Experimental and Molecular Pathology 100 (2016) 167–176

Contents lists available at ScienceDirect

Experimental and Molecular Pathology

journal homepage: www.elsevier.com/locate/yexmp

Reduced expression of adherens and gap junction proteins can have a fundamental role in the development of heart failure following cardiac hypertrophy in rats

Daniele O. dos Santos [a], Valdecir Blefari [a], Fernanda P. Prado [a], Carlos A. Silva [b], Rubens Fazan Jr [b], Helio C. Salgado [b], Simone G. Ramos [a], Cibele M. Prado [a,*]

[a] Department of Pathology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
[b] Department of Physiology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

ARTICLE INFO

Article history:
Received 5 September 2015
and in revised form 12 December 2015
Accepted 15 December 2015
Available online 17 December 2015

Keywords:
Cardiac hypertrophy
Heart failure
Intercalated disk
Adherens junction
Gap junction
Desmosome

ABSTRACT

Hypertension causes cardiac hypertrophy, cardiac dysfunction and heart failure (HF). The mechanisms implicated in the transition from compensated to decompensated cardiac hypertrophy are not fully understood. This study was aimed to investigate whether alterations in the expression of intercalated disk proteins could contribute to the transition of compensated cardiac hypertrophy to dilated heart development that culminates in HF. Male rats were submitted to abdominal aortic constriction and at 90 days post surgery (dps), three groups were observed: sham-operated animals (controls), animals with hypertrophic hearts (HH) and animals with hypertrophic + dilated hearts (HD). Blood pressure was evaluated. The hearts were collected and Western blot and immunofluorescence were performed to desmoglein-2, desmocollin-2, N-cadherin, plakoglobin, B-catenin, and connexin-43. Cardiac systolic function was evaluated using the Vevo 2100 ultrasound system. Data were considered significant when $p < 0.05$. Seventy percent of the animals presented with HH and 30% were HD at 90 dps. The blood pressure increased in both groups. The amount of desmoglein-2 and desmocollin-2 expression was increased in both groups and no difference was observed in either group. The expression of N-cadherin, plakoglobin and B-catenin increased in the HH group and decreased in the HD group; and connexin-43 decreased only in the HD group. There was no difference between the ejection fraction and fractional shortening at 30 and 60 dps; however, they were decreased in the HD group at 90 dps. We found that while some proteins have increased expression accompanied by the increase in the cell volume associated with preserved systolic cardiac function in the HH group, these same proteins had decreased expression even without significant reduction in the cell volume associated with decreased systolic cardiac function in HD group. The increased expression of desmoglein-2 and desmocollin-2 in both the HH and HD groups could work as a protective compensatory mechanism, helping to maintain the dilated heart. We can hypothesize that inappropriate intercellular mechanical and electrical coupling associated with necrosis and/or apoptosis are important factors contributing to the transition to HF.

1. Introduction

Cardiac hypertrophy is an adaptive response of the heart to increased workload; however, patients with left ventricular hypertrophy have an increased risk of cardiovascular morbidity and mortality. In fact, numerous studies have reported on its association with the development of coronary heart disease, cardiac failure, ventricular dysrhythmias and sudden death (Frohlich, 1999; Frohlich et al., 1992; Kannel et al., 1961). The transition from compensated hypertrophy to heart failure is still incompletely understood and some events have been highlighted, including myocyte hypertrophy (Frey et al., 2004; Swynghedauw, 1999), changes in myocyte phenotype resulting from the re-expression of fetal gene programs (Chien et al., 1991; Chien et al., 1993; Rajabi et al., 2007), alterations of proteins involved in excitation–contraction coupling (Bers, 2002; Crossman et al., 2011; Spann et al., 1967) and fibrosis (Hein et al., 2003; Mujumdar and Tyagi, 1999; Weber, 2000). Although myocyte hypertrophy and fibrosis are standard markers for myocardial abnormalities in heart failure, the mechanical properties of the myocytes might themselves be altered (Monreal et al., 2008).

Intercalated disks (ICDs) are essential structures unique to cardiac muscle; they enable mechanical coupling and chemical communications among adjacent cardiomyocytes to achieve the regulated contraction essential for cardiac function (Wang et al., 2012). Because of its

* Corresponding author: Department of Pathology, School of Medicine of Ribeirão Preto, University of São Paulo, Av. Bandeirantes, 3900, Monte Alegre, Ribeirão Preto, SP CEP: 14049-900, Brazil.
E-mail address: cibeleprado@usp.br (C.M. Prado).

http://dx.doi.org/10.1016/j.yexmp.2015.12.009
0014-4800/

integral function in mechanical force transmission as well as intercellular communication in the heart, ICDs can be considered key structures in heart failure. An ICD seems to act as a sensor for inadequate functional output at the level of the cardiomyocytes and can respond by increasing the myofibrillar attachment sites in cardiomyocytes (Perriard et al., 2003). Three cell junction types for ICDs – gap junction, adherens junction and desmosomes – physically connect the disk membranes, acting in concert to integrate cardiac electromechanical function. The gap junction provides intercellular communication via an electrical stimulus and small molecules that pass through a channel generated by a family of proteins called connexins. The adherens junction provides strong cell–cell adhesion that is mediated by the cadherin/catenin complex via linkage to the actin cytoskeleton. Also, it is the site of attachment of the myofibrils, enabling transmission of the contractile force across the plasma membrane. The desmosome provides structural support through the interactions of desmosomal cadherins (desmocollin and desmoglein) with the intermediate filament system (Green and Gaudry, 2000; Severs, 2000; Sheikh et al., 2009; Wang et al., 2012).

It has been demonstrated that absence of ICD proteins contributes to the cardiomyopathy observed in the Naxos disease and Carvajal syndrome, resulting from mutations in plakoglobin and desmoplakin, respectively (McKoy et al., 2000; Nehme et al., 2012; Protonotarios and Tsatsopoulou, 2004). Studies on the absence of ICDs proteins in knockout mice have revealed devastating consequences to the heart development and fetal viability (Bierkamp et al., 1996; Haegel et al., 1995; Radice et al., 1997; Ruiz et al., 1996), while studies in humans have demonstrated that alterations and/or mutations in the ICD components are associated with a spectrum of human cardiomyopathy (Asimaki et al., 2009; Basso et al., 2009; Harada et al., 2002; Masuelli et al., 2003). There are few data available on changes in the expression and distribution of these proteins at the intercalated disk sites during the progression from compensated cardiac hypertrophy to heart failure.

The aim of this work was to investigate whether alterations in the expression of ICDs proteins could contribute to the transition of compensated cardiac hypertrophy to dilated heart development, culminating in heart failure in Wistar rats submitted to aorta abdominal constriction, and correlate these alterations with cardiac function. Our results point to a reduced expression of adherens junction (cadherin, beta-catenin and plakoglobin) and gap junction proteins (connexin-43) in hypertrophic + dilated hearts associated with decreased cardiac function. The animals with increased expression of these proteins remained hypertrophied with preserved cardiac function.

2. Materials and methods

All the experimental protocols used in the current study were reviewed and approved by the Committee of Ethics in Animal Research of Ribeirão Preto School of Medicine, University of São Paulo, SP, Brazil (Protocol no. 204/2009). Experiments were performed in male Wistar rats supplied by the Animal Facility of Ribeirão Preto School of Medicine, University of São Paulo, SP, Brazil. The animals were housed with 5 rats per cage with free access to food and water and were maintained on a 12 h light/dark cycle. All efforts were made to minimize animal suffering and to decrease the number of animals used.

2.1. Experimental protocol

Male Wistar albino rats, weighing 149.10 ± 1.30 g, were used (n = 80). The animals were randomly divided into an operated group with animals submitted to surgical abdominal aorta stenosis and sham-operated group with animals submitted to sham operation to simulate abdominal aorta stenosis, named the control group.

The rat model of abdominal aortic constriction was generated as previously described by Béznak (1995) and modified by Rossi and Peres (1992). The animals were lightly anesthetized with inhaled isoflurane (1.5%) vaporized in medical O_2. In brief, a midline abdominal incision was performed to expose the aorta. Once the aorta was isolated above the renal arteries, a 0.85 mm diameter blunted probe was placed alongside the vessel. The aorta and needle were tied tightly using a 4–0 cotton thread. The needle was quickly removed, leaving the abdominal aorta with an 80% reduction in the lumen diameter. A similar surgery was performed in the sham-operated rats with the exception of aortic

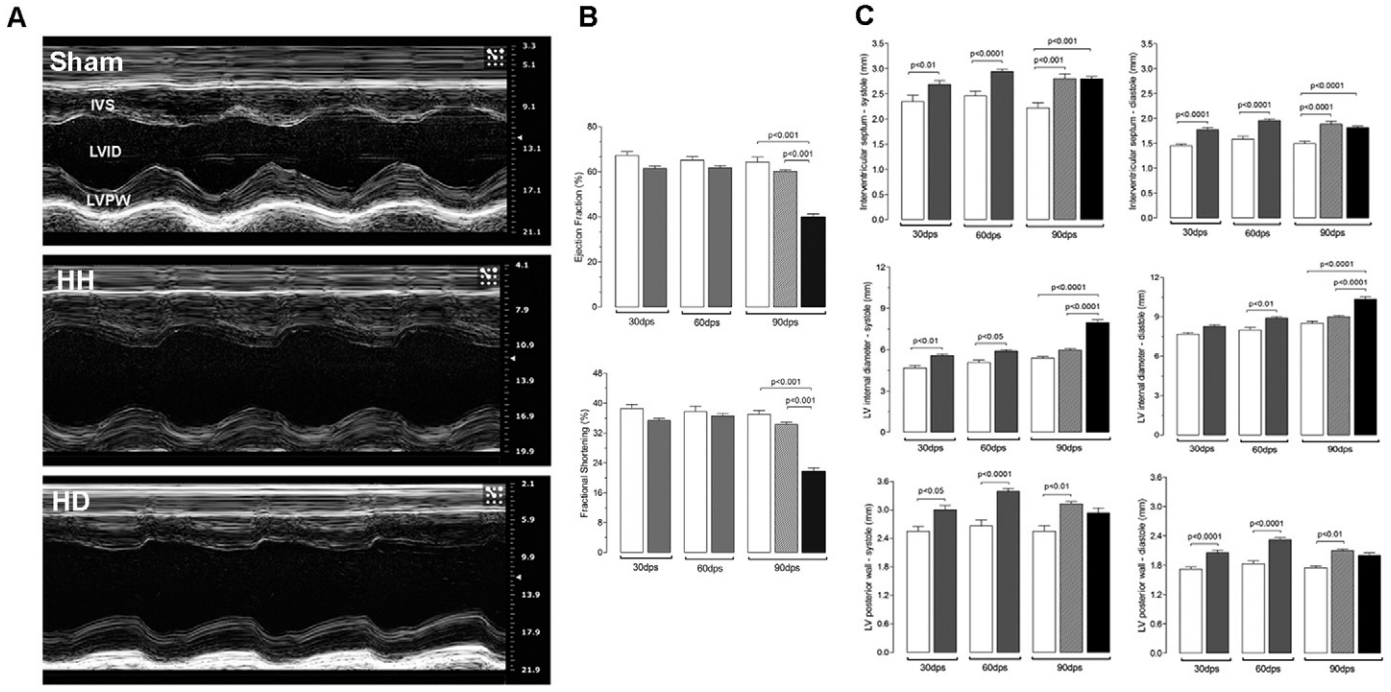

Fig. 1. Echocardiography data. A) Representative images showing the M-mode long-axis ultrasound from sham-operated, HH and HD groups. The waves represent the cardiac cycle in systole (minor diameter) and diastole (major diameter). B) Systolic function (ejection fraction and fractional shortening). C) The graphs represent the analysis of 10 cycles per animal (n = 15–20/group) at 30, 60 and 90 days post surgery (dps). LV = left ventricle; IVS = interventricular septum; LVID = left ventricle internal diameter; LVPW = left ventricle posterior wall. White bars = sham-operated, gray bars = operated; gray bar with stripes = HH group; and black bars = HD group. The values represent the means ± SE.