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The effect of blood flow restriction along with low-intensity exercise on cardiac structure and function in aging rat: Role of angiogenesis

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ABSTRACT

Aims: Low-intensity aerobic training along with limbs blood flow restriction can improve mass and strength of skeletal muscle, but its effects on aging heart structure and performance is unidentified. We investigated the effects of this model of training on myocardial function, histology and angiogenesis in old male rats.

Main methods: Animals randomly were divided into control (Ctl), sham-operated (Sh), limbs blood flow restriction (BFR), sham-operated plus 10 weeks low intensity treadmill exercise (Sh + Ex), and BFR plus exercise (BFR + Ex) groups. Finally, blood pressure, heart physiological and stereological parameters, myocardial oxygen consumption index and expression of vascular endothelial growth factor (VEGF) and its receptors (Flt-1 and kdr) were assessed.

Key findings: BFR + Ex group had significantly lower heart rate ($P < 0.05$ vs. Ctl and Sh groups), rate-pressure product (RPP) and left ventricular end diastolic pressure ($P < 0.05$ and $P < 0.01$ vs. untrained groups, respectively). BFR + Ex group also had greater $+dp/dt$ max ($P < 0.01$) and $-dp/dt$ max ($P < 0.05$) than untrained groups. A significant increase in volumes of left ventricle and myocytes ($P < 0.05$, vs. Ctl and Sham), ventricular hypertrophy index and capillaries length density ($P < 0.05$ vs. untrained groups) were observed in BFR + Ex group.

The level of VEGF and Flt-1 proteins and their mRNAs increased in the BFR + Ex group compared to Ctl, Sh and BFR ($P < 0.01$) and Sh + Ex ($P < 0.05$) groups. The kdr mRNA and its protein level were significantly higher in the BFR + Ex group.

Significance: Findings suggest that BFR plus exercise through improving the angiogenesis, physiological cardiac remodeling and oxygen demand/supply matching can promote cardiac performance in the elderly rats.

1. Introduction

Intrinsic cardiac aging is determined by a number of slowly progressive changes in physiological and morphological characteristics of the heart that make it more susceptible to stress and lead to ventricular dysfunction, heart failure, and arrhythmias in the elderly and increase cardiovascular mortality and morbidity [1]. These age-related changes include alteration of the diastolic filling pattern and heart rhythm [2], degenerative changes in myocytes, as well as alterations in structure and composition of the extracellular matrix, fibrosis, loss of ventricular compliance [1] and down-regulation of angiogenesis [3].

According to the recommendation of World Health Organization (WHO), older adults should do at least 150 min of moderate-intensity aerobic physical activity or do at least 75 min of vigorous-intensity aerobic physical activity throughout the week [4]. Chronic aerobic exercise can improve cardiovascular performance in aged people and those with cardiovascular risk factors [5]. The KAATSU training is a new model of short-term and low-intensity exercise in which muscle blood flow is restricted during exercise by binding the proximal portion of lower or upper extremities in an invasive (by partial closing of the

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desired arteries) or non-invasive (by binding a specially-designed tourniquet around the interest limb) way [6]. This kind of exercise reduces the muscle blood flow during contractions and treadmill running but does not limit normal resting muscle flow [7]. In this regard, animal and human studies have reported that the use of low-intensity resistance exercise with blood flow restriction (BFR) can improve muscle mass, strength, perfusion conditions and oxygen delivery of skeletal muscles in aging subjects [8]. The elderly usually does not tolerate heavy exercise, and mild exercise such as walking alone have little effect on their muscle mass and strength, so the KAATSU model is recommended for these people. However, despite its importance, the positive or negative effects of this method especially its aerobic endurance model on aging hearts and its plausible mechanisms have been less paid attention to in previous literatures. This is while, as noted above, aging is accompanied by some degree of cardiovascular disability that various sports models can exacerbate or recover the degree of cardiovascular insufficiency.

Vascular endothelial growth factor A (VEGF-A), usually known as VEGF, is a member of VEGFs family and plays a key role in angiogenesis. VEGF-A exerts its biologic effect through interaction with cell surface receptors, VEGFR-1(VEGF receptor-1) or Flt-1(fms-like tyrosine kinase-1) and VEGFR-2, kdr (Kinase insert domain receptor) or Flk-1(fetal liver kinase-1) [9,10]. It has been demonstrated that exercise training is able to improve age-associated down-regulation of angiogenesis through VEGF angiogenic signaling cascade and increase new vessels formation in the aging myocardium [12], as well as in the skeletal muscle [12].

We hypothesized that low-intensity endurance exercise along with blood flow restriction may interfere with age-related cardiac remodeling, angiogenesis process and cardiac dysfunction.

Therefore, present experimental study was designed to determine whether the model of BFR aerobic training, that is particularly tolerable and is done by elderly, physically limited and cardiovascular patients, can improve the age-associated changes in cardiac structure and function in aged rats.

2. Material and method

2.1. Material

Chemical materials were prepared as sodium thiopental from Sandoz (Austria), Ketamine and Xylazine from Alfasan Company (Holland), the cDNA PrimeScript™ RT reagent kit (Takara, # RR037A; Japan), SYBR Green master mix (1×) (Takara, #RR820L; Japan), monoclonal primary antibodies against VEGF, Flt-1, kdr and GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) and the secondary antibody from Santa Cruz Biotechnology Co (Beverly, MA, USA), PVDF membrane and enhanced chemiluminescence (ECL) detection kits from Roche (Mannheim, Germany). Specific forward and reverse primers selected and designed by using the Gene Runner software (Version: 5.0.999 Beta).

2.2. Ethical approval

This study was conducted in accordance with national laboratory practice guidance and approved by the Ethics Committee (permission No IR.KMU.REC.1394.495) of the Kerman University of Medical Sciences, Kerman, Iran.

2.3. Animals

Male Wistar rats (22–24) months-old weighing 380–450 g were obtained from Tehran University, Tehran, Iran. Animals were housed in a 21–23 °C environment on a 12-h light–dark cycle with free access to water and food.

2.4. Experimental design

Animals were divided randomly into 5 groups of 14 animals. The groups included untrained control, sham-operated, and blood flow restriction (BFR) and their trained counterparts; sham-operated plus exercise (Sh + Ex), and blood flow restriction along with exercise (BFR + Ex) respectively. Blood flow restriction was induced by the bilateral partial closing of the femoral arteries have been explained previously in details [13–15]. In sham-operated rats, the same surgery was performed without closing the artery. After a week of recovery of animals undergoing surgery, the 10-week treadmill running program was started for training groups. The low-intensity exercise program started with 15-min/day sessions at the speeds of 7.5 m/min and zero-degree incline as the learning phase. Animals lacking the ability and cooperation needed for exercise were excluded from the study and the new animals were replaced. Duration of exercise sessions and treadmill speed were gradually increased so that at the onset of the tenth week, animals run for 60 min per day at the speed of 15 m/min while maintaining a 0% grade [13–15]. For the purpose of familiarizing, untraining rats were placed on the treadmill at least 20 min in each session.

2.5. Hemodynamic measurement and heart monitoring

48 h after a 10-week intervention period, half of animals from all groups were weighed and then anesthetized with i.p. injection of sodium thiopental (50 mg/kg). To evaluate the left ventricular performance, a polyethylene catheter (PE-50) filled with heparin saline (15 units/mL) was inserted into the right carotid artery and advanced into the left ventricle (LV) through the aortic valve [16]. After a 10-min period of stabilization, left-ventricular systolic pressure (LVSP), left-ventricular end diastolic pressure (LVEDP), the maximal positive and negative rate of changes in left ventricular pressure ($+dP/dt$ max and $-dP/dt$ max), as indices of cardiac contractility and cardiac relaxation velocity, respectively, were recorded on an 8-channel PowerLab Physiograph system (ADInstruments, Australia). Another catheter was placed in the left carotid artery and used for arterial blood pressure recordings. The mean arterial pressure (MAP) was calculated by "MAP = DAP + (SAP − DAP) / 3 formula," where DAP is the diastolic arterial pressure and SAP is the systolic arterial pressure. To estimate the heart-energy demand and oxygen consumption, rate-pressure product (RPP), was determined as the product of the heart rate and mean arterial pressure ($(MAP * \text{heart rate}) * 1000^{-1}$) [17]. The left ventricular hypertrophy index was estimated using the following formula: Left ventricular weight (mg) / body weight (g). Throughout the experiment, the body temperature was maintained at 37 °C by a thermostat connected to a metal plate placed in the center of the surgical table. Following the hemodynamic measurement animals were sacrificed, hearts were excised quickly, washed in cold PBS, frozen in liquid nitrogen, and stored at −80 °C until the real-time PCR and Western blot analysis.

2.6. Real time RT-PCR

Quantitative Real Time Polymerase Chain Reaction (QPCR) method was used to quantify gene expression with QIAGEN's real-time PCR cycler (the Rotor-Gene Q, USA). About 30 mg of samples obtained from the hearts apex were homogenized and total RNA was extracted with Trizol reagent according to the RNA Isolation Protocol of Manufacturer's instruction (Roche, USA) [18]. After determining the concentration and purity of total RNA by a Nano drop spectrometer (HELLA; USA), extracted RNA was reverse transcribed using PrimeScript™ RT reagent kit (Takara, # RR037A; Japan) in gradient thermal cycler (ASTEC, BL-516H; Japan) in a final volume of 10 μL according to the manufacturer's protocol. For preparation of QPCR array, the synthesized cDNA was directly used in PCR by addition of 2 μL of the cDNA reaction mixture to a final volume of 20 μL PCR reaction composed of