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Inhibition of autophagy recovers cardiac dysfunction and atrophy in response to tail-suspension

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ABSTRACT

Aims: Physical inactivity during space flight or prolonged bed rest may cause cardiac dysfunction and atrophy, but the exact mechanism that governs the regulation of myocardial dysfunction and cardiac atrophy remains poorly understood. Autophagy, a protein degradation pathway, has recently been shown to be involved in the regulation of cardiac dysfunction and atrophy. In this study, we investigated the relationships between dysfunction and inactivity-induced atrophy and autophagy in rat cardiac tissue.
Main methods: Physical inactivity was simulated by a tail suspension model, and cardiac function was examined by echocardiography. Cardiac atrophy was measured by wheat germ agglutinin staining and autophagic activity was detected by Western blot analysis and immunofluorescence staining.
Key findings: We demonstrated that cardiac function, especially contractility, declined and the area of cardiac atrophy increased in the tail-suspended cardiac tissue. Additionally, the cross-sectional area of myocardial cells decreased; however, apoptosis did not increase with tail suspension. Similarly, the expression of autophagy-related proteins and the number of autophagosomes were elevated in the tail-suspended cardiac tissue. Moreover, the administration of chloroquine, an autophagy inhibitor, reversed cardiac dysfunction and atrophy via the suppression of autophagic activity during suspension. Our results indicate that autophagy facilitates the development and progression of cardiac dysfunction and atrophy induced by tail suspension.
Significance: Our studies hint that the components of the autophagy-related signaling pathway are potential therapeutic targets for the treatment of cardiac diseases induced by physical inactivity.

Introduction

Physical inactivity during prolonged bed rest or tail suspension (TS) induces a number of adaptive changes in all physical functions, especially cardiac functions [24,30,32]. Numerous studies indicate that myocardial atrophy is a critical factor in the development and progression of depressed cardiac function caused by physical inactivity [19,32]. Myocardial atrophy, rather than apoptosis, plays an important role in the reduction of contractile function during physical inactivity [32]. However, the exact mechanism that governs the regulation of cardiac atrophy and myocardial dysfunction in physical inactivity remains poorly understood.

Autophagy, a self-catabolic process that maintains intracellular homeostasis under various stimuli, has recently been demonstrated to be involved in the regulation of myocardial tissue atrophy [7,21]. Colon adenocarcinoma causes cardiac atrophy via induction of autophagy in a sexually dimorphic manner [7]. Autophagic activity is augmented in atrophic myocardial tissue [21]. BCL2 Adenovirus E1B 19 kDa Interacting Protein 3, a BH3 only protein, induces autophagy via the stimulation of the autophagic core complex [27]. Cao et al. determined that cardiac atrophy was significantly blunted in Bnip3 null animals [6]. Accordingly, autophagic activity may be overactive and the suppression of autophagy may attenuate myocardial dysfunction and cardiac atrophy in response to physical inactivity.

In this study, we examined the autophagic activity involved in the regulation of myocardial dysfunction and atrophy during physical inactivity by using a TS model. We determined that autophagy is overactive in cardiac muscle tissue after TS, which promotes atrophy in cardiac tissue and a reduction of systolic functions. This study provides insight into the molecular mechanisms of cardiac atrophy and cardiac function decline during physical inactivity. It is suggested that controlling autophagy may represent a promising therapeutic strategy for physical inactivity-induced myocardial atrophy and cardiac function decline.

Materials and methods

Animals and reagents

Male Sprague Dawley rats that weighed 300 ± 20 g (10-week-old) were purchased from the Laboratory Animal Center, Academy of Military Medicine (certificate number: SCX-2007–004). Chloroquine (CQ) (C6628) and FITC conjugated wheat germ agglutinin (FITC-WGA)

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(L4895) were purchased from Sigma (St. Louis, MO). Alexa Fluor 488 (A-11,055) and 647 (A-31573) antibodies (Abs) were purchased from Invitrogen (San Diego, CA). Anti-rat Bcl-2 (2870), Beclin-1 (3495), Vps34 (4263), mTOR (2972), p-mTOR (S2448) (2971), caspase-3 (9665), cleaved caspase-3 (9661), caspase-9 (9506), cleaved caspase-9 (9607), and GAPDH (3683) Abs were purchased from Cell Signaling Technology (Danvers, MA). LC3B (L7543) and p62 (P0067) Abs were purchased from Sigma. Anti-rat LAMP-1 (ab24170) were purchased from Abcam (Cambridge, UK). Anti-rat cathepsin D (sc-377299) was purchased from Santa Cruz Biotechnology (Dallas, Texas). TUNEL assay kits (12156792910) were purchased from Roche (Basel, Switzerland). Other materials were purchased from commercial sources.

TS

The Sprague Dawley rats were randomly assigned to three groups with 10 rats per group; a control group (without TS), TS group 1 (with TS for 4 weeks), and TS group 2 (with TS for 8 weeks). The rats in the control group were housed in standard cages in the same room as the TS groups. The TS followed the methodology of Wronski and Morey-Holton [26]. The rats were suspended by using a tail harness to suspend the hindlimbs above the floor of the cage. Following TS for 4 weeks or 8 weeks, the rats were sacrificed via excessive doses of anesthesia.

To measure the autophagic flux in the rats who underwent TS for 8 weeks, the CQ (60 mg/kg/day) was administered via an intraperitoneal (*i.p.*) injection [10] into the suspended rats from weeks 5 to 8. Following TS for 8 weeks, the rats were sacrificed via excessive doses of anesthesia.

All animal protocols conformed to the Guidelines for the Care and Use of Laboratory Animals, which were prepared and approved by the Animal Care and Use Committee of the China Astronaut Research and Training Center.

Echocardiography

Following TS for 4 or 8 weeks, echocardiography was performed with the Visual Sonics Vevo 770 system using a 30 MHz image transducer (RMV710B). The rats were anesthetized with chloraldurate (400 mg/kg, *i.p.*). After a good quality two-dimensional image was obtained, M-mode images of the left ventricle's internal dimension-diastole (LVIDd), left ventricular posterior wall dimensions (LVPWd), left ventricular posterior wall systole (LVPWs), left ventricular end-diastolic volume (LVEDV), interventricular septal thickness at diastole (IVSd), and stroke volume (SV) were recorded. The percent fractional shortening (FS%), left ventricular ejection fraction (EF%) and cardiac output (CO) were automatically calculated on a cardiac ultrasound machine. The formula for the left ventricular mass (LV mass) was as follows [11]:

$$\text{LV mass} = 1.05 \times \left((\text{LVIDd} + \text{LVPWd} + \text{IVSd})^3 - \text{LVIDd}^3\right)\text{g}.$$

Heart weight (HW) index

Following TS for 4 or 8 weeks, the body weight (BW) of the rats was measured. The hearts were subsequently eviscerated and weighed to determine the HW. The formula for HW index was as follows [21]:

$$\text{HW index} = \text{HW} \div \text{BW} \times 1000.$$

Histomorphology

The hearts were rapidly excised, fixed with 4% paraformaldehyde, and embedded in paraffin for histopathological examination as previously described [29]. The heart tissue sections (5 μm thick) were prepared and stained with hematoxylin and eosin (HE). The grade of cardiac inflammation was blindly assessed by a professional pathologist. The pathologic pictures of the heart tissue were examined using Image-Pro Plus image analysis software.

Confocal assay

Standard protocols for immunofluorescence microscopy were used as previously described [20]. The heart sections (5 μm thick) were prepared and stained with the indicated primary Abs overnight at 4 °C. The sections were washed twice, incubated with fluorochrome-labeled secondary Abs (1:200) for 30 min, and then washed three times after staining. Images were obtained with a Leica SP2 confocal microscope (Leica Microsystems, Exton, PA) and analyzed with Leica confocal software. Cell death was identified via TUNEL staining, and the cross-sectional area of the myocardial cells was identified by WGA [21]. Autophagolysosomes were identified by the coexpression of LC3 and LAMP-1 [16]. The statistical analysis of LC3 and LAMP-1 coexpression was processed as previously described [23]. To determine the percentage of colocalization between LC3 and LAMP-1, the amount of LC3 and LAMP-1 co-staining was compared with the cross-sectional area of myocardial cells.

Western blot analysis

Proteins were extracted from papillary muscles in the rats' left cardiac ventricles as previously described [14]. Western blot analyses were performed as previously described [5]. Specific antibody binding was visualized via ECL (Amersham Biosciences).

Statistics

All results are expressed as the mean ± SE. Statistical analyses using Student's t-test were performed for two-group comparisons. Multiple comparisons among three or more groups were performed using one-way ANOVA (S–N–K method). The *P* value was set at 0.05. All statistics were analyzed using SPSS 17.0 software.

Results

Physical inactivity impairs systolic function of rat myocardial tissues

Numerous studies have demonstrated that cardiac function is impaired by physical inactivity during space flight or prolonged bed rest [1,2,24,32]. We detected the effects of physical inactivity on cardiac function. Rats who underwent TS exhibited significantly declined systolic function regarding the SV, fraction shortening (FS), EF, CO, and LVEDV (Fig. 1A–E). Moreover, as indicated in the sections stained with HE, the degeneration of myofibril fiber particles and the infiltration of inflammatory cells were obvious in the heart tissues of rats suspended for 4 weeks; TS for 8 weeks further promoted pathological changes (Fig. 1F). These findings suggest that physical inactivity induced by TS weakened the systolic function in rat heart tissue.

Physical inactivity induces the atrophy of myocardial cells following TS

Previous research indicates that cardiac atrophy of papillary muscles occurs in the left cardiac ventricles of rats following TS or short-term space flight [13,19]. We further tested whether cardiac atrophy could occur in the papillary muscles of the left cardiac ventricle in response to TS. TS resulted in a reduced HW to BW ratio (Fig. 2A). In addition, TS also decreased the LV mass, LVPWd and LVPWs in cardiac muscle tissue (Fig. 2B-D). A comparison of tissues stained with WGA between the control and TS animals revealed that the myofiber cross-sectional area in the hearts exposed to TS was greatly reduced (Fig. 2E). A myofiber