Iranian Journal of Health and Physical Activity (2011) 2 (1), 1-5

The Role of Exercising and Curcumin on the Treatment of lead-induced Cardiotoxicity in Rats

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Received 23 October 2010 Accepted 30 January 2011

Abstract

The present study aims to evaluate Cardioprotection effects of exercise training and curcumin on myocardial damage induced by lead acetate. Forty-eight rats were randomly divided into six groups of the base, sham, lead, exercise, curcumin and exercise+curcumin (EC). The rats in the exercise and (EC) groups performed the progressive treadmill running of 15 to 22 m/min for 25 to 64 min, 5 times a week for 8 weeks. Lead, exercise, curcumin and (EC) groups received lead acetate (20 mg/kg), and sham, curcumin and (EC) groups received curcumin solvent (ethyloleat) and curcumin solution (30 mg/kg). Cardiac tissue was removed of aorta hiratus and homogenized for the estimation of troponin I, using ELISA. CK-MB and lead was determined in serum by immunological DGKE method and atomic absorption Spectrophotometry methods, respectively. Injection of lead acetate into intra-peritoneal resulted in a significant increase of the CK-MB levels. However, treadmill running exercise and curcumin supplementation resulted in a significant decrease of CK-MB levels while there was no significant difference in troponin I levels. The results of this study suggest the cardioprotective potential of administration of exercise and curcumin in ameliorating the lead-induced cardiotoxicity in rats through a decrease of myocardial damage markers.

Keywords: Aerobic training, Antioxidant, Pollution, Cardiac damage, Rat

Introduction

The epidemiological association between exposure to air pollution and cardiovascular morbidity and mortality has been well documented in previous studies. The underlying mechanisms linking pulmonary exposure to air pollution with increased risk of cardiovascular events have also been investigated in the last decade [1].

Lead is one of the worldwide-used metals, which has been used since the ancient time. It is also a toxin, known to have adverse effects on the body, even at low level of exposure, inducing a broad range of physiological, biochemical and behavioral dysfunctions. Studies have shown that this metal has harmful effects on tissues such as nervous system, blood tissues, cardiovascular system, reproductive and urinary system [2]. Recent studies suggest that one of the mechanisms by which lead can exert some of its toxic effects is through the disruption of the delicate prooxidant/antioxidant balance that exists within mammalian cells. In vivo studies have suggested that lead exposure is capable of generating reactive oxygen species (ROS) and so altering antioxidant defensive systems in animals [3].

Curcumin{1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione}(diferuloyl methane), the principal coloring agents present in the rhizomes of Curcuma longa (zingiberaceae), possesses many therapeutic properties including antioxidant, anti-inflammatory and anticancer properties [3]. Curcumin has a wide range of therapeutic effects in numerous diseases including neoplastic and chronic inflammatory diseases (Alzheimer's disease, Parkinson's disease, multiple sclerosis, epilepsy, cerebral injury, cardiovascular disease, cancer, allergy, asthma, bronchitis, colitis, rheumatoid arthritis, renal ischemia, psoriasis, diabetes, obesity, depression, fatigue and AIDS) [4]. Recent research has shown curcumin to be a powerful scavenger of the superoxide anion, the hydroxyl radical and nitrogen dioxide [3]. Exercise is a deterrent of cardiovascular disease, and its antiatherogenic effects have been described in different animal models. Exercise can also positively influence risk factors that are associated

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with cardiovascular disease: hypertension, diabetes mellitus, obesity, increased plasma lipids, and endothelial dysfunction. However, the mechanism(s) by which exercise might be beneficial to cardiovascular disease is not known [5]. Therefore, the present study was designed to evaluate the cardioprotective role of exercise training and curcumin against lead-induced myocardial damage in rats.

Materials and methods

Animals

The experimental protocol was approved by the Department of Physiology, University of Mazandaran and was performed according to Guiding procedures in the Care and Use of Animals, prepared by the Council of the American Physiological Society. 48 wistar male rats, weighing between 200 and 250g, were procured from Iran Pasture Institute and acclimatized under standard laboratory conditions at 25 ± 2 °C, 50 ± 15% relative humidity and normal photoperiod (12 h light:dark cycle) for 7 days. Rats were fed with a standard rat chow provided by Pars institute for animal and poultry factory with a daily regimen of 10 gr/100 body weight for every rat. Also, water was available ad libitum.

Treatment of animals

After acclimatization, 48 animals were divided into six groups of eight animals each, and treated as follows: Group 1 (control): rats received nothing, Group 2 (sham): rats received ethyl oleate (30 mg/kg) 3 days a week, for 8 weeks. Group 3 (lead acetate or (pb)): rats received lead acetate (20mg/kg) 3 days a week, for 8 weeks. Group 4 (curcumin + pb): rats received lead acetate (20mg/kg) and curcumin (30 mg/kg) 3 days a week, for 8 weeks. Group 5: (exercise training+ pb): rats received lead acetate, as well they performed the progressive running exercise of 15 to 22 m/min for 25 to 64 min, 5 times a week. Group 6 (curcumin+ exercise training+ pb): rats received lead acetate (20mg/kg) and curcumin (30 mg/kg), in addition, they performed physical training protocol.

Aerobic training protocol

Rats in the aerobic training groups were trained by running on a level motorized rodent treadmill, 5 days a week, for 8 weeks. The speed of the treadmill and duration of the training sessions was gradually increased from 15 to 22 m/min to 25 to 64 min, 5 times a week.

Induction of myocardial injury

Myocardial injury was induced by subcutaneous injection of Lead acetate at a dose of 20 mg/kg, 3 days a week, for 8 weeks.

Estimation of cardiac biochemical markers

All groups were anesthetized with ketamine and Xaylozine and decapitated after 12-14 hours overnight fasting. Moreover, blood samples were collected from the heart of the participants in all the groups, 24 hours after the last dose of treatment was recieved as mentioned above. These blood samples were first centrifuged by a refrigerated centrifuge at 3,000 rpm for 15 minutes, within 30 minutes after collection, and then stored at -80 C before assay and serum were separated for biochemical estimations of CK-MB. Then Cardiac tissue was removed and perfused for 2 min by phosphate buffer saline (PBS, pH 7.2) to remove the remaining blood. Cardiac tissue at 0.2 g was homogenized on ice in 2 ml PBS, and the filtrate was collected. These samples were first centrifuged by a refrigerated centrifuge at 3,000 rpm for 15 minutes, within 30 minutes after collection and then stored at -80 C before assay and extract were separated for estimations of troponin I. CK-MB and troponin were measured by immunological DGKE method and sandwich-linked immunoassay (ELISA), respectively [6]. Lead was measured in serum atomic absorption Spectrophtometry [7].

Statistical analysis

Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). Results are expressed as means ± SE. The Data for troponin I and CK-MB markers were normally distributed after log- transformation. A one-way ANOVA was used to detect statistical difference between groups. Furthermore, a Post-Hoc test (Tukey test) was performed to establish change differences in markers mentioned above, between groups. The differences were considered significant at $p<0.05$.

Results

Lead acetate administration induced damage to the myocardium. Changes in cardiac tissue troponin I and serum CK-MB levels are presented in Table 1. We found that CK-MB and troponin I levels, tissue damage markers, increased in lead-induced myocardial injury in rats. As a result, serum marker enzymes (CK-MB) levels were significantly ($P < 0.0001$) increased in the lead group as compared to

other groups, whereas curcumin, exercise training and composed of both treatment significantly ($P < 0.0001$) reversed these elevated levels, but it was still higher than the CK-MB level of control group (Fig. 1).

On the other hand, troponin I level increased insignificantly by the end of the 8-week period in the lead group as compared to other groups. But There was an insignificant decrease in troponin I level in the lead group when compared with groups ($P > 0.05$) (Fig. 1).

Table 1: Troponin I and CK-MB levels in lead-induced myocardial injury in rats (mean ± SEM for eight rats)

Markers / Groups	CK-MB	Troponin I
Control	55.57 ± 1.54	0.1614 ± 0.0680
Sham	58.23 ± 1.73	0.1670 ± 0.0700
Lead	86.31 ± 3.56	0.1857 ± 0.0710
Exercise training+ lead	67.95 ± 2.32	0.1750 ± 0.0707
Curcumin+lead	65.00 ± 1.01	0.1571 ± 0.0786
Curcumin+Exercise training+ lead	57.45 ± 1.30	0.1500 ± 0.0755

Statistical significance $p < 0.05$: ⁎ more significant than in the control group: + more significant than in the sham group: # more significant than in lead group.

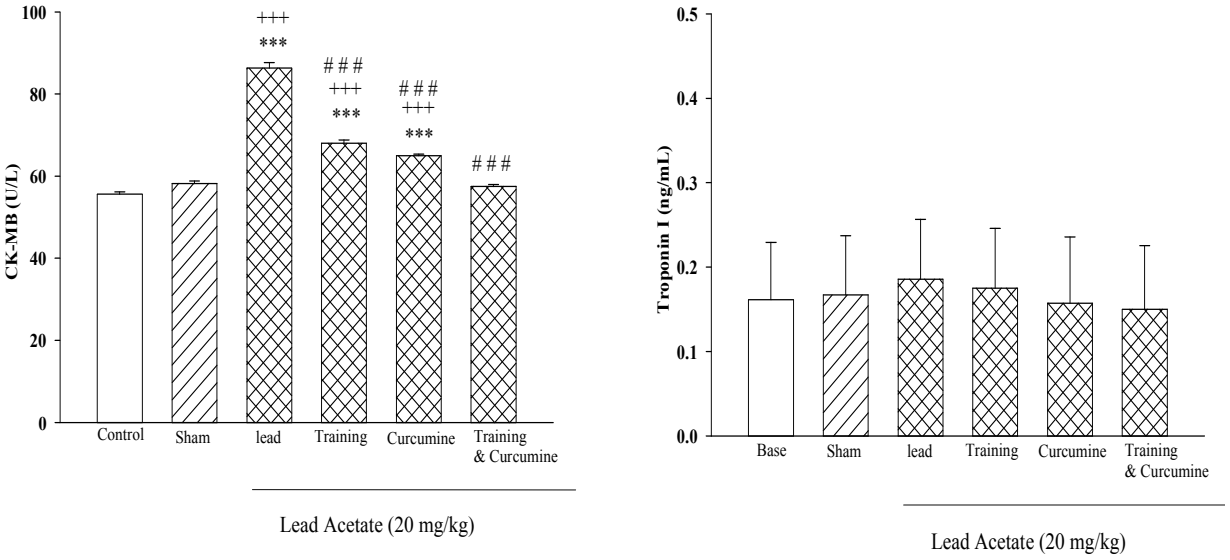

Figure 1: Shows CK-MB and troponin I concentrations in experimental animals. Statistical significance $p < 0.05$: ⁎more significant than in the control group: + more significant than in the sham group: # more significant than in the lead group.

Discussion

Creatin kinase-MB (CK-MB) mass concentration and troponin I are the recent biochemichal markers for the diagnosis of acute myocardial infarction or cardiac tissue damage markers [6]. The present study indicated that there appears to be a proximate correlation between lead-induced cardiotoxicity and cardiac tissue damage markers. We found that there was higher troponin I and CK-MB levels after subcutaneous lead injection as compared to the control group. But CK-MB and troponin I levels that increased after subcutaneous lead injection, decreased by regular training and curcumin supplementation for eight weeks.

Lead toxicity has been known since ancient times and many studies have explored the mechanisms and symptoms of this toxicity through the years [9]. Because the known mechanisms have not been successful in explaining some of the symptoms of lead poisoning, alternative mechanisms are now being investigated. Recent studies have reported lead's potential for inducing oxidative stress and evidence is accumulating in

support of the role of oxidative stress in pathophysiology of lead poisoning [9, 10]. The pathogenesis of lead toxicity is multifactorial, as lead directly interrupts enzyme activation, competitively inhibits trace mineral absorption, binds to sulfhydryl proteins (interrupting structural protein synthesis), alters calcium homeostasis, and lowers the level of available sulfhydryl antioxidant reserves in the body [10]. Recent research examining the etiology of lead toxicity-induced hypertension reveals that the free radical production and lowering of inherent antioxidant reserves resulting from lead toxicity are directly related to vasoconstriction underlying lead-induced hypertension. Many of the mechanisms of lead-related pathologies are a direct result of the oxidant effect of lead on tissues and cellular components [9, 10]. on the other hand, cardiovascular diseases, the most dreaded sequel among the diseases, are invariably followed by several biochemical alterations, such as lipid peroxidation, free radical damage, hyperglycemia and hyperlipidemia, leading to qualitative and quantitative alterations of myocardium[8]. In cardiovascular diseases, major injury is caused by free radical generation; hence, free radical scavengers (antioxidants) form an important therapeutic [8].

Ansari et al. have shown that CK-MB is inactivated by the curcumin supplementation [8]. Yeh et al. have shown that troponin I also decreases by the curcumin supplementation [11]. These results agree with our findings that Curcumin reduces troponin I and CK-MB levels in rat. The mechanisms of Protective curcumin is multifactorial, for: I) its unique conjugated structure, which includes two methoxylated phenols and an enol form of b-diketone[13] II) prevent lipid peroxidation and inhibit the generation of ROS[8] III) The effective antioxidant property of curcumin decreases the utilization of vitamin C and vitamin E in the liver and thus maintains their level[12] IV) interactions between curcumin and lead metal and metal–curcumin complex.Bboth the hydroxyl groups and the b-diketone moiety of curcumin are involved in metal–ligand complexation, either directly bonding to the metal, or in intermolecular hydrogen bonding[3].

Recep Aslan et al. have shown that CK-MB levels, by regular training for five weeks, returned to sedentary [14]. Frederico et al. have shown that Creatine Kinase - MB levels were markedly increased in hearts from isoproterenol -treated animals by twelve weeks of treadmill training, reduced and prevented the deleterious effects of isoproterenol [15]. Ascensao et al. reported that thetroponin I levels increased in doxorubicin - treated animals, by fourteen weeks of Endurance exercise training [16]. These findings are in agreement with our findings that exercise training reduces troponin I and CK-MB levels in rats. The mechanisms of Protective exercise training on cardiac tissue damage increases antioxidant enzymes (Superoxide dismutase, catalase, glutathione peroxidase) and antioxidant non-enzymes, and decreases oxidative damage, [5, 15, 17,18]. Therefore, it seems that the training-induced increases in both myocardial glutathione and SOD activity are potential mechanisms to explain the training- induced reduction in myocardial injury [18].

In conclusion, results from the present study suggest the cardioprotective potential of administration of curcumin and performed aerobic training protocol in ameliorating the lead-induced cardiotoxicity in rats through decreasing cardiac tissue damage markers.

References

1-Chuang K., Chang-Chuan Chan, Ta-Chen Su, Chung-Te Lee, and Chin-Sheng Tang (2007). The Effect of Urban Air Pollution on Inflammation, Oxidative Stress, Coagulation, and Autonomic Dysfunction in Young Adults. American Journal of Respiratory and Critical Care Medicine 176: 370-376

2-Gholam hosseini B, Khaki A, Khaki AA, Kachabi H, Radsaeed F (2009). Ultra structure study of lead acetate cytotoxic effects on heart tissue in rabbit. Ofogh-E-Danesh 15(4): 5-12

3-Sheril Daniel, Janice LL, Amichand D, Gareth MW, Santy D (2004). Through mental binding, curcumin protects against lead- and cadmium- induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain. Biochemistry 98: 266-275

4-Shamsara, J, Ramezani, M, Mohammadpour AH (2009). Curcumin as a novel plaque stabilizing agent in prevention of acute coronary syndrome. Iranian Journal of Medical Hypotheses and Ideas 3: 9

5-Meilhac M, Ramachandran S, Chiang K, Santanam S, Parthasarathy S (2001). Role of Arterial wall Antioxidant Defense in Beneficial Effects of Exercise on Atherosclerosis in Mice. Arterioscler Thromb Vasc Biol. 21(10): 1681-1688

6-Chiu, Chan WK, Cheng SH, Leung CK, Chol CH (1999). Troponin I, myoglobin, and mass concentration of creatine kinzase-MB in acute myocardial infarction. Q J Med 92: 711-718

7-Kulikowska-Karpinska, Moniuszko-Jakoniuk (2001). Lead and Zinc Influence on Antioxidant Enzyme Activity and Malondialdehyde Concentrations. Polish Journal of Environmental Studies 3: 161-165.

8-Ansari N, Bhandari U, Pillai KK (2007). Protective role of curcumin in myocardial oxidative damage induced by isoproterenol in rats. Human &

Experimental Toxicology 26: 933–938
9-Gurer H, Ercal N (2000). Can antioxidants be beneficial in the treatment of lead poisoning? Free Radic Biol Med. 29 (10): 927–945
10-Lyn Patrick, ND (2006). Lead Toxicity Part II: The Role of Free Radical Damage and the Use of Antioxidants in the Pathology and Treatment of Lead Toxicity. Altern Med Rev 11(2): 114-127
11-Yeh CH, Chen TP, Wu YC, Lin YM, Jing LP (2005). Inhibition of NFkappaB activation curcumin attenuates plasma inflammatory cytokines surge and cardiomyocytic apoptosis following cardiac ischemia/reperfusion. J Surg Res 125: 109–116
12-Kalpana C, Sudheer, AR, Rajasekharan, KN, Menon, VP (2007). Comparative effects of curcumin and its synthetic analogue on tissue lipid peroxidation and antioxidantstatus during nicotine-induced toxicity. Singapore Med J 48 (2): 124
13-Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK (2004). Turmeric and curcumin: Biological actions and medicinal applications . current science 87(1): 10
14-Aslan R, Sekeroglu MR, Tarakcioglu M, Bayiroglu F, Meral I (1998). Effect of acute and regular exercise on antioxidative enzymes, tissue damage markers and membran lipid peroxidation of erythrocytesin sedentary students. Tr. J. of Medical Sciences 28: 411-414
15-Frederico MJS, Justo SL, Da Luz G, Da Silva S, Medeiros C, Barbosa VA, Silva LA, Boeck CR, De Pinho RA, De Souza CT (2009). Exercise training provides cardioprotection via a reduction in reactive oxygen species in rats submitted to myocardial infarction induced by isoproterenol. Exercise Biochemistry and Physiology Laboratory 43: 957 - 964.
16-Ascensao A, Magalhaes J, Soares J, Ferreira R, Neuparth M, Marques F, Duarte J (2006). Endurance exercise training attenuates morphological signs of cardiac muscle damage induced by doxorubicin in male mice. Basic Appl Myol 16 (1): 27 – 35
17-Li Li Ji (1999). Antioxidants and Oxidative Stress in Exercise**.** Proceedings of the Society for Experimental Biology and Medicine **2**22: 283-292
18-Powers SK, Demirel HA, Vincent HK, Coombes JS, Naito H, Hamilton KL, Shanely RA, Jessup J (1998). Exercise training improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. Am J Physiol Regulatory Integrative Comp Physiol 275: 1468-1477