou maior instabilidade, deslocando-se dentro do TP em 3 animais, durante a movimentação dos mesmos, desencadeando alterações hemodinâmicas importantes. Essas alterações hemodinâmicas foram acompanhadas, clinicamente, de sinais de baixo débito, com irritabilidade, taquidispnéia acentuada e síncope (1 dos animais apenas). Sempre que isso ocorreu, foi realizada a desinsuflação do balão, aguardando-se a estabilização hemodinâmica do animal. Assim que o animal apresentasse os parâmetros hemodinâmicos estáveis, realizava-se novo ajuste do balão, impondo uma sobrecarga sistólica menor que a anterior, porém superior à inicialmente encontrada.

A comparação das médias dos gradientes VD-TP mostrou um comportamento diferente dos grupos ao longo do experimento. O gradiente VD-TP inicial foi significativamente maior no grupo II e esta diferença ainda permaneceu no momento final. O grupo I apresentou um aumento do gradiente VD-TP progressivamente maior, ao longo dos momentos avaliados e o mesmo foi observado no grupo II (Gráfico 1).

As médias das razões VD/VE do grupo I mostraram-se significativamente menores que as observadas no grupo II, ao longo dos momentos avaliados. O grupo II apresentou média inicial significativamente maior que o grupo I e esta diferença foi

GRÁFICO 1

EVOLUÇÃO DAS MÉDIAS DOS GRADIENTES VD-TP (GVD/TP) DOS GRUPOS I E II

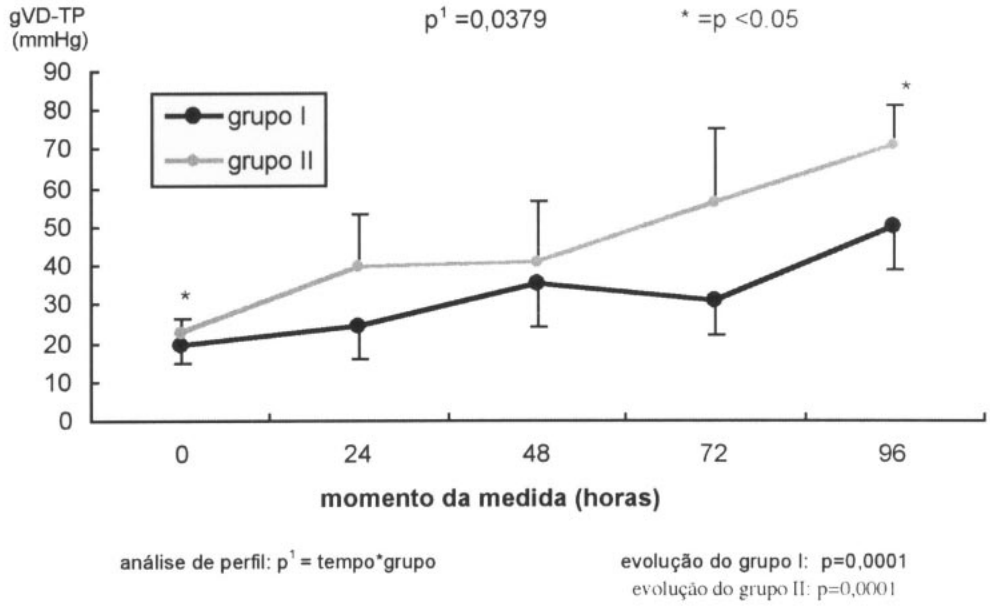


TABELA 1

COMPARAÇÃO DAS ESPESSURAS DAS PAREDES DO VD, VE E SEPTO INTERVENTRICULAR AVALIADAS PELO ECOCARDIOGRAMA DURANTE O EXPERIMENTO

ESPESSURA	GR	0h	24h	48h	72h	96h	VALORES DE p
VD (mm)	I	3,86±0,69	3,86±0,69	4,57±1,40	5,71±2,29	6,86±1,95	0,99983[a]/0,3554[b]/0,0004[c]
	II	4,43±0,53	4,43±0,53	5,14±0,90	6,14±1,07	7,29±1,70	
VE (mm)	I	5,29±0,49	5,14±0,38	5,14±0,38	5,14±0,38	5,14±0,38	0,5788[a]/0,6695[b]/0,2752[c]
	II	5,43±0,53	5,29±0,49	5,14±0,38	5,29±0,49	5,14±0,38	
Septo (mm)	I	5,29±0,49	5,29±0,49	5,14±0,69	5,29±0,49	5,29±0,49	0,2671[a]/0,2766[b]/0,2671[c]
	II	4,86±0,38	4,86±0,38	4,86±0,38	5,29±0,49	5,14±0,38	

mantida durante todo o experimento. Tanto para o grupo I, como para o grupo II, há um aumento progressivo da razão VD/VE ao longo dos momentos avaliados.

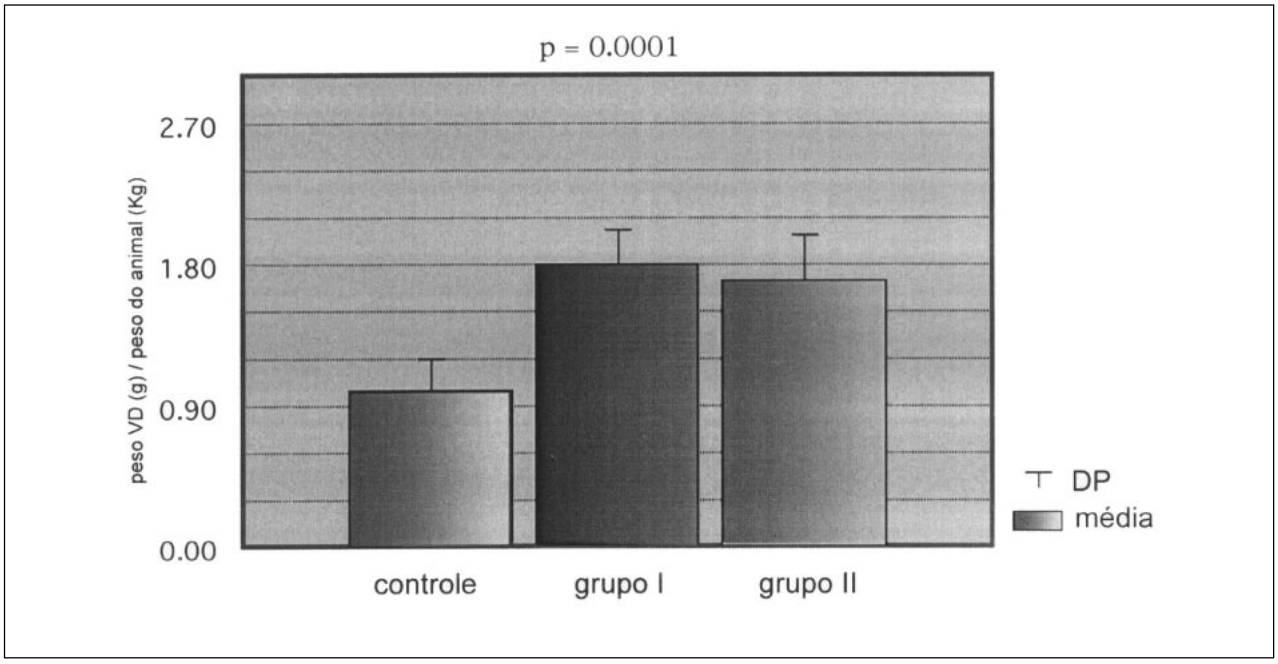
As médias das pressões sistólicas do VD do grupo I e do grupo II se comportaram de forma diferente ao longo dos momentos avaliados. Os grupos não apresentaram diferença significativa das médias no momento inicial, mas esta diferença ocorreu ao longo do tempo e, também, no momento final. Observamos um aumento progressivo dessas pressões em ambos os grupos ao longo dos momentos avaliados (p=0,0001).

A evolução das curvas das médias das pressões sistólicas do TP dos grupos I e II, se comportou de forma semelhante ao longo dos momentos avaliados. As médias dos 2 grupos não apresentaram diferença significativa durante todo o experimento, mas observamos um efeito de tempo, isto é, houve uma diferença significativa das médias ao longo dos momentos avaliados para os 2 grupos (p=0,0285).

Na evolução das médias dos gradientes VD-TP dos grupos I e II, avaliadas ao ecocardiograma, podemos observar que os grupos se comportaram de forma diferente ao longo dos momentos avalia-

GRÁFICO 2

COMPARAÇÃO DAS MÉDIAS DOS PESOS DO VD INDEXADO DOS GRUPOS I, II E CONTROLE



dos (p=0,0045), porém não apresentaram diferença entre os momentos 0, 24 e 96. Por outro lado, houve diferença significativa nos momentos 48 e 72. Houve um aumento progressivo das médias dos gradientes VD-VE ao longo dos momentos avaliados, em ambos os grupos (p=0,0001).

As espessuras das paredes livres do VD, VE e septo interventricular medidas ao ecocardiograma podem ser vistas na Tabela 1.

As espessuras das paredes do VD se comportaram de forma semelhante, nos grupos I e II ao longo dos momentos avaliados, as médias de ambos os grupos não apresentaram diferença significativa durante todo o experimento, porém existiu um efeito de tempo, isto é, houve aumento progressivo da espessura da parede do VD ao longo dos momentos avaliados para os 2 grupos (p=0,0004).

A evolução da espessura do VE, nos grupos I e II se comportou de forma semelhante ao longo dos momentos avaliados. As médias dos 2 grupos não apresentaram diferença significativa nos momentos avaliados e não ocorreu efeito de tempo, isto é, não houve diferença significativa das médias ao longo do experimento para os grupos I e II (p=0,2752).

O mesmo comportamento foi observado na evolução das espessuras do septo interventricular, isto é, as médias dos grupos I e II foram semelhantes ao longo do tempo. Não houve diferença significativa entre os grupos e não houve aumento significativo da espessura do septo em nenhum dos 2 grupos, durante todo o experimento (p=0,2671).

O peso do VD indexado ao peso do animal foi de 1.79±0.22 g/kg no grupo I, 1.69±0.30 g/kg no grupo II e 1.02±0.21 g/kg no grupo controle. A análise simples de variância mostra um significativo aumento do peso médio do VD indexado ao peso do animal dos grupos I e II quando comparados ao peso médio do VD do grupo controle (p=0,0001), não havendo diferença significativa entre os grupo I e II (p>0,05), (Gráfico 2).

Quando comparamos o peso do septo indexado ao peso do animal não observamos diferença significativa entre os grupos. O mesmo acontece com os pesos médios do VE indexado ao peso do animal, que não apresentam diferenças significativas entre os grupos.

O peso seco do VD foi significativamente maior nos grupo I (2.62±0.53 g) e grupo II (2.42±0.19 g) que no grupo controle (1.63±0.31 g) (p=0,0006), não havendo diferença significativa entre os grupos I e II. O peso seco da parede livre do VE e o peso seco do septo interventricular não apresentaram diferença significativa entre os grupos I, II e controle.

O perímetro médio e a área dos miócitos nos grupos I e II observado no final do treinamento ventricular mostraram um aumento significativo quando comparados aos da amostra retirada no momento do implante dos dispositivos.

COMENTÁRIOS

Muitos defeitos congênitos são, atualmente, corrigidos no período neonatal e início da infância. Em todos esse casos, o tratamento cirúrgico promove uma melhora importante da função cardiovascular após a correção, quando comparado ao quadro clínico no período pré-operatório. Por exemplo, um recém-nascido portador de TGA é submetido, atualmente, de forma rotineira à operação de Jatene, com melhora imediata da cianose e, na maioria dos casos, com um período pós-operatório sem intercorrências [4,33,34]. Em contraste com os casos onde realizamos a correção do defeito congênito, a bandagem do TP empregada como procedimento preparatório para a operação de Jatene não resulta em uma melhora clínica da criança. Na verdade, impõe ao ventrículo uma sobrecarga de pressão importante, muitas vezes não bem tolerada pelo paciente. O período pós-operatório imediato à bandagem é bastante crítico para o paciente, onde observamos disfunção ventricular aguda, baixo débito, acidose metabólica e necessidade de suporte de drogas inotrópicas. Isto ocorre até o terceiro dia após a bandagem do TP, quando observamos um aumento significativo da massa do VE [35]. A disfunção cardiovascular observada se deve à sobrecarga de pressão agudamente imposta ao ventrículo subpulmonar (VE) e, quando associamos uma comunicação sistêmico-pulmonar, a sobrecarga volumétrica e de pressão imposta ao ventrículo sistêmico (VD) contribui com essa disfunção [10]. Existem vários modelos experimentais de aquisição de massa tanto do VD como do VE, através da sobrecarga aguda de pressão. Entretanto, nesses modelos animais de bandagem aórtica ou do TP, o débito cardíaco depende diretamente da manutenção do volume sistólico pelo ventrículo conectado ao vaso onde foi realizada a bandagem, o que limita a severidade da estenose que pode ser agudamente imposta ao mesmo [35]. No modelo proposto neste estudo, o ajuste progressivo da bandagem do TP, através dos dispositivos ajustáveis, permitiu a imposição de uma sobrecarga de pressão ao ventrículo conectado ao vaso onde foi realizada a bandagem, suficiente para obter a hipertrofia ventricular, sem causar disfunção do mesmo. Com o passar do tempo o ventrículo foi ganhando massa e suportando cada vez mais pressões intraventriculares maiores, como podemos observar na curva de pressão do VD e na curva da razão VD/VE.

Estudos clínicos prévios [7,36,37], utilizando a bandagem convencional, demonstraram hipoxemia e acidose metabólica e a necessidade de realização de uma comunicação sistêmico-pulmonar em grande parte dos casos. KATAYAMA et al. [25], em 1993, demonstraram pequena hipoxemia nos animais estudados, bastante tolerada e rapidamente corrigida com a desinsuflação do cateter balão, utilizado em seu estudo. Com o ajuste progressivo dos dispositivos de bandagem ajustáveis, sem comprometimento do débito cardíaco, o fluxo pulmonar mantém-se adequado, e nossos dados sugerem que a comunicação sistêmico-pulmonar pode ser dispensada.

Vários tipos de cateter balão foram utilizados para a obstrução ao fluxo do ventrículo pulmonar. Dentre eles, o mais utilizado foi o cateter angiográfico de Berman, disponível comercialmente, e bastante comum nos laboratórios de hemodinâmica. Os estudos experimentais de BONHOEFFER et al. [23], KRZESKI et al. [24] e KATAYAMA et al. [25], mais tarde, os estudos clínicos de BONHOEFFER et al. [38] utilizaram o cateter de Berman, que simulou a bandagem do TP com bastante eficiência. Esse cateter dispõe de um orifício apenas, abaixo do balão, que permite a monitorização da pressão proximal, após a oclusão do vaso, com a insuflação do balão. BONNET et al. [26], em 1997 e ASSAD et al. [27], em 1998 apresentaram um cateter especialmente desenhado para preparar o ventrículo subpulmonar para a operação de Jatene. Esses cateteres apresentam várias vias, que permitem a monitorização simultânea das pressões do VD e do TP, além da insuflação do balão. O cateter apresentado por BONNET et al. [26] não está comercialmente disponível. Não encontramos maiores detalhes sobre o fabricante (Numed), de maneira que não foi possível considerar a sua utilização. Já o cateter apresentado por ASSAD et al. [27] foi desenvolvido na Divisão de Experimentação do InCor-HC-FMUSP, a partir de um cateter de Swan-Ganz, disponível comercialmente (Baxter Healthcare Co., Irvine, CA, USA), e apresenta as mesmas características atrativas do apresentado por BONNET et al. [26] :as 2 vias de monitorização das pressões distal e proximal. A disponibilidade desse cateter nos permitiu a sua utilização com grande facilidade, ao mesmo tempo que se mostrou eficiente no cumprimento do objetivo proposto neste estudo.

Dentre os vários modelos descritos na literatura, o dispositivo de bandagem externa, utilizado nesse estudo, foi descrito por DIAS [13] em 1998, que mostrou ser de fácil colocação, biocompatível e bastante simples. Os projetos baseados em torniquetes com metal [14] ou com fios ou fitas registraram acidentes causados por lesão do TP [16,17,21,39]. O desenho do dispositivo, utilizado neste experimento no grupo II, está baseado no conceito

de manguito idealizado pela primeira vez por JACOBSON & MACALLISTER [40], em 1957.

Além do risco de lesar o TP, outro grande problema descrito com esses dispositivos foram os vazamentos apresentados nas conexões das partes que compõem o sistema. O dispositivo que utilizamos apresenta duas peças soldadas em silicone medicinal, e uma única conexão: a do tubo extensor com o botão de insuflação. Esta conexão pode ser amarrada, o que diminui bastante a chance de ocorrer vazamentos. Diferencia-se também dos projetos anteriores por apresentar um reforço deliberado na espessura do anel externo do manguito. Isto faz com que o manguito, quando insuflado, ocupe o espaço interno do anel e a bandagem seja efetiva com a injeção de pequenas quantidades de líquido, como observado em nossos experimentos. Esse dispositivo foi desenvolvido, também, em nosso laboratório de experimentação, o que nos permitiu a sua utilização com grande facilidade.

O cateter balão ainda está em fase de experimentação, necessitando de algumas melhorias no seu desenvolvimento, para sua utilização na clínica. O dispositivo de bandagem externa se encontra totalmente pronto para ser implantado clinicamente. Existe a possibilidade de se utilizar o anel de bandagem com diferentes diâmetros, o que permite a sua implantação nos mais variados diâmetros do TP.

Tanto o dispositivo de bandagem externa quanto o balão de oclusão do TP foram ajustados com a introdução de água destilada, para a insuflação do manguito ou do balão. A quantidade de líquido usada para insuflar o manguito foi de 0,1 a 0,4 ml e de 0,5 a 2,0 ml para insuflar o balão. Esta variação é explicada pelos diferentes calibres do TP encontrados entre os animais.

PARK et al. [18], em seu projeto, utilizaram uma válvula unidirecional para reter o líquido no manguito e evitar vazamentos. Este recurso retira uma das melhores virtudes dos dispositivos utilizados em nosso estudo: a reversibilidade da bandagem.

Ao realizarmos as medidas de pressão durante as 96 horas de implante do dispositivo, encontramos algumas vezes medidas menores que as observadas no final do ajuste, no dia anterior. Para explicar esses dados, podemos considerar quatro possibilidades:

1. disfunção ventricular aguda causada pelo aumento súbito de pressão no VD.
2. adaptação fisiológica das resistências sistêmica e pulmonar
3. dilatação do TP no grupo I ou afrouxamento da fixação do manguito em torno do TP no grupo II.
4. possível vazamento do balão ou da conexão do botão-autoselante com o tubo extensor. Mesmo considerando esta última possibilidade, as variações foram facilmente corrigidas com o ajuste subseqüente dos dispositivos.

O objetivo do estudo foi obter hipertrofia do ventrículo subpulmonar, utilizando o VD do animal, simulando o comportamento do VE nos caso de TGA. A avaliação dessa hipertrofia foi feita através das medidas das espessuras das paredes do VD, VE e septo interventricular pelo ecocardiograma e pelos parâmetros morfológicos e histológicos, após o sacrifício do animal.

Nesse experimento optamos pela utilização de animais jovens, porém com VE já significativamente mais espesso que o VD. Para isso realizava-se um exame ecocardiográfico na chegada do animal ao biotério, onde as espessuras VE, VD e septo interventricular eram avaliadas. O animais onde a espessura do VD se mostrava semelhante à do VE, eram devolvidos ao criador. Não sabemos se a resposta ao estímulo de sobrecarga de pressão do VD, aqui observada, seria a mesma utilizando animais mais velhos, pois essa não foi a proposta deste estudo. O grupo controle foi utilizado apenas como parâmetro de peso dos ventrículos e septo interventricular em condições normais, e nas demais situações os animais foram controles de si mesmos.

Os critérios iniciais de ajuste foram: manter a pressão sistólica do VD entre 70 a 80% do VE, desde que a queda da pressão sistólica da aorta não diminuísse mais que 10 a 15% ou o animal apresentasse algum sinal de baixo débito (irritabilidade do animal, taquidispnéia ou síncope). Esses critérios, inicialmente utilizados por DIAS [13], foram escolhidos no intuito de estabelecer números exatos e objetivos no protocolo. SOLIS et al. [41], em 1987 usaram como critério o máximo de estenose tolerável. Outros autores [25,27] usaram critérios como o bem estar do animal, ou sinais clínicos de disfunção ventricular, ambos de caráter subjetivo, por vezes difíceis de quantificar. Num projeto piloto, realizado por nosso grupo, observou-se que diminuições de até 15% da pressão sistólica do VE estavam associadas à melhor adaptação do VD à bandagem do TP. Além desses critérios objetivos, alguns sinais subjetivos, como taquidispnéia, agitação do animal ou outro sinal clínico de baixo débito foram observados, e caso presentes realizou-se a desinsuflação do dispositivo.

O regime de treinamento utilizado, neste estudo foi o de resistência contínua por um período de 96 horas, com ajustes a cada 24 horas. Os estudos de biologia molecular da hipertrofia miocárdica [42-44] mostraram que, em poucos minutos de sobrecarga,

diversos mecanismos desencadeiam a síntese proteica, para promover a adaptação do músculo cardíaco ao novo sistema de pressão. Através desse conceito, o ventrículo submetido a uma sobrecarga de pressão seria capaz, em poucos dias, de se adaptar ao novo regime imposto. Observamos, em nosso experimento, que alguns animais do grupo II foram capazes de desenvolver pressão sistêmica já com 48 horas de treinamento. Outros protocolos de treinamento foram propostos, como KATAYAMA et al. [25], em 1993, que utilizaram um regime de sobrecarga intermitente, por um período de quatro dias, em vez de um regime de sobrecarga contínua. Demonstraram a ocorrência de hipertrofia do VD, neste regime, utilizando um cateter insuflado no TP. Parece-nos que o regime utilizado, em nosso estudo, impõe uma sobrecarga progressiva ao ventrículo, que, com o aumento progressivo de sua massa, passa a tolerar progressivamente pressões maiores, sem apresentar disfunção ventricular. Ambos os dispositivos foram capazes de aumentar a massa do VD de forma eficiente nas 96 horas de treinamento, o que reforça a importância do protocolo de treinamento ventricular, muito mais que o tipo de dispositivo utilizado. Observamos também que, com o dispositivo utilizado no grupo II, conseguimos obter pressões do VD maiores que no grupo I, mas as curvas de evolução das espessuras das paredes livres do VD foram paralelas. Ou seja, embora a capacidade de estenose do dispositivo externo seja superior à do cateter balão, no final de 96h, o grau de hipertrofia ventricular foi semelhante em ambos os grupos. Isto nos faz crer que não é necessário uma sobrecarga sistólica excessiva para preparar o ventrículo subpulmonar.

Os sinais de baixo débito observados nos 3 animais do grupo I, desencadeados com a movimentação do animal, podem ser explicados pelo deslocamento do cateter balão dentro do TP, levando a obstrução aguda e distal das artérias pulmonares. A possibilidade de deslocamento do cateter no interior do TP foi descrita, anteriormente, por KATAYAMA et al. [25], que sugeriram uma modificação no formato do balão, desenhando-o em espiral, para que essa obstrução distal fosse evitada. Sabendo da dificuldade em manter bem posicionado esse tipo de dispositivo dentro do TP, o cateter balão foi, no nosso experimento, introduzido diretamente na via de saída do VD, como descrito em trabalhos anteriores [24,25,27]. Com isso evitamos o mau posicionamento do cateter e não tivemos nenhum caso de deslocamento do mesmo fora do TP. Não conseguimos, porém, evitar a sua movimentação no interior do TP, que levou à oclusão aguda das artérias pulmonares, como descrito anteriormente. Esse problema não foi observado com o dispositivo de bandagem externa, que permaneceu bem posicionado durante todo o experimento e em todos os animais em que foi implantado.

A capacidade de causar a estenose do TP pôde ser avaliada através dos parâmetros hemodinâmicos obtidos durante o treinamento ventricular dos animais. Analisando a evolução dessas curvas, notamos maior eficiência do dispositivo de bandagem externa, que pôde ser evidenciada nas curvas de pressão sistólica da aorta, nos gradientes VD-TP observados e na curva da pressão sistólica do VD. A diminuição mais acentuada, no final do experimento, das pressões sistólicas da aorta no grupo II, quando comparadas às observadas no grupo I, indica o maior comprometimento da função ventricular imposta pelo dispositivo de bandagem externa. O gradiente sistólico entre o VD e o TP, a pressão sistólica do VD e a razão VD/VE significativamente maior no grupo II, reforçam o maior grau de estenose observado com o dispositivo de bandagem externa, onde a sobrecarga sistólica imposta ao VD foi nitidamente maior.

A pressão sistólica do TP teve um comportamento semelhante em ambos os grupos, com diminuição progressiva ao longo do treinamento ventricular. Mesmo com a diminuição da pressão sistólica do TP, os animais não apresentaram cianose, mostrando que o fluxo sanguíneo foi o suficiente para manter a saturação sistêmica. A avaliação de hipoxemia neste estudo foi subjetiva, pois não realizamos gasometria arterial durante os ajustes dos dispositivos. Em estudos anteriores [24,25], onde a gasometria arterial foi documentada, demonstrou-se hipoxemia discreta nos animais, bem tolerada e facilmente revertida através da desinsuflação do dispositivo de bandagem utilizado. Considerando esses dados experimentais, talvez a realização de uma comunicação sistêmico-pulmonar, como a realizada na experiência clínica [6,7,10,12,36], possa não ser necessária com os dispositivos ajustáveis de bandagem. Um regime de treinamento intermitente, com desinsuflação do dispositivo quando necessário, seria bem tolerado pelo paciente.

Após o implante dos dispositivos, o exame ecocardiográfico realizado, além de avaliar o correto posicionamento do dispositivo no TP, tinha o objetivo de identificar a presença de gradiente transvalvar pulmonar. A existência de um gradiente inicial, significativamente maior em um dos grupos, poderia promover o preparo ventricular num período de tempo menor, uma vez que os animais aguardavam 1 a 3 dias para o início da primeira insuflação dos dispositivos de bandagem. A identificação de um gradiente inicial maior implicaria na não aceitação desse animal para a continuação do estudo. O gradiente VD-TP inicial, avaliado ao ecocardiograma, não apresentou diferença significativa no início do experi-

mento e nenhum dos animais foi descartado do estudo. A medida direta das pressões do VD e TP, porém, mostrou um gradiente VD-TP inicial significativamente maior no grupo II, só identificado no primeiro ajuste, quando os cateteres implantados foram conectados ao sistema de monitorização de pressão. Esses dados não confirmaram os valores anteriormente observados ao ecocardiograma. Contudo, a evolução da espessura da parede livre do VD, avaliada ao ecocardiograma durante o experimento, não mostrou diferença significativa entre os grupos, nos diversos momentos avaliados, com um aumento progressivo de sua espessura através do tempo. Analisando esses dados, podemos concluir que o gradiente inicial possivelmente maior no grupo II não resultou em diferença significativa na evolução da espessura da parede do VD, pelo menos identificável pelo método utilizado.

No plano macroscópico, a medida simples do peso dos ventrículos é adotada pela maioria dos pesquisadores. Neste experimento, o aumento do peso do VD foi muito evidente. Utilizamos a indexação do peso dos ventrículos pelo peso do animal, conforme proposto por BISHOP& COLE [29], no intuito de diminuirmos as diferenças entre os mesmos. Observamos diferença significativa no peso do VD dos animais treinados, e os animais do grupo controle, não havendo diferença entre os grupos I e II, enquanto os pesos do VE e septo interventricular não apresentaram diferença entre os grupos. O peso seco dos ventrículos treinados foi calculado com o intuito de evidenciar o quanto do aumento do peso do VD observado se deu por edema. KATAYAMA et al. [25] realizaram a medida do peso seco e constataram aumento real de massa nos ventrículos treinados, demonstrando que o aumento de água não é determinante no aumento de peso do VD. FRANK & LARGER [45], em 1974, documentaram o aumento do conteúdo de água no miócito submetido à sobrecarga aguda como um fenômeno temporário. Em nosso estudo, observamos que houve significativo aumento do peso seco do VD dos animais treinados, o que não se observa nos pesos secos do VE e septo interventricular, ou seja, apenas o VD, submetido à sobrecarga sistólica de pressão, apresentou aumento significativo de peso, e que esse aumento não foi por acúmulo de água.

No plano da microscopia óptica foi utilizado um método indireto de medida de hipertrofia: a medida do perímetro e da área do miócito em corte transverso no nível do núcleo celular. Esta metodologia foi baseada nos estudos de ANVERSA et al. [30]. Em nosso estudo, os miócitos apresentaram seus perímetros e áreas significativamente maiores nos grupos I e II quando comparados à amostra inicial retirada antes do início do treinamento, confirmando as informações já descritas em literatura. Os miócitos foram avaliados em cortes transversos ao nível do núcleo, pois a dificuldade técnica de avaliar o miócito no sentido longitudinal é maior. Embora exista muita crítica a essa metodologia, devemos lembrar que usamos um estudo comparativo entre o miócito, antes do treinamento, e o mesmo, no final do treinamento, ou seja os valores são comparativos, não absolutos e isolados.

CONCLUSÃO

Podemos concluir que o dispositivo de bandagem externa é mais estável e promove maior grau de estenose do tronco pulmonar, embora ambos os dispositivos têm a capacidade de preparar o ventrículo subpulmonar. Apesar do maior grau de estenose do tronco pulmonar obtida com o dispositivo de bandagem externa, o preparo do ventrículo subpulmonar realizado no período de 96h, com o protocolo proposto, teve a mesma eficiência nos 2 grupos

RBCCV 44205-528

Canêo L F, Dias C A, Assad R S, Abduch M C D, Aiello V D, Moreira L F P, Lourenço Filho D D, Stolf N A G - Preparation of the pulmonary ventricle for arterial switch operation using two adjustable devices for pulmonary trunk obstruction: an experimental study. *Rev Bras Cir Cardiovasc* 2001; **16**(1):35-48.

ABSTRACT: Two adjustable pulmonary artery banding devices were implanted in 14 young goats to evaluate their efficacy for the training of the pulmonary ventricle (right ventricle). An obstructing balloon catheter was placed in the lumen of the pulmonary artery in 7 animals (group I) and other 7 animals (group II) underwent implantation of an external pulmonary banding device. As a control group for septum, left and right ventricle weights a third group of 7 animals was used. Right ventricle (RV) load was increased gradually, at 24 hours intervals, for 96 hours. RV muscle mass evolution was assessed by echocardiography and by optical microscopy. All animals completed the protocol. RV to PT pressure gradient, RV to LV ratio, and RV systolic pressure were significantly higher in group II ($p<0.05$). A significant increase in the RV wall thickness was observed in groups I and II. RV dry weight and indexed weight were higher in groups I and II ($p<0.05$), with no difference between them ($p>0.05$). Myocyte perimeter and cross-area increased at the end of the training interval. We concluded that both devices are able to induce a similar degree of pulmonary ventricle hypertrophy, despite higher pressure gradients with the external banding device.

DESCRIPTORS: Heart ventricle, surgery. Pulmonary artery, surgery. Cardiovascular surgical procedures, methods. Hypertrophy, left ventricular, etiology. Heart ventricle, physiology. Hipertrophy, left ventricular, surgery. Ventricular outflow obstruction. Transposition of great arteries, surgery.

REFERÊNCIAS BIBLIOGRÁFICAS

1 Di Donato R M, Fujii A M, Jonas R A et al. - Age-dependent ventricular response to pressure overload: considerations for the arterial switch operation. *J Thorac Cardiovasc Surg* 1992; **104**: 713-22.

2 Baño-Rodrigo A, Quero-Jiménez M, Moreno-Granado F et al. - Wall thickness of ventricular chambers in transposition of the great arteries: surgical implications. *J Thorac Cardiovasc Surg* 1980; **79**: 592-7.

3 Brawn W J & Mee R B - Early results for anatomic correction of transposition of the great arteries and for double-outlet right ventricle with subpulmonary ventricular septal defect. *J Thorac Cardiovasc Surg* 1988; **95**: 230-8.

4 Norwood W I, Dobell A R, Freed M D et al. - Intermediate results of the arterial switch repair: a 20-institution study. *J Thorac Cardiovasc Surg* 1988; **96**: 854-63.

5 Wernovsky G, Mayer Jr. J E, Jonas R A et al. - Factors influencing early and late outcome of the arterial switch operation for transposition of the great arteries. *J Thorac Cardiovasc Surg* 1995; **109**: 289-302.

6 Yacoub M H; Radley-Smith R; Maclaurin R - Two-stage operation for anatomical correction of transposition of the great arteries with intact interventricular septum. *Lancet* 1977; **1**: 1275-8.

7 Jonas R A, Giglia T M, Sanders S P et al. - Rapid, two-stage arterial switch for transposition of the great arteries and intact ventricular septum beyond the neonatal period. *Circulation* 1989; **80**: I-203-8.

8 Mee R B - Severe right ventricular failure after Mustard or Senning operation. Two-stage repair: pulmonary artery banding and switch. *J Thorac Cardiovasc Surg* 1986; **92**: 385-90.

9 Yacoub M, Bernhard A, Lange P et al. - Clinical and hemodinamic results of the two-stage anatomic correction of simple transposition of the great arteries. *Circulation* 1980; **62**: I-190-6.

10 Wernovsky G, Giglia T M, Jonas R A et al. - Course in the intensive care unit after 'preparatory' pulmonary artery banding and aortopulmonary shunt placement for transposition of the great arteries with low left ventricular pressure. *Circulation* 1992; **86**: II-133-9.

11 Iyer K S, Sharma R, Kumar K et al. - Serial echocardiography for decision making in rapid two-stage arterial switch operation. *Ann Thorac Surg* 1995; **60**: 658-64.

12 Bosisio I B J - Avaliação do desempenho do ventrículo esquerdo na Operação de Jatene em transposição das grandes artérias com septo ventricular íntegro após preparo rápido. [Tese. Doutorado]. São Paulo: Faculdade de Medicina, Universidade de São Paulo, 1977. 83 p.

13 Dias C A - Modelo experimental de bandagem ajustável do tronco pulmonar: novo dispositivo para preparo rápido do ventrículo. [Tese. Doutorado]. São Paulo: Faculdade de Medicina, Universidade de São Paulo, 1998. 92 p.

14 Shane R A, Kimmell G O, Jaques W E et al. - Adjustable prosthesis for pulmonary artery banding: comparinson with umbelical tape and teflon bands. *Circulation* 1967; **35** e **36**: I-148-51.

15 Edmunds J R, Rudy L W, Heymann M A et al. - An adjustable pulmonary arterial band. *Trans. Amer. Soc. Artif. Int. Organs* 1972; **18**: 217-25.

16 Muraoka R, Yokota M, Aoshima M et al. - Extrathoracically adjustable pulmonary artery banding. *J Thorac Cardiovasc Surg* 1983; **86**: 582-6.

17 Dajee H, Benson L, Laks H - An improved method of pulmonary artery banding. *Ann Thorac Surg* 1984; **37**: 254-7.

18 Park S C, Griffith B P, Siewers R D et al. - A percutaneously adjustable device for banding of the pulmonary trunk. *Intern J Cardiol* 1985; **9**: 477-84.

19 Solis E, Heck C, Seward J et al. - Percutaneously adjustable pulmonary artery band. *Ann Thorac Surg* 1986; **41**: 65-9.

20 Higashidate M, Beppu T, Imai Y et al. - Percutaneously adjustable pulmonary artery band: an experimental study. *J Thorac Cardiovasc Surg* 1989; **97**: 864-9.

21 Ahmadi A, Rein J, Hellberg K et al. - Percutaneously adjustable pulmonary artery band. *Ann Thorac Surg* 1995; **60**: S520-2.

22 Tatooles C J, Kittle C F - Transvenous balloon catheter implant for partial pulmonary occlusion. *Ann Thorac Surg* 1968; **5**: 560-5.

23 Bonhoeffer P, Carminati M, Parenzan L et al. - Non-surgical left ventricular preparation for arterial switch in transposition of the great arteries [letter]. *Lancet* 1992; *340*: 549-50.

24 Krzeski R, Katayama H, Bonhoeffer P et al. - Obstructing balloon catheter for the induction of systolic hypertension in the pulmonary ventricle - an acute hemodynamic study in the piglet. *Cardiol Young* 1992; **2**: 89-94.

25 Katayama H, Krzeski R, Frantz E G et al. - Induction of right ventricular hypertrophy with obstructing balloon catheter. Nonsurgical ventricular preparation for the arterial switch operation in simple transposition. *Circulation* 1993; **88**: 1765-9.

26 Bonnet D, Maillard A, Chetboul V et al. - Non-surgical ventricular training before arterial switch: focus on an animal model in lambs. *Arch Mal Coeur Vaiss* 1997; **90**: 707-12.

27 Assad R S, Cardarelli M, Abduch M C D et al. - Bandagem reversível do tronco pulmonar: modelo experimental para preparo rápido do ventriculo pulmonar. *Rev Bras Cir Cardiovasc* 1998; **13**: 239-48.

28 Fulton R M, Hutchinson E C, Jones A M - Ventricular weight in cardiac hypertrophy. *Br Heart J* 1952; **14**: 413-20.

29 Bishop S P & Cole C R - Production of externally controlled progressive pulmonic stenosis in the dog. *J Appl Physiol* 1969; **26**: 659-63.

30 Anversa P, Vitali-Mazza L, Gandolfi A et al. - Morphometry and autoradiography of early hypertrophic changes in the ventricular myocardium of adult rat: a light microscopic study. *Lab Invest* 1975; **33**: 125-9.

31 ROSNER B. *Fundamentals of biostatistics.* 2.ed. Boston: PWS Publishers, 1986.

32 TIMM N H - *Multivariate analysis with applications in educations and psychology.* . Monterrey, CA: Brooks/ Cole, 1975.

33 Wernovsky G, Hougen T J, Walsh E P et al. - Midterm results after the arterial switch operation for transposition of the great arteries with intact ventricular septum: clinical, hemodynamic, echocardiographic, and electrophysiologic data . *Circulation* 1988; **77**: 1333-44**.**

34 Di Donato R M, Wernovsky G, Walsh E P et al. - Results of the arterial switch operation for transposition of the great arteries with ventricular septal defect. Surgical considerations and midterm follow-up data *Circulation* 1989; **80**: 1689-705.

35 Boutin C, Jonas R A, Sanders S P et al. - Rapid two-stage arterial switch operation. Aquisition of left ventricular mass after pulmonary artery banding in infants with transposition of the great arteries. *Circulation* 1994; **90**: 1304-9.

36 Yasui H, Kado H, Yonenaga K et al. - Arterial switch operation for transposition of the great arteries, with special reference to left ventricular function. *J Thorac Cardiovasc Surg* 1989; **98**: 601-10.

37 Nakazawa M, Oyama K, Imai Y et al. - Criteria for two-staged arterial switch operation for simple transposition of great arteries. *Circulation* 1988; **78**: 124-31.

38 Bonhoeffer P, Henry G, Katayama H et al. - Preparation of the “pulmonary ventricle” for arterial switch by adjustable intravascular balloon outflow obstruction-an experimental approach in a lamb model. *Cardiol Young* 1992; **2**: 85-8.

39 Nahas R, Mundth E D, Ross B et al. - Adjustable instrument for pulmonary artery banding. *J Thorac Cardiovasc Surg* 1972; **63**: 732-4.

40 Jacobson J H & Macallister F F - A method for the controlled occlusion of larger blood vessels. *Ann Surg* 1957; **145**: 334-43.

41 Solis E, Bell D, Alboliras H et al. - Left ventricular preparation with an extrathoracically adjustable balloon occluder. *Ann Thorac Surg* 1987; **44**: 58-61.

42 Izumo S; Nadal-Guinard B; Mahdavi V - Protooncogenese induction and reprogramming of cardiac gene expression produced by pressure overload. *Proc Natl Acad Sci* 1988; **85**: 339-43.

43 Sadoshima J & Izumo S - The cellular and molecular response of cardiac myocytes to mechanical stress. *Annu Rev Physiol* 1997; **59**: 551-71.

44 Cooper IV G, Kent R L, Uboh C E et al. - Hemodynamic versus adrenergic control of cat right ventricular hypertrophy. *J Clin Invest* 1985; **75**: 1403-14.

45 Frank J S & Larger G A - The myocardial interstitium: its structure and its role in ionic exchange. *J Cell Biol* 1974; **60**: 586-90.

Discussão *(Transcrições de fita gravada)*

D.R FERNANDO ANTÔNIO FANTINI
Nova Lima, MG

Gostaria de agradecer a oportunidade de comentar este trabalho do Dr. Caneo, inicialmente pela importância do tema, mas, também, pela excelente estruturação técnica, pela metodologia usada no experimento, assim como a apresentação, para os quais não temos reparos a fazer. Muito embora algumas observações a respeito do modelo precisem ser realizadas. Os estudos de treinamento experimental do ventrículo subpulmonar, não só do presente trabalho como de outros da literatura, têm como base o ventrículo direito, que, obviamente, é diferente do ventrículo esquerdo, tanto do ponto de vista anatômico como funcional. O crescimento pós-natal do ventrículo direito é comparável a uma hipertrofia secundária à sobracarga de volume, enquanto que o crescimento do ventrículo esquerdo é considerado uma combinação de hipertrofia por sobrecarga de volume e de pressão. Além disso, as alterações subcelulares e microvasculares também ocorrem mais rapidamente no ventriculo esquerdo do que no direito. Parece-me, ainda, que a capacidade de sintetizar proteínas, estímulo necessário para o desenvolvimento da hipertrofia miocárdica, é mais rapidamente perdida pelo ventrículo direito. Além disso, as alterações presentes na transposição das grandes artérias, como a hipertrofia crônica e a acidose, podem provocar modificações na resposta singular. Estes detalhes não invalidam o método que foi utilizado pelos autores, mesmo porque deve ser bastante dificil a obtenção de carneiros com transposição. Do ponto de vista prático, tenho algumas considerações a fazer em relação aos 2 métodos que, como o próprio autor menciona, existem dificuldades na estabilização do cateter balão no interior da artéria pulmonar, o que provocou instabilidade hemodinâmica nos carneiros estudados. Este fato já havia sido relatado por Katayama, em um trabalho publicado em 1994, tendo este autor, inclusive, sugerido modificações no desenho do balão, com o intuito de torná-lo mais estável. Além disso, numa criança com transposição das grandes artérias, o caminho a ser percorrido é mais longo e complicado se queremos utilizar a principal vantagem do balão intravascular, que seria a implantação sem toracotomia, o que causa ainda maiores dificuldades na estabilização. Quanto aos dispositivos de bandagem externa, existem relatos na literatura de falhas no funcionamento dos mesmos a médio prazo, problemas que acreditamos possam ser solucionados pela moderna tecnologia. Assim, a vantagem de se conseguir uma cerclagem gradual, externamente ajustável ao ventrículo esquerdo, é extemamente atrativa. Além disso, ao ler o trabalho que nos foi enviado previamente pelo autor,pudemos vislumbrar outras aplicações do método bastante interessantes, como a possibilidade de cerclagem de cardiopatias complexas com fisiologia univentricular e fluxo pulmonar livre, e, ainda, em pacientes portadores de CVI e de hipertensão pulmonar, com resultados hemodinâmicos *borderline,* onde a cerclagem gradativa pode resultar na transformação de muitos pacientes considerados inoperáveis em candidatos à correção total.

DR. CÂNEO
(*Encerrando)*

Na literatura o melhor modelo ainda é o ventrículo direito, uma vez que não temos transposição nos animais para estudar este tipo de aplicação. Este trabalho fecha um ciclo de estudos de nosso grupo, no qual nós testamos vários tipos de cateteres, o balão externo. Ele traz uma importante mensagem, a idéia de que não é o cateter que importa e sim a forma de treinamento. Este tipo de treinamento deve ser considerado, seja por toracotomias repetidas, com o Dr. Roger Minn vem fazendo nos casos de preparo dos ventrículos falidos com correção a nível atrial. Eu acho que esta é a grande idéia; se nós conseguirmos fazer uma bandagem progressiva, sem causar disfunção, como vimos nesses animais, teremos um outro tipo de preparo e, com certeza, um melhor resultado deste tipo de opção técnica.