RESEARCH ARTICLE

Pretreatment Effects of Regular Aerobic Training on the IGF System and Hepatotoxicity Induced by Doxorubicin in Rats

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Abstract

Aims: To examine the pretreatment effects of regular aerobic training on the IGF system (IGF-I, IGFBP-3 and IGF/IGFBP) and doxorubicin(DOX) induced hepatotoxicity in rats. Materials and Methods: Forty-eight male rats were divided into groups:(1) control+placebo (2)control+DOX_{10} mg.kg^{-1} (3)control+DOX_{20} mg.kg^{-1} (4) training+placebo (5) training+DOX_{10} mg.kg^{-1} (6) training+DOX_{20} mg.kg^{-1}. Hepatotoxicity was induced by DOX with dosages of 10 and 20 mg.kg^{-1}. The rats in groups 4, 5 and 6 performed treadmill running of 25-54 min/day and 15-20 m/min, 5 days/wk for 6 wks. At the end of the aerobic training protocol, rats in the 1 and 4 groups, in the 2 and 5 groups and in the 3 and 6 groups received saline solution, DOX_{10} mg.kg^{-1} and DOX_{20} mg.kg^{-1}, respectively. Results: Administration of DOX_{20} mg.kg^{-1} caused a significant increase in IGF-1 and IGF-1/IGFBP-3, an insignificant decrease in IGFBP-3, as compared to the control+placebo group. However, after six weeks of aerobic training and DOX treatment with 10mg.kg^{-1} and or/ 20mg.kg^{-1} an insignificant decrease in IGF-1, an insignificant increase in IGFBP-3 and a significant decrease in IGF-1/IGFBP-3 were detected, in comparison to C+DOX_{10} and C+DOX_{20}. Conclusions: Hepatotoxicity of doxorubicin is dose-dependent and pretreatment with regular aerobic training may improve DOX-induced hepatotoxicity by up-regulation of IGFBP3.

Keywords: Insulin-like growth factor system - aerobic training - hepatotoxicity - rats - doxorubicin

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Introduction

Over the years, doxorubicin (DOX), as an anti-tumor antibiotics and anticancer (Dragojevic-Simic et al., 2004; Chicco et al., 2005; 2006; Kelishomi et al., 2008; Wonders et al., 2008; Sheng, 2010), has become a routinely and widely used agent in various cancers treatment (Ludke et al., 2009; Wang et al., 2012). Unfortunately, the clinical use of these drugs is limited, because of severe toxic effects of the drug on the body's tissues, including heart, liver, kidneys and nervous system.The mechanisms of DOX-mediated cytotoxicity in cancer cells and normal tissues are different. Cytotoxicity may play a central role in DOX-induced cardiotoxicity or hepatotoxicity (Yen et al., 1996; Chicco et al., 2006; Wang et al., 2012). In this regard, there has been considerable effort by researchers in medicalfield (Dragojevic-Simic et al., 2004; Kelishomi et al., 2008; Sheng, 2010), and sports (Kalyanaraman et al., 2002; Chicco et al., 2005; 2006; Wonders et al., 2008; Kavazis et al., 2010) to develop strategies to prevent DOX-induced toxicity, especially in heart tissue. Kelishomi et al. (2008) reported the protective effect of morphine on DOX-induced cardiotoxicity. However, few studies have been conducted on the toxicity of DOX in liver tissue.

The insulin-like growth factor (IGF) signaling system is an essential regulator of growth and development (Aleem et al., 2012). The IGF system is viewed as a complex multifactorial system in both physiological and pathophysiological conditions. In normal conditions, the levels of the components reach a balance, so that the IGF axis plays a critical role in cellular proliferation as well as cell survival (Zumkeller et al., 2001; Cao et al., 2012). While in case the original balance is broken, it plays a predominant role in pathogenesis, of which neoplasia is currently attracting substantial interest (Cao et al., 2012). The bioavailability of IGFs is modulated by high-affinity binding proteins known as insulin-like growth factor binding proteins (IGFBPs) (from 1 to 7), that are mainly synthesized in liver. Most of the IGF-1 in circulation is bound by IGFBP-3, whose circulating levels are more than 10-fold higher than any of the other binding proteins (Aleem et al., 2012). There is growing evidence to suggest that the IGF system as a panel of tumor markers for histological diagnosis of various diseases associated with cancer are used (Ascensao et al., 2005). In this regard, several studies have reported an elevated level of circulating IGF-1 and/or a lower level of IGFBP-3 was related to the increased risk of cancers, and this was supported by some studies in breast, prostate and colorectal cancers (Cao et al., 2012). Although, there are few longitudinal studies regard the effect of training on IGF-1 proteins, it is not clear whether regular

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aerobic training could affect the IGF system in the liver tissue.Furthermore, it is not clear that DOX-induced hepatotoxicity by two doses of 10 and 20 mg.kg^{-1} affects the hepatic IGF system and whether pretreatment with regular aerobic training can affect changes in these parameters.

While, many recent studies have focused on the treatment effect of endurance exercise in induce DOX cardiotoxicity (Hhydock et al., 2008; Ascensão et al., 2012), the present study was carried out to investigate effect of prior (pretreatment) regular aerobic training against doxorubicin-induced hepatotoxicitywith various dosages (10 and 20 mg.kg^{-1}) on insulin-like growth factor (IGF) signaling system (IGF-I, IGFBP-3 and IGF/IGFBP).

Materials and Methods

Experimental design

The experimental protocols of current study approved by Department of Physiology, University of Mazandaran and were performed according to guiding procedures in the Care and Use of Animals, prepared by the Council of the American Physiological Society. The experiments were carried out with forty-eight Wistar male rats (8-week-old, initially weighing 257±28 g), which were obtained from the laboratory of animal bearing and multiplying at the Pasture institute of Iran. Rats were housed in standard cages of polycarbonate (20×15×15 cm), made at the Pasture institute of Iran, in a large air-conditioned room with a controlled temperature of 22±2°C, light- dark cycles of 12: 12 hours and humidity of 50±5%. The pollutant standard index (PSI) was in the acceptable range as determined by the Iranian meteorological organization. Rats were fed with a standard rat chow provided by Pars Institute for animals and poultry with a daily regimen of 10 g per 100 g body weight for each rat. Water was available ad libitum.

Familiarization and aerobic training protocols

Animals were habituated to treadmill running for one week (once a day for 10 min/session at 10 m/min, 0% grade). Because rats are more active in darkness, the front portion of the treadmill lines was covered with a dark thick paper to darken this area. At the rear of the lines, an electric grid provided a stimulus for running. An electric stimulus (30 V, 0.5 A) was manually turned on for less than 2 s when the animals stayed on the electric grid for longer than 10 s. Rats quickly learned to stay on the belt and avoid shock, except for one rat, which would not stay on the moving belt, and thus was quickly removed from familiarization process. Following this familiarization period, they were randomly assigned into control and trained groups. Exercise training protocol was performed on treadmill with zero slopes between 25-54 min.session^{-1} and 15 to 20 m.min^{-1}, 5 days/wk for 6 wks (Table 1). We replicated the aforesaid exercise training protocol that was previously reported by Dabidi Roshan et al. (2011).

At the end of the exercise training protocol, rats from the control and trained groups were again randomly separated into subgroups; the DOX (10, 20 mg.kg^{-1}) and placebo treatment. Thus, the control rats were distributed into control+placebo (C+P, n=8), control+DOX (C+DOX$_{10}$ mg.kg^{-1}, n=8) and control+DOX (C+DOX$_{20}$ mg.kg^{-1}, n=8) groups and rats in the trained group into trained+placebo (T+P, n=8), trained+DOX (T+DOX$_{10}$ mg.kg^{-1}, n=8) and trained+DOX (T+DOX$_{20}$ mg.kg^{-1}, n=8) groups.

Table 1. Aerobic Training Protocol in the Current Study

Training sessions and variables	Weeks of training 1	2	3	4	5	6
1. Speed* (duration**)	15 (25)	16 (30)	17 (35)	18 (40)	19 (45)	20 (50)
2. Speed (duration)	15 (26)	16 (31)	17 (36)	18 (41)	19 (46)	20 (51)
3. Speed (duration)	15 (27)	16 (32)	17 (37)	18 (42)	19 (47)	20 (52)
4. Speed (duration)	15 (28)	16 (33)	17 (38)	18 (43)	19 (48)	20 (53)
5. Speed (duration)	15 (29)	16 (34)	17 (39)	18 (44)	19 (49)	20 (54)

*meter/min; **min/session

Doxorubicin treatment

Doxorubicin hydrochloride (EBEWE Pharma Ges.m.b.H.Nfg.KG) was dissolved in saline and administered by i.p injection at two dosages of 10 mg.kg^{-1} (Wonders et al., 2009) and 20 mg.kg^{-1} (Ascensao et al., 2006), and control animals received saline with comparable volume. Both treatments were carried 24h after the last exercise bout and animals were sacrificed 24h after DOX and placebo injections.

Liver tissue collection and preparation

Rats in all groups were anesthetized with ketamine and xylazine following 24h of DOX injection and 12 h fasting. The abdominal cavity was opened to expose the liver tissue. Then liver tissue were rapidly excised, rinsed, carefully dried, weighed and it was placed into Petri dishes containing cold isolation medium (0.1 mol/L K_2HPO_4, 0.15 mol/L NaCl, pH 7.4) to remove the blood and were frozen immediately in liquid nitrogen and stored at -80ºC. liver tissue was squashed in liquid nitrogen, homogenized in a lysis buffer (5 ml/g of tissue) with a protease inhibitor cocktail for mammalian cell and tissue extracts (Sigma-Aldrich, St. Louis, U.S.A) 100 ul/1 ml, and 10 mM Tris base (Sigma-Aldrich, St. Louis, U.S.A), pH 7.4 and centrifuged at 1500 g at 4ºC for 15 min. Hepatic supernatant was diluted 1:30. Plasma was diluted 1:10 homogenized in doubly distilled water. Homogenates were centrifuged (2 min at 2,000 g, 4°C) to eliminate cellular debris, and the resulting supernatant was stored at liquid nitrogen (-80°C) for later determination of insulin-like growth factor (IGF) system include insulin-like growth factor (IGF-1) and insulin-like growth factor binding protein-3 (IGFBP-3).

Quantitative detection of the IGF system

Markers of the IGF system (IGF I and IGFBP 3) were measured using the following ELISA kits according to the manufacturer's instructions: IGF 1 ELISA kit (DRG International, USA) and IGFBP 3 Quantikine ELISA Kit (R&D Systems, USA). Absorbance was read at 450 nm for the three kits in a microplate reader. In summary, 100μl of standard, Blank, or Sample added per well. The liquid was removed from each well and 100μl of Biotin-antibody working solution added to each well. Then, aspirated each

well and washed and repeated these step for three times. In addition, 100μl of HRP-avid in working solution added to each well and the aspiration and washing repeated five times as step 4. Moreover, 90μl of TMB Substrate added to each well. Also, 50 μl of stop solution added to each well when the first four wells containing the highest concentration of standards developed obvious blue color. Finally, the optical density of each well was determined within 30 minutes; a microplate reader set to 450nm was used.

Statistical analysis

All data have been expressed as mean±SD. Statistical analysis was performed using a commercial software package (SPSS version 20 for Windows). Data of the IGF system were normally distributed after log-transformation. A one-way analysis of variance (Statistics software, Stat Soft, Inc., Tulsa, OK) was used to detect statistical differences between groups. A post-hoc test (Tukey test) was performed to determine differences in the various biomarkers between groups. Differences were considered statistically significant at p value<0.05.

Results

At first, no differences existed in the age and weight values between rats in the various groups. Changes in IGF-1, IGFBP-3 and IGF-1/IGFBP-3 levels following doxorubicin treatment (DOX_{10} mg.kg^{-1}, DOX_{20} mg.kg^{-1}) in the various groupsshowed in Table 2. Rats in the control group, showed an insignificant increase in IGF-1 and IGF-1//IGFBP-3 (6.8% and 6.41%, respectively), an insignificant decrease in IGFBP-3 (0.04%), following DOX_{10} mg.kg^{-1} administration. However, administration of DOX_{20} mg.kg^{-1} in control group caused a significant increase in IGF-1 and IGF-1//IGFBP-3 (14% and 14.1%, respectively), an insignificant decrease in IGFBP-3 (0.3%) in comparison to rats in the C+P group. Also, there was no significant difference between DOX_{10} mg.kg^{-1} and DOX_{20} mg.kg^{-1} treatments in IGF-1, IGFBP-3 and IGF-1/IGFBP-3 levels.

Table 3 shows changes in IGF-1, IGFBP-3 and IGF-1/IGFBP-3 levels following pretreatment with 6 weeks of aerobic training in the rats exposed to DOX-induced hepatotoxicity. Six weeks of the regular aerobic training led to a significant decrease of IGF-1 (16.2%) anda significant increase in IGFBP-3 and IGF-1/IGFBP-3 levels (1.61%, 3.12%, respectively), ascompared to C+P group (p<0.05).

Changes in the IGF-1, IGFBP-3 and IGF-1/IGFBP-3 levels, are shows in Figure 1, 2 and 3, respectively.After six weeks of aerobic training and DOX treatment with 10mg.kg^{-1}, a significant decrease in IGF-1 and IGF-1//IGFBP-3 (12.4% and 13.25%, respectively), and a significant increase in IGFBP-3(0.9%),were detected in comparison to C+DOX_{10} group (p<0.05). In addition,pretreatment with regular aerobic training and DOX treatment with 20mg.kg^{-1} resulted in a significant decrease in IGF-1 and IGF-1//IGFBP-3 levels (14.2% and 14.6%, respectively), an insignificant increase in IGFBP-3(0.8%),as compared to C+DOX_{20} group p <0.05).

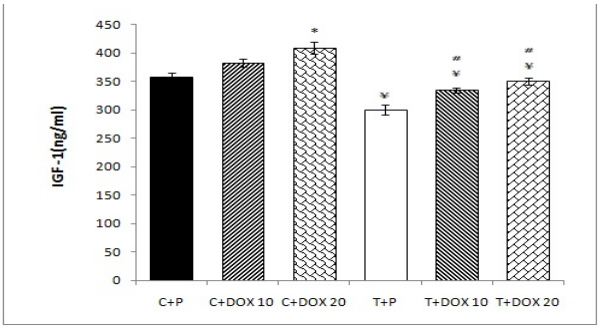

Figure 1. Insulin Like Growth Factor (IGF-1) Levels After Pretreatment with Six Weeks of Aerobic Training and DOX Treatment. C+P (Control+Placebo), C+DOX_{10} (Control+Doxorubicin 10mg.kg^{-1}, C+DOX_{20} (Control+Doxorubicin20mg. kg^{-1}), T+P (Training+Placebo), T+DOX_{10} (Training+Doxorubicin 10mg.kg^{-1}), T+DOX_{20} (Training+Doxorubicin 20mg.kg^{-1}). Data are presented as the mean±SD for 8 Rats. *Significantly different with the C+P group (p<0.05), ¥Significantly different with similar control group (p<0.05), #S ignificantly different with T+P group (p<0.05)

Table 2. Effect of Doxorubicin Treatment on the IGF System Levels in Liver Tissue

Groups and markers	C+P	C+DOX_{10}	C+DOX20
IGF^{-1}(ng/ml)	358.62± 6.09	383±7.12	409.12±10.48
IGFBP^{-3}(ng/ml)	4541.5±12.9	4530.6±7.5	4527.6±6.8
IGF-1/IGFBP^{-3}	0.078±0.003	0.083±0.004	0.089±0.006

*Data are presented as the mean±SD for 8 Rats. Abbreviations: Insulin-like growth factor-I (IGF-I), Insulin-like growth factor binding protein (IGFBP-3), C+P (Control+Placebo), C+DOX_{10} (Control+ Doxorubicin 10mg.kg^{-1}), C+DOX20 (Control+Doxorubicin 20mg.kg^{-1})

Table 3. Effect of Endurance Training and Doxorubicin Treatment on the IGF System Levels in Liver Tissue

Groups and markers	T+P	T+DOX10	T+DOX20
IGF^{-1}(ng/ml)	300.62±8.70	335.37±4.40	351±6.24
IGFBP^{-3}(ng/ml)	4614.8±11.1	4583.2±6.46	4564.2±5.3
IGF-1/IGFBP^{-3}	0.064±0.005	0.072±0.002	0.076±0.003

*Data are presented as the mean±SD for 8 Rats.Abbreviations: Insulin-like growth factor-1 (IGF-1), Insulin-like growth factor binding protein (IGFBP-3), T+P (Training+Placebo), T+DOX_{10} (Training+Doxorubicin 10mg.kg^{-1}), T+DOX20 (Training+Doxorubicin 20mg.kg^{-1})

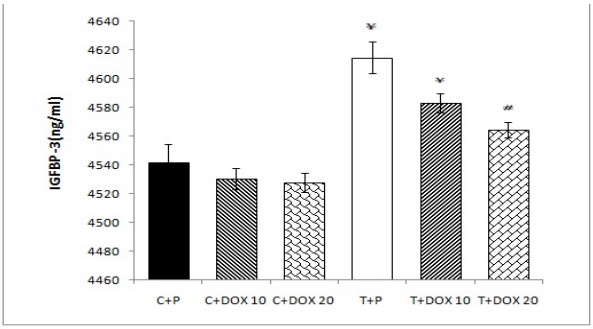

Figure 2. Insulin Like Binding Protein-3 (IGFBP-3) Levels After Six Weeks of Aerobic Training and Dox Treatment. C+P (Control+Placebo), C+DOX_{10} (Control+Doxorubicin 10mg.kg^{-1}, C+DOX_{20} (Control+Doxorubicin 20mg.kg^{-1}), T+P (Training+Placebo), T +DOX_{10} (Training+Doxorubicin 10mg.kg^{-1}), T+DOX_{20} (Training+Doxorubicin 20mg.kg^{-1}). Data are presented as the mean±SD for 8 Rats. ¥Significantly different with similar control group (p<0.05), #Significantly different with T+P group (p<0.05)

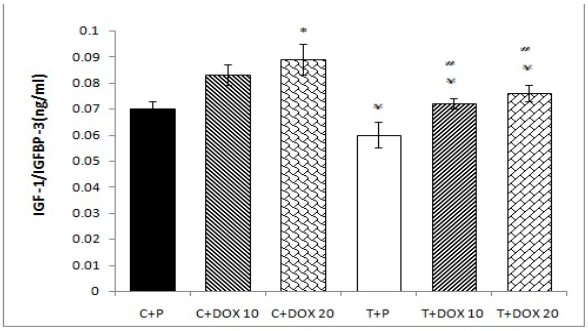

Figure 3. IGF1/IGFBP3 Ratio Levels After Six Weeks of Aerobic Training and DOX Treatment. C+P (Control+Placebo), $C+DOX_{10}$ (Control+Doxorubicin 10mg.kg^{-1}, $C+DOX_{20}$ (Control+Doxorubicin 20mg.kg^{-1}), T+P (Training+Placebo), $T+DOX_{10}$ (Training+Doxorubicin 10mg.kg^{-1}), $T+DOX_{20}$ (Training+Doxorubicin 20mg.kg^{-1}). Data are presented as the mean±SD for 8 Rats. * Significantly different with the C+P group ($p<0.05$), ¥Significantly different with similar control group ($p<0.05$), #Significantly different with T+P group ($p<0.05$)

Furthermore, six weeks of aerobic training and Doxorubicin treatment (10mg.kg^{-1}), a significant increase in IGF-1 and IGF-1/IGFBP-3 levels (11.56% and 12.5%, respectively), an insignificant decrease in IGFBP-3(0.7%), were detected in comparison to T+P group ($p<0.05$). After six weeks of aerobic training and Doxorubicin treatment (20mg.kg^{-1}), a significant increase in IGF-1 and IGF-1/IGFBP-3 levels (16.75% and 18.75%, respectively), a significant decrease in IGFBP-3 (1.1%), were detected in comparison to T+P group ($p<0.05$).However, after six weeks of aerobic training and DOX_{10} mg.kg^{-1} and DOX_{20} mg.kg^{-1} treatments there was no significant difference between in IGF-1, IGFBP-3 and IGF-1/IGFBP-3 levels.

Discussion

The present study was designed to determine pretreatment effects of regular aerobic training on the IGF system (IGF-1, IGFBP-3 and IGF-1/IGFBP-3) in the rats exposed to DOX-induced hepatotoxicity with the various dosages (10 and 20 mg.kg^{-1}). The primary novel finding in present study was that, administration of DOX_{10} mg.kg^{-1} and DOX_{20} mg.kg^{-1} led to imbalance in the IGF signaling system in rats. This result indicates potent role of DOX administration in induce oxidative stress. Doxorubicin is an anthracyclin antibiotic that is considered as one of the most effective antitumor agents. The clinical use of DOX is limited by its toxicity to normal tissues, such as the heart and liver (Ibrahim, 2010; Wang et al., 2012). The mechanisms of DOX-mediated cytotoxicity in cancer cells and normal tissues are different (Wang et al., 2012). DOX acts through DNA intercalation, alteration of membrane function, and free radical formation (King et al., 2001). On the other hand, DOX-induced cardiotoxicity or hepatotoxicity mainly occurs by generating oxygen free radicals, which is inhibited by free radical scavengers (Wang et al., 2012). DOX is extensively metabolized in the liver which is the cause of liver damage (King et al., 2001). There is growing evidence to suggest that the IGF system as a panel of tumor markers for histological diagnosis of various diseases associated with cancer are used (Ibrahim et al., 2010). Guo et al. (1998) reported IGF-1 as a potential mitogen for various types of cells including cancer cells. In a study of IGF and IGFBP as an indicator for the detection of tumor growth was introduced. The researchers said these factors play a pivotal role in tumor formation. Epidemiological data suggest that the risk of cancer is associated with high levels of IGF-1. Lack of regulation of IGF system can cause problems in some tissues is the correct pattern IGF and its binding proteins thus appears as a new tumor marker for cancer detection and evaluation may be useful (Zumkeller, 2001). We also confirmed that acute administration of DOX increased IGF-1 levels and IGF-1/IGFBP-3 ratio and decreased IGFBP-3 values in liver tissue of male rats. Our results are consistent with several published reports on the levels of IGF signaling system in the rats exposed to DOX-induced hepatotoxicity. Also, our data demonstrated that acute administration of DOX increased serum indices of liver function, including ALT and ALP . This increase in ALT and ALP is attributed to the hepatocellular damage and decreased liver functions. The present results indicate that there is a potent relationship between IGF system and DOX-induced hepatotoxicity.

No studies have been previously to determine pretreatment effects of regular aerobic training on the IGF system (IGF-1, IGFBP-3 and IGF-1/IGFBP-3) in the rats exposed to DOX-induced hepatotoxicity with the various dosages (10 and 20 mg.kg^{-1}). Our study confirmed that that pretreatment of regular aerobic training prevented down-regulation of IGFBP-3 and up-regulation of IGF-1 in liver tissue. In the present study, a significant decrease in the IGF-1 level and IGF/IGFBP-3 ratio in rats treated with T+DOX treated groups, suggests the protective and pretreatment effect of regular aerobic training against DOX-induced hepatotoxicity. While, previous researchers have reported physical activity as a non-pharmacological strategy in various cancers, we are the first to investigate the pretreatment effect of regular aerobic training before the various dosages (10 and 20 mg.kg^{-1}) of DOX on markers of related to hepatotoxicity in liver tissue. Our study demonstrated that, 20 mg.kg^{-1} of DOX can lead to down-regulation of the IGF signaling system, regular aerobic training increase IGFBP-3, decrease IGF-1 and IGF-1/IGFBP-3 which is induced by DOX administration. In contrast with these results, Andrea and et al examine the effect of 16 weeks of aerobic training on IGF axis proteins in active young women. They reported the baseline values in subjects IGFBP-3 had little significant increase. Researchers concluded that 16 weeks of aerobic training in young women has no effect on IGF proteins (Arikawa et al., 2010; Sprod et al., 2012) found an inverse relationship between changes in IGF-1 and changes in overall health related quality of life, physical role limitations, and social functioning. Changes in IGFBP-1 and IGFBP-3, binding proteins for IGF-1, were correlated with changes in physical role limitations and physical functioning, respectively. Early epidemiological evidence does not support an inverse association between chronic exercise and circulating levels of IGF1 and IGFBP3, particularly in older women. A recent review also suggested that long

term exercise may actually increase circulating IGF1. Therefore, the evidence is weak that chronic exercise lowers IGF-1 levels (Friedenreich et al., 2011).

In conclusion, the present findings demonstrate that the hepatotoxicity induced by DOX_{20} $mg.kg^{-1}$, may be related to imbalance of IGF system in liver tissue. Moreover, pretreatment of 3 weeks aerobic training effectively improve the toxic effects of DOX in liver, so that it was associated with up-regulation of IGFBP-3 and down-regulation of IGF-1. Overall, our study suggests that shot-term regular aerobic training in before administration of DOX may be considered as a potentially useful strategy to limit hepatotoxicity before DOX therapy and to improve in IGF system in liver tissue.

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