International Journal of Cardiology 215 (2016) 384–387

Contents lists available at ScienceDirect

International Journal of Cardiology

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

Myocardial myostatin in spontaneously hypertensive rats with heart failure

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ARTICLE INFO

Article history:
Received 7 April 2016
Accepted 11 April 2016
Available online 15 April 2016

Keywords:
Myostatin
Follistatin
Spontaneously hypertensive rat
Cardiac failure
Myocardium
Cardiac remodeling

ABSTRACT

Background: Myostatin has been shown to regulate skeletal and cardiac muscle growth. However, its status on long-term hypertrophied myocardium has not been addressed. The purpose of this study was to evaluate the expression of myocardial myostatin and its antagonist follistatin in spontaneously hypertensive rats (SHR) with heart failure.

Methods: Eighteen-month-old SHR were evaluated to identify clinical features of heart failure such as tachypnea/labored respiration and weight loss. After heart failure was detected, rats were subjected to echocardiogram and euthanized. Age-matched normotensive Wistar-Kyoto (WKY) rats were used as controls. Myostatin and follistatin protein expression was assessed by Western blotting. Statistical analysis was performed by Student's t test.

Results: All SHR (n = 8) presented right ventricular hypertrophy and five had lung congestion. SHR had left chambers hypertrophy and dilation (left atrial diameter: WKY 5.73 ± 0.59; SHR 7.28 ± 1.17 mm; p = 0.004; left ventricular (LV) diastolic diameter/body weight ratio: WKY 19.6 ± 3.1; SHR 27.7 ± 4.7 mm/kg; p = 0.001), and LV systolic dysfunction (midwall fractional shortening: WKY 34.9 ± 3.31; SHR 24.8 ± 3.20%; p = 0.003). Myocyte diameter (WKY 23.1 ± 1.50, SHR 25.5 ± 1.33 μm; p = 0.004) and myocardial interstitial collagen fraction (WKY 4.86 ± 0.01; SHR 8.36 ± 0.02%; p < 0.001) were increased in the SHR. Myostatin (WKY 1.00 ± 0.16; SHR 0.77 ± 0.23 arbitrary units; p = 0.035) and follistatin (WKY 1.00 ± 0.35; SHR 0.49 ± 0.18 arbitrary units; p = 0.002) expression was lower in SHR. Myostatin and follistatin expression negatively correlated with LV diastolic diameter-to-body weight ratio and LV systolic diameter, and positively correlated with midwall fractional shortening.

Conclusion: Myostatin and follistatin protein expression is reduced in the long-term hypertrophied myocardium from spontaneously hypertensive rats with heart failure.

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1. Introduction

Myostatin, also known as growth and differentiation factor (GDF) 8, is a member of the transforming growth factor-β superfamily of secreted growth factors that regulates muscle differentiation and growth [1]. Although its main expression site is skeletal muscle, myostatin can also be found in heart muscle and fat tissue [2–5]. In skeletal muscle, myostatin acts as a major negative regulator of muscle growth [1,6]. Considering the effects of myostatin on skeletal muscle, anti-hypertrophic myocardial effects could be anticipated. However, in contrast to skeletal muscle, the cardiac effects of myostatin are still controversial [7].

Under physiological conditions lower levels of myostatin are expressed in cardiomyocytes than skeletal muscle [5]. However, studies have shown that myostatin is upregulated in cardiac remodeling induced by different pathological conditions [2,4,6,8]. Despite the potential role of myostatin to inhibit cardiac hypertrophy, we have not identified any studies evaluating myocardial expression of myostatin after long-term pressure-overload induced cardiac hypertrophy and heart failure. In this study, we evaluated the myocardial protein expression of myostatin and its antagonist follistatin in spontaneously hypertensive rats (SHR) with heart failure.

2. Methods

Male SHR (n = 8) and normotensive Wistar-Kyoto (WKY, n = 9) rats were housed in a temperature controlled room at 21 °C and kept on a 12-hour light/dark cycle. Food and water were supplied ad libitum. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National

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http://dx.doi.org/10.1016/j.ijcard.2016.04.101
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Institutes of Health (NIH publication 85–23, revised 1996), and study protocols complied with and were approved by the Ethics Committee of Botucatu Medical School, UNESP, Botucatu, SP, Brazil.

Systemic arterial hypertension was confirmed by the tail-cuff method [9] at fifteen months of age (systolic blood pressure: WKY 118 ± 10; SHR 221 ± 35 mm Hg; $p = 0.001$). Beginning at 18 months old, all rats were observed twice weekly to identify clinical heart failure features. Animals were studied after heart failure had been detected, which was clinically characterized by tachypnea and/or labored respiration [10]. WKY rats were studied at comparable ages. After diagnosing heart failure, rats were subjected to transthoracic echocardiography and euthanized two days later. During euthanasia, we evaluated pathological evidence of heart failure such as pulmonary congestion (lung weight-to-body weight ratio > 2 standard deviations above the mean for the WKY group), and right ventricular hypertrophy (right ventricle weight-to-body weight ratio > 0.8 mg/g) [11].

2.1. Echocardiographic study

Cardiac structures and left ventricular (LV) function were evaluated by transthoracic echocardiogram using a commercially available echocardiograph (General Electric Medical Systems, Vivid S6 model, Tirat Carmel, Israel) equipped with a 5–11.5 MHz multifrequency transducer as previously described [12–14]. Rats were anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (1 mg/kg) intramuscularly. A two-dimensional parasternal short-axis view of the left ventricle (LV) was obtained at the level of the papillary muscles. M-mode tracings were obtained from short-axis views of the LV at or just below the tip of the mitral-valve leaflets, and at the level of the aortic valve and left atrium. M-mode images of the LV were printed on a black-and-white thermal printer (Sony UP-890MD) at a sweep speed of 100 mm/s. All LV structures were manually measured by the same observer (KO). Values obtained were the mean of at least five cardiac cycles on M-mode tracings. The following structural variables were measured: left atrium diameter (LA), LV diastolic and systolic diameters (LVDD and LVSD, respectively), and LV diastolic posterior wall thickness (PWT). Left ventricular function was assessed by the midwall fractional shortening (MFS) and ejection fraction.

2.2. Morphologic evaluation

Cardiomyocyte diameters from each left ventricle (LV) were measured as the shortest distance between borders drawn across the nucleus as previously described [15]. Other slides were stained with Sirius red F3BA and used to quantify interstitial collagen fraction [16]. Perivascular collagen was excluded from this analysis. Measurements were taking using a compound microscope (Leica DM LS; Nussloch, Germany) attached to a computerized imaging analysis system (Media Cybernetics, Silver Spring, MD, USA).

2.3. Western blotting analysis

Myocardial protein levels were analyzed by Western blotting as previously described [17–19] with specific anti-myostatin (N-19-R sc-6885-R) or anti-follistatin (H-114 sc-30194) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Protein levels were normalized to those of GAPDH (6C5, sc-32233, Santa Cruz Biotechnology). Myocardium protein was extracted using Tris-Triton buffer (10 mM Tris pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate). Supernatant protein content was quantified by the Bradford method. Samples were separated on a polyacrylamide gel and then transferred to a nitrocellulose membrane. After blockage, membrane was incubated with the primary antibody. Membrane was washed with TBS and Tween 20 and incubated with secondary peroxidase-conjugated antibody. Super Signal® West Pico Chemiluminescent Substrate (Pierce Protein Research Products, Rockford, USA) was used to detect bound antibodies.

2.4. Statistical analysis

Comparisons between groups were performed by Student's-t test. The association between variables was assessed with Pearson's correlation coefficient. The significance level was set at 5%.

3. Results

All SHRs ($n = 8$) had tachypnea and right ventricular hypertrophy (right ventricle weight-to-body weight ratio: WKY 0.62 ± 0.08; SHR 1.70 ± 0.35; $p < 0.001$), and 5 had lung congestion. No WKY rat ($n = 9$) presented any heart failure feature. Anatomical and echocardiographic data from all rats have been previously published [20]. In short, SHR presented left chambers hypertrophy and dilation (left atrial diameter: WKY 5.73 ± 0.59; SHR 7.28 ± 1.17 mm; $p = 0.004$; LV diastolic diameter/body weight ratio: WKY 19.6 ± 3.1; SHR 27.7 ± 4.7 mm/kg; $p = 0.001$; LV systolic diameter: WKY 3.68 ± 0.92; SHR 5.08 ± 1.51 mm; $p = 0.043$), and LV systolic dysfunction (midwall fractional shortening: WKY 34.9 ± 3.31; SHR 24.8 ± 3.20%; $p = 0.003$).

Myocyte diameter (WKY 23.1 ± 1.50, SHR 25.5 ± 1.33 μm; $p = 0.004$) and myocardial interstitial collagen fraction (WKY 4.86 ± 0.01; SHR 8.36 ± 0.02%; $p < 0.001$) were increased in the SHR.

Myocardial myostatin and follistatin expression was lower in SHR (Fig. 1). The myostatin expression negatively correlated with LV diastolic diameter-to-body weight ratio, LV systolic diameter, and LV diastolic posterior wall thickness, and positively correlated with midwall fractional shortening (Fig. 2). The follistatin expression negatively correlated with LV diastolic diameter-to-body weight ratio (r: −0.79; $p = 0.002$) and LV systolic diameter (r: −0.73; $p = 0.007$), and positively correlated with midwall fractional shortening (r: 0.79; $p = 0.002$).

4. Discussion

SHR present early arterial hypertension and LV hypertrophy [21], which evolve to cardiac decompensation in senescence, usually at 18–22 months of age [22]. Due to the slow development of heart failure, as usually observed in clinical settings, SHR are widely used as a model of long-term compensated cardiac hypertrophy. In SHR, heart failure is better characterized by the right ventricular hypertrophy [23], which was found in all SHR. Then, we can conclude that all SHR had heart failure.

To the best of our knowledge, this is the first study to evaluate myocardial protein expression of myostatin and its antagonist follistatin in SHR with heart failure. During chronic hemodynamic overload, the myocardium can produce and secrete myostatin into systemic circulation, which may have effects on myocardial cells and also induce skeletal muscle atrophy [5]. It was therefore unexpected that we observed lower myostatin expression in the hypertrophied and failing SHR myocardium than in the normotensive rats. This result diverges from previous studies, which have shown increased myostatin expression during cardiac remodeling in different experimental models [2,4,6,8]. Also in clinical setting, myostatin transcript levels were significantly upregulated in hearts with dilated cardiomyopathy [24] and in plasma of heart failure patients [25].

The role of cardiac myostatin in heart failure is still under debate. Both beneficial and deleterious effects have been described. Hearts of aging myostatin knockout mice are characterized by increased left ventricular fractional shortening, higher phosphorylated phospholamban, and less interstitial fibrosis [26]. Furthermore, myostatin overexpression in cardiomyocytes leads to interstitial fibrosis and cardiac dysfunction in older mice [27]. In contrast, it has recently been observed that genetic inactivation of myostatin was associated with impaired cardiac energy homeostasis, cardiac hypertrophy, heart failure, and increased

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