Effect of Resistance Exercise on Cardiac Apoptosis Following of Ischemic/Reperfusion

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Abstract: Researchers studied the cardioprotective effect of resistance training against ischemia reperfusion induced injury. The 40 male Wistar rats were divided into trained and sedentary groups (n = 20 for each). Trained rats were exercise in squat-training apparatus (12 repetitions set^{-1}, 4 sets day^{-1} and 5 days week^{-1} for 4 weeks). After last training session, transient regional ischemia of left anterior descending coronary artery (40 min) was followed by 80 min of reperfusion. Coronary flow, left ventricular developed and diastolic pressures, infarct size and apoptosis rate were measured. Baseline developed and diastolic pressures and coronary flow were similar in two groups. While diastolic pressure increased and developed pressure and coronary flow decreased both in ischemia and perfusion periods (as indices of cardiac damage), there were no differences between trained and sedentary groups in these parameters statistically. Resistance training did not change the infarct size and apoptosis rate statistically. The researchers did not see cardioprotective effect of resistance exercise against ischemia-reperfusion induced injury in this study. Precise conclusion about this issue needs more investigations.

Key words: Exercise, heart, infarction, ischemia, reperfusion, Iran

INTRODUCTION

Ischemic heart disease remains a worldwide problem affecting all economic groups of the society (Hansen, 1995). The primary pathological manifestation of ischemic heart disease is myocardial infarction due to Ischemia-Reperfusion (IR) injury (Rao and Viswanath, 2007). Preservation cardiac performance and reduction of infarct size are the main goals in the management of IR-induced complications (Rao and Viswanath, 2007). In this regard, many approaches to provide cardioprotection against IR-induced injury have been studied. Until now, regular exercise has been confirmed as a pragmatic and sustainable countermeasure for cardioprotection (Powers *et al.*, 2008). While convincing evidence indicates that both short-term (3-5 consecutive days) and long-term (months) endurance exercise training (i.e., running and swimming) improves myocardial tolerance to IR-induced injury in both male and female animals as well as young and old animals (Powers *et al.*, 2008), there is no clear understanding about cardioprotective effect of resistance exercise training (such as body building and weight lifting) against IR-induced injury. Resistance exercise training is a specialized method of conditioning designed to increase strength and muscle endurance (Barauna *et al.*, 2007). Similar to endurance training, it has shown that resistance training has beneficial effects on some physiologic and pathologic processes such as physical fitness, quality of life and chronic heart failure (Zavorsky, 2000). While the risk of cardiovascular complications is the primary concern with resistance training in some cardiac patients (due to blood pressure elevation during this type of exercise), resistance training can positively influence quality of life, cardiovascular risk factors and cardiovascular function in healthy persons and in selected patients with cardiovascular disease (Bjarnason-Wehrens *et al.*, 2004; Zavorsky, 2000). Although, several investigators have studied the impact of resistance training on cardiac structure and function, the cardioprotective effect of resistance exercise training

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against IR-induced injury has not been understood. The purpose of this study was to investigate cardiac performance during ischemia and reperfusion period as well as to determine cardiac infarct size and apoptosis rate after IR-induced injury in rats undergoing resistance exercise training for a short period of 4 weeks.

MATERIALS AND METHODS

Animals: The 40 male Wistar rats (220-240 g, 3 months old) were obtained from laboratory animal house of Tabriz University of Medical Sciences and they were randomly divided into trained (EXT) and Sedentary (Sed) groups (n = 20 for each group). Animals were housed at room temperature (23±1°C) with 12 h light/dark cycles and had free access to food and water. The study protocol was designed in accordance with the guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, revised 1996) and approved by the Ethics Committee for the Use of Animals in Research of the Tabriz University of Medical Sciences.

Training program: Trained rats were exercised according the model described by Tamaki *et al.* (1992) with some modification. Rats were placed vertically in squat-training apparatuscylinder (Rat WLI009, Tajhiz Azmaye Pooya Co, Iran) as they could stand on their hind limb in response to electrical stimulation and raise the piston which was located above their heads. An electrical stimulation (20 V, 0.3 sec duration at 3 sec intervals) was applied to the rat's tail through a surface electrode. After 1 week of adaptation, trained group rats exercised 4 sets of 12 repetitions day^{-1} with a 90 sec rest period between each set, 5 times week^{-1} for 4 weeks (Barauna *et al.*, 2007). Each rat in train group was weighed daily and 120% of its body weight (approximately 70% of maximum load that the rats were able to raise following electrical stimulation) was considered for weight of piston. The piston movement for each rat was recorded by a distance sensor which had been located above the piston and work performed by each rat was calculated daily by multiplying of piston weight and piston movement.

Heart preparation: According to the method of Brown *et al.* (2003), after anesthetization with pentobarbital sodium (35 mg kg^{-1} ip injection) hearts were excised, placed in ice-cold saline and rapidly hung by the aorta on the cannula of Langend orff apparatus. Hearts were perfused with 37.5°C Krebs buffer (76.5 mm Hg perfusion pressure with 95% O_2 and 5% CO_2) containing 117.4 mM NaCl, 4.7 mM KCl, 1.9 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 5 mM pyruvate, 11 mM glucose, 0.5 mM EDTA, 25 mM $NaHCO_3$ and 1200 U lheparin^{-1}. A

pressure-transducing catheter was placed through the cannula and aortic valve in to the chamber of the Left Ventricle (LV) and developed pressure was acquired with a computer connected to the transducer (Power Lab, AD Instruments, Australia). After a 5 min stabilization period, base line pressure was measured and coronary flow rate was obtained by collection of the coronary effluent for 1 min.

Ischemia-reperfusion protocol: After baseline record, a suture was threaded through the left anterior descending coronary artery 3-5 mm distal to the aorta in 14 rats of each group. Both ends of the suture were inserted into a small polyethylene tube that was used as a snare and ischemia was induced by tightening the snareso that the artery was fully compressed. Pressure and coronary flow measurements were recorded at 5, 15 and 30 min after onset of ischemia. After 40 min, the snare was loosened and re-perfusion ensued for 80 min. Coronary flow and pressured at were recorded at 5 min after the on set of reperfusion and then every 15 min until the end of the 80 min reperfusion period.

Measurement of infarct size: Infarct size was measured using methods similar to those previously described (Brown *et al.*, 2003). After the re-perfusion period, the snare was retightened around the left anterior descending coronary artery in 6 hearts of each group and 100 µL of 0.05% evans blue solution was injected into the aortic cannula and for 3 min perfused through the heart. Then the heart was sliced transversely from base to apex in to four slices of equal width. Each slice was immersed in phosphate buffer and was photographed with a digital camera. After both sides of each slice were photographed, each slice was placed in 100 mM phosphate buffer with 0.1% triphenyl tetrazolium chloride and incubated for 10 min at 37°C. After incubation, each side of every slice was again photographed and the slices were weighed. Heart weight was obtained by summation of the slice weights for each heart. To avoidance experimenter bias, Images of the slices were analyzed in a single-blind manner by Scion image 4.0 software. Total slice Area (TA), Zone at risk (ZAR; the area of each slice that did not turn blue after perfusion with the solution containing evans blue dye) and Infarct Area (IA; the portion of the ZAR that did not turn red in response to triphenyltetrazolium chloride incubation and remained white) were measured. ZAR and IA were obtained from each side of a single slice and the mean of both sides was used as the representative ZAR and IA for that slice.

Finally, IA was expressed as a fraction of all ZAR by taking the sum of all infarcts and was reported as percentage.

Quantification of apoptosis: The left ventricle was immersion-fixed in 10% neutral formalin and embedded in paraffin n = 5 for controls and n = 6 for exercised rats). Serial sections of 4 µm thicknesses were prepared. Apoptosis was evaluated via the terminal deoxynucleotidyl Transferase-mediated dUTP Nick-End Labeling (TUNEL) methodwith the use of In Situ Cell Death Detection Kit, POD (1684817, Roche, Germany) according to manufacturer's instructions with some modifications (Hansen, 1995). Briefly, the tissue sections were dewaxed and rehydrated by heating at 60°C followed by washing in xylene and rehydration through a graded series of ethanol and double distillated water. Then, the sections were incubated for 30 min at 21-37°C with Proteinase K working solution (20 µg mL^{-1} in 10 mM Tris-Cl, pH 7.6). The sections were rinsed with PBS and incubated with the TUNEL reaction mixture for 1 h at 37°C in a humidified chamber. As a positive control, sections were treated with DNase I (1 mg mL^{-1}; Sigma) for 10 min to introduce nicks in the genomic DNA. After converter Peroxidase (POD) was added, the sections were incubated for 30 min at 37°C in a humidified chamber. Then, the 3, 3 diaminobenzidine substrate was added for the visualization of nuclei with DNA nick end labeling. The sections were counter-stained with toluidine blue to show normalnuclei. The percentage of myocytes with DNA nick end labelingwas analyzed by counting the cells exhibiting brown nuclei at x40 magnification in 5 randomly chosen fields (1 mm^2) in triplicateplates. The number of TUNEL-positive cardiomyocytes was counted by double-blinded observation.

Data analysis: All statistical comparisons were made using SPSS 16.0 software (Chicago, IL) and were expressed as mean±SD. Work performed, pressures and flow data were analyzed using repeated measures ANOVA. When a significant p-value was obtained, a post hoc Bonferroni test was employed to determine the differences between the groups. Between-group comparisons of heart rate, infarct size, body weight, heart weight and apoptosis rate data were made using a Student's t-test. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Infarct size and apoptosis rate: Figure 1 and 2 show the size of infarction and apoptosis rate, respectively in the heart of EXT and sed groups. Resistance exercise training did not change the infarct size and apoptosis rate statistically.

The previous study depicted that 12 weeks resistance exercise training preserves the heart against IR-induced injury (Soufi *et al.*, 2011). Although, there are some documents about effect of resistance training on cardiac

Fig. 1: Effect of resistance exercise on the heart infarct size; a): Representative digital images of stained heart. Non necrotic viable tissue is red and infracted tissue is white. b): Quantification of average infarct size expressed as percentage of ischemic ZAR (Zone At Risk). Values are mean±SD (n = 6 rats); Sed: Sedentary and EXT: Exercise Trained rats

structure and function, to the best of the knowledge, this is the first study which has focused on role of short-term
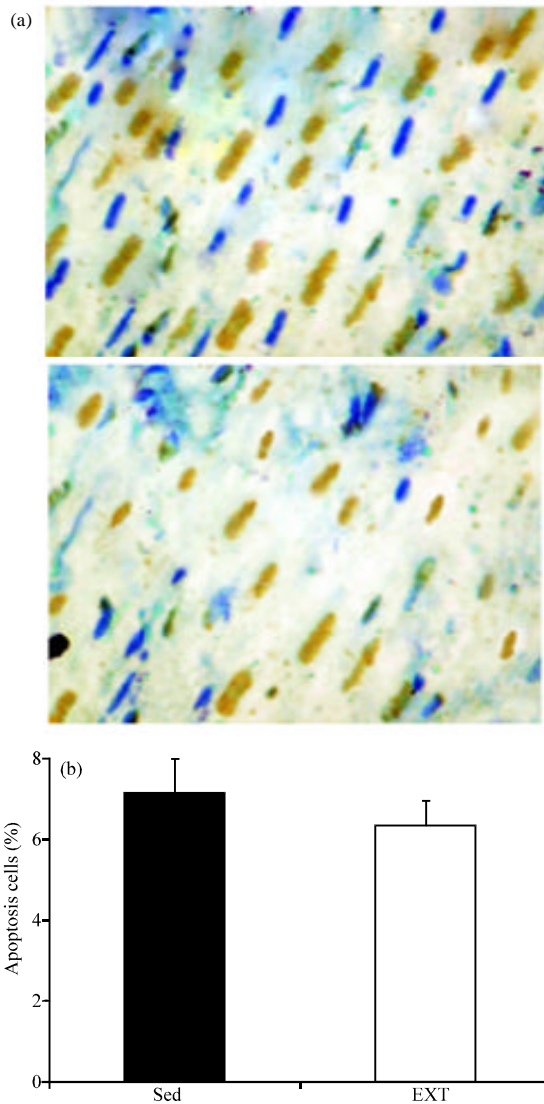
Fig. 2: Effect of resistance exercise on the heart apoptosis rate; a): Cell death detection by TUNEL method at x40 magnification (brown nuclei are the apoptotic cells). b): Comparison of apoptotic cells ratio in different groups. Sed: Sedentary and EXT: Exercise Trained rats. Values are mean±SD (n = 6 for trained and n = 5 for sedentary rats)

resistance training in preserving the heart against IR-induced injury. The main findings of the present study are that 4 weeks resistance training:

- Increases the weight lifting ability
- Induces cardiac hypertrophy without any change in cardiac function statistically
- Do not preserve the heart against IR-induced injuries as evidenced by no change in infarct size and apoptosis rate

Weight loss, cardiac hypertrophy and work performed are some indices to characterize training efficiency. Previously Barauna *et al.* (2007) reported that 4 weeks resistance trainingincreases weight lifting ability and also it induces cardiac hypertrophy with no change in cardiac function in the rats. Progression in weight lifting ability indicates training efficacy and development. While maximum heart rate or VO_{2max} are used to prescribe endurance exercise training (Zavorsky, 2000), work performed may be good indicator of resistance training efficacy. Moritani and de Vries (1979) depicted that neuronal and muscular adaptationsinvolved in training induced enhancement of the rat muscular strength.

Resistance training is a known stimulus for cardiac hypertrophy due to pressure overload imposed on the heart during training (Barauna *et al.*, 2008). The results are in agreement with pervious researches (Barauna *et al.*, 2008). Precise underlying mechanism of the resistance training induced-cardiac hypertrophy needs to be elucidated. In this regard it has been suggested that induction of Angiotensin receptor Type 1 (AT_1) expression in the heartand elevation of circulating anabolic hormonesmay be involved (Barauna *et al.*, 2008; Goto *et al.*, 2008).

In this study, coronary flow, left ventricular developed pressure and diastolic pressure did not differ between trained and untrained rats statistically. There are several published documents about the beneficial effect of resistance exercise on cardiac performance in the patients with heart failure (Hambrecht *et al.*, 2000; Levinger *et al.*, 2005; Palevo *et al.*, 2009). In this regard it has proposed that resistance training could improve stroke volume and ejection fraction without enhancement of cardiomegaly or cardiac deterioration (Hambrecht *et al.*, 2000; Levinger *et al.*, 2005; Palevo *et al.*, 2009). But few studies have investigated the effect of this type of exercise on cardiac function in healthy individuals and most of them did not report changes in heart function after resistance training (Colan *et al.*, 1987; Longhurst *et al.*, 1980; Pluim *et al.*, 2000). Moreover Barauna *et al.* (2007) reported that 4 weeks of resistance training did not change cardiac function in rats. The results are in agreement with the results of these studies.

Growing evidence indicates that IR-induced myocardial cell death is not limited to necrosis but also includes apoptotic cell death (Quindry *et al.*, 2005). For this reason we measured ventricular apoptosis rate and infarct size in this study. The results show that short-term resistance training neither induces excessive damage to the heart nor preserves it against IR-induced injury because apoptosis rate and infarct size did not change

between our trained and control animals hearts. While it has shown that short to long term endurance exercise can protect the heart against IR-induced injuries (Powers *et al.*, 2008), some investigations did not report the beneficial effects of endurance exercise (up to 12 weeks) on cardiac performance, antioxidant defense and cell death rate (Moran *et al.*, 2004; Soufi *et al.*, 2008). It has proposed that these controversies could result from methological differences such as type and duration of endurance exercise (swimming, treadmill or wheel running), time between the end of the training program and sacrifice of the animals and so on (Ascensao *et al.*, 2007).

CONCLUSION

In this study, the researchers saw that 12 weeks resistance exercise training preserved the heart against IR-induced injury but the results of present study show that 4 weeks resistance training unable to do it. Nevertheless, this is the first study with this purpose and precise conclusion about this issue needs more investigations.

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