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Cardiovascular Pathology 21 (2012) 199–205

CARDIOVASCULAR PATHOLOGY

Original Article

Age is a risk factor for maladaptive changes of the pulmonary root in rats exposed to increased pressure loading

Vladimiro L. Vida[a,*], Stefania Rizzo[b], Nicola Maschietto[c], Giovanna Boccuzzo[d], Ornella Milanesi[c], Gaetano Thiene[b], Giovanni Stellin[a], Cristina Basso[b]

[a]*Pediatric and Congenital Cardiac Surgery Unit, Department of Cardiac, Thoracic and Vascular Sciences, University of Padua Medical School, Padua, Italy*
[b]*Department of Medical Diagnostic Sciences and Special Therapies, University of Padua Medical School, Padua, Italy*
[c]*Pediatric Cardiology Unit, Department of Pediatrics, University of Padua Medical School, Padua, Italy*
[d]*Department of Statistics, University of Padua Medical School, Padua, Italy*

Received 11 November 2010; received in revised form 30 June 2011; accepted 10 July 2011

Abstract

Introduction: Pulmonary artery root does not adapt properly when exposed to increased pressure stress, with progressive dilatation. The aim of this study was to evaluate, in an animal model, the histologic changes of the pulmonary root wall under increased pressure load. **Methods and Results:** To increase the systolic pressure in the pulmonary root, a banding of the pulmonary artery (PAB) was performed in 10 adult Sprague–Dawley rats and in 10 weanlings, using 7 adults and 8 weanlings as controls. We analyzed the structural changes of the pulmonary artery root after 30 days of increased pressure load. The mean pressure gradient across the banded pulmonary trunk was 53.57 ± 10 mmHg in the adult rats and 86.73 ± 15 mmHg in the weanlings. The pulmonary artery wall was significantly thicker in both age groups of PAB rats when compared to age-matched controls, showing also architectural structural changes, as a higher degree of mucoid degeneration, medionecrosis, and fibrosis as well as elastic fibers fragmentation. The apoptotic index was also increased in both PAB age groups. We also confirmed the physiologic higher degree of elastic fibers disarray in adult rats when compared to weanlings. **Conclusions:** The pulmonary artery wall seems to present maladaptive architectural changes in the media when exposed to systemic pressure. The PAB-related increase of the apoptotic index seems to reflect an accelerated involution of the pulmonary root's media. The physiologic higher degree of elastic fibers disarray in adult rats can possibly influence the worst adaptation of the pulmonary arterial wall to a systemic pressure load.

Keywords: Pulmonary root; Histology; Pressure overload

1. Introduction

The increased pressure in the pulmonary artery district leads inevitably to the dilation of the pulmonary arteries [1–4]. The presence of pulmonary hypertension is supposed to be the driving force to dilate the pulmonary arteries; however, the influence of other parameters, such as the degree of pulmonary artery hypertension and the age of patients at its onset, is still unknown [5,6].

Similar data on pulmonary artery dilatation have also been reported in young and adult patients where the pulmonary root has been exposed to acute systemic pressure load when transferred into the systemic position during the surgical repair of complex cardiac lesions [7–12].

To investigate these problems, we have established an animal model of pressure loading of the pulmonary root by pulmonary artery banding (PAB).

Our goals were to evaluate the structural changes of the pulmonary root wall under increased pressure load in comparison to sham-operated animals and also to establish

* Corresponding author. Pediatric and Congenital Cardiac Surgery Unit, Department of Cardiac, Thoracic and Vascular Sciences, University of Padua, Via Giustiniani, 2-35128, Padua, Italy. Tel.: +39 049 8212410; fax: +39 049 8212409.
E-mail address: vladimiro.vida@unipd.it (V.L. Vida).

1054-8807/11/$ – see front matter
doi:10.1016/j.carpath.2011.07.007

whether the age of animals at the time of PAB could influence the mechanism of adaptation of the pulmonary root's vascular wall to increased pressure load.

2. Methods

2.1. Establishment of the model

We obtained 40 male Sprague–Dawley rats from Harlan Laboratories, San Pietro al Natisone, Udine, Italy; 20 of the rats were 10 weeks old, and, hence, adults, whereas the other 20 were 3-week-old weanlings. We randomly divided these animals into experimental groups, 10 rats each (animals undergoing banding PAB), and control groups, 10 rats each (animals undergoing sham operations). The animals were housed, treated, and handled in accordance with the recommendations stated in the National Institute of Health's Guide for the Care and Use of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources, National Research Council, as stated in the National Institute of Health Publication number 85-23, revised 1996. The protocol was approved by the Animal Care Committee of the University of Padua Veterinary School and by the Italian Government Committee for Animal Care. The operative procedures were carried out under general anesthesia using 5% sevoflurane (Abbott Spa, Latina, Italy) and tiletamine chlorohydrate (Zoletil, Virbac, France) at 50 mg/kg. Rats were intubated utilizing a 16- and 20-gauge polyethylene cannula in adults and in weanlings, respectively. The endotracheal cannula was connected to a ventilator for small animals (Rodent Ventilator 7500; Ugo Basile, Varese, Italy), and anaesthesia was maintained by a mixture of oxygen and sevoflurane. The pulmonary trunk was exposed through a limited left lateral thoracotomy in the fourth intercostal space and was banded proximal to its bifurcation using a 4.0 silk suture, which was calculated from previous pilot studies to produce a 60% decrease in the diameter, both in the adults and in the weanlings. The PAB was maintained for 30 days. To reduce the potential of pneumothorax, the chest was closed under positive pressure ventilation, and a chest tube was placed during the time of the closure to evacuate all air, which was being removed after chest closure. Intraoperative and postoperative pain was controlled with the nonsteroidal anti-inflammatory drug tramadol, given at 2 mg/kg intramuscularly, every 12 h, for 5 days. The animals undergoing sham procedures of both age groups had the same intraoperative and postoperative procedures as did the rats undergoing PAB, except for the PAB itself. Rats were subsequently weighed and inspected twice a week.

All rats had an echocardiogram on the 30th postoperatives days, performed using a Hewlett-Packard Sonos 5500 echocardiographic apparatus equipped with a 12.5-MHz probe. Rats were sedated by tiletamine chlorohydrate at 50 mg/kg, and the pressure gradient across the band was obtained. On the 13th postoperative day, surviving animals underwent a second operation to obtain hemodynamic measurements and to harvest their hearts. Rats were anesthetized, placed on mechanical ventilation, and treated for pain as described above. During the operation, the animals were inspected for hydrothorax and ascites. Pressures in the systemic left ventricle and right ventricle were obtained by direct puncture of cardiac chambers with an 18-gauge needle connected to a fluid-filled polyethylene tubing to a pressure transducer and a digital monitor (Roche, Monza, Italy).

2.2. Preparation of samples for morphological analysis

Hearts were then quickly perfused with an antegrade cold cardioplegic solution to obtain a diastolic arrest and were removed from the thorax and washed with phosphate-buffered saline. We measured the body weight and wet heart weight of all the animals. A total of 35 of the 40 sampled pulmonary roots were analyzed: 10 adults with PAB, 7 adults undergoing sham operations, 10 weanlings with PAB, and 8 weanlings after sham operations. The remaining 5 rats (all controls) were not included in the final analysis due to technical problems during tissue processing.

2.3. Morphometric analysis

2.3.1. Histology and histochemistry

The pulmonary roots were fixed in 10% formalin–phosphate-buffered saline solution and subsequently embedded in paraffin. Seven-micron-thick sections were processed for histology by using hematoxylin and eosin, a modified Azan-Mallory trichrome, Alcian–periodic acid-Schiff stain, and elastic van Gieson stainings. The histologic evaluation of the media of the arterial wall was based on the presence and degree of the following variables: elastic fragmentation, fibrosis, mucoid degeneration, and medionecrosis, which were graded from 1 to 3 according to the severity of the process on the basis of the modified criteria from Jonas [9] when examined at a magnification of ×200 with an Olympus microscope (variables listed in Table 1).

Table 1
Variables analyzed at the histologic analysis

1. Elastic lamellae disarray (scored from 1 to 3)
2. Elastic lamellae fragmentation (scored from 1 to 3; 1: small area with fragmented elastic lamellae; 2: 1/3 –1/2 of the medial thickness with fragmented elastic lamellae; 3: elastic lamellae severely fragmented)
3. Fibrosis (scored from 1 to 3; 1: increase in collagen in <1/3 of the medial thickness; 2: 1/3–2/3 of the medial thickness; 3: >2/3 of the medial thickness)
4. Mucoid degeneration (scored from 1 to 3; 1: minute foci cysts; 2: size or number of cysts increased; 3: large cysts)
5. Medionecrosis (scored from 1 to 3; 1: loss of smooth muscle cells in <1/3 of medial thickness; 2: loss in 1/3–2/3 of the medial thickness; 3: loss in >2/3 of the medial thickness)
6. Apoptotic index (TUNEL) as % of apoptotic cells