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8-weeks training program attenuates mitochondrial oxidative stress in the liver of emotionally stressed rats

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Summary. In recent years it has been shown that emotional stress induced by immobilization may change the balance between pro-oxidant and antioxidant factors inducing oxidative damage. On the other hand, contradictory views exist concerning the role of physical activity on redox metabolism. Consequently, the present work was designed to assess the influence of an 8-week moderate swimming training program in emotionally stressed rats.

Sixty 1-month-old male albino Wistar rats weighing 125-135 g were used in this experimental study. They were divided into three groups, as Control (lot A; n=20), Stressed (lot B; n=20) and Stressed & Exercised (lot C; n=20). Rats were stressed by placing the animals in a 25 x 7 cm plastic bottle 1 h/day, 5 days a week for 8 weeks.

Protein carbonyl content values in liver homogenates were significantly increased in stressed animals (0.58±0.02 vs 0.86±0.03; p=0.018) which clearly indicated that emotional stress was associated with oxidative stress. Ultrastructural alterations, predominantly mitochondrial swelling and the decrease of cristae number observed by electron microscopy represented direct evidence of membrane injury.

The most striking feature of our study was that we also found differences between stressed rats and stressed rats that performed our 8 week training program. Consequently our results highlight the potential benefit of a moderate training program to reduce oxidative damage induced by emotional stress since it attenuated protein oxidation and mitochondrial alterations.

Key words: Oxidative stress, Exercise, Emotional stress, Mitochondria

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Introduction

In recent years it has been shown that emotional stress may change the balance between pro-oxidant and antioxidant factors, inducing oxidative damage. Immobilization is widely employed to provoke psychological stress since it is less harmful than other stressors such as electric foot shocks (Retana-Martquez et al., 2003). Further, it was reported that immobilization was also associated with oxidative damage in rats (Fontella et al., 2005).

Mitochondria play a central role in the cellular metabolism; they are responsible for cellular respiration coupled with the generation of ATP from ADP and inorganic phosphate. In this respect, many studies indicate that mitochondria are a main intracellular source of reactive oxygen species. In fact, under physiological conditions, 1-4% of oxygen reacting with the mitochondrial respiratory change is incompletely reduced to superoxide anion and, consequently, hydrogen peroxide (Inoue et al., 2003). As a consequence, the importance of the assessment of oxidative stress by means of the determination of mitochondria alterations has been increasing in the last years (Le Bras et al., 2005).

Physical activity is recognized as an important component of a healthy life style and consequently is highly recommended by scientists and clinicians (Donaldson, 2000). However, to date, contradictory views exist concerning the role of physical activity on redox metabolism. Published data suggest that exhausting and acute activity should be prooxidant (Ogonovszky et al., 2005) whereas moderate and chronic exercise should be antioxidant (Chang et al., 2004).

Based on these observations we hypothesyze that moderate exercise may reduce oxidative damage induced by emotional stress. This finding would be of interest since oxidative stress plays an important role in the pathogenesis of aging, neurodegeneration, cancer and

auto-immune diseases, among others (Ando et al., 1998; Galli et al., 2005; Valyi-Nagi and Dermody, 2005).

Materials and methods

Sixty 1-month-old male albino Wistar rats weighing 125-135 g were used in this experimental study. They were divided into three groups, as Controls (n=20), Stressed (n=20) and Stressed & Exercised (n=20). All rats were given standard rat chow and tap water ad libitum and were housed at 23±2°C on a 12 h dark and 12 h light cycle.

Rats were stressed by placing the animals in a 25x7 cm plastic bottle and adjusting it with plater tape on the outside so that the animal was unable to move. There was a 1-cm hole at one end for breathing. Animals were stressed 1 h/day, 5 days a week for 8 weeks (Ely et al., 1997) .

Swimming, used as the exercise for the trained rats was administered 5 days a week for 8 weeks. To familiarize them with the water immersion, the duration of each session was increased progressively as was reported previously (Hu et al., 2002) . In any case, it was performed in a steel tank of 100x50 cm with a depth of 40 cm filled with tap water maintained at a temperature between 32±2°C. It should be emphasized that all animals received human care since we followed the recommendations of the Helsinki Declaration for research involving animals during the whole experience.

Upon completion of the experience, ether-anesthetized animals were sacrificed by decapitation. Protein oxidation was assessed as carbonyl group content in liver homogenates reading the absorbance at 366 nm according to Levine et al. (1990).

Liver samples were also collected and prepared for ultrastructural examination by conventional electron microscopy. In this respect, a block of liver tissue (1mm thick) was cut from the center of each lobe and diced into 1mm cubes. All the samples for transmission electron microscopy were fixed initially in 0.25 g/l glutaraldehyde, then postfixed with 0.1 g/l OsO_4 dehydrtaed in a graded series of alcohols and embedded in araldite. Ultrathin sections were obtained with an ultramicrotome (60nm) and counterstained with uranyl acetate and lead citrate.

Protein carbonyl group results were expressed as mean ± sd and 95% confidence interval. To compare mean values, we performed Student t test for unpaired data. The significance level was ascertained at $p<0.05$. They were all performed using the software SPSS 11.0.

Results

Protein carbonyl content values in liver homogenates were 2.87±0.11 [2.77–2.97], 4.09±0.18 [3.96 – 4.22] and 3.14±0.09 [3.12–3.16] in control (lot A), stressed (lot B) and stressed plus exercised (lot C) specimens respectively. It should be noted that they were all expressed as nmol/mg proteinas.

Protein carbonyl groups were significantly decreased in rats stressed by immobilization when compared to controls (2.87±0.11 vs 4.09±0.18; p=0.021). Furthermore, we also found significant differences between stressed rats and stressed rats that performed our 8-wk training program (4.09±0.18 vs 3.14± 0.09 p=0.020).

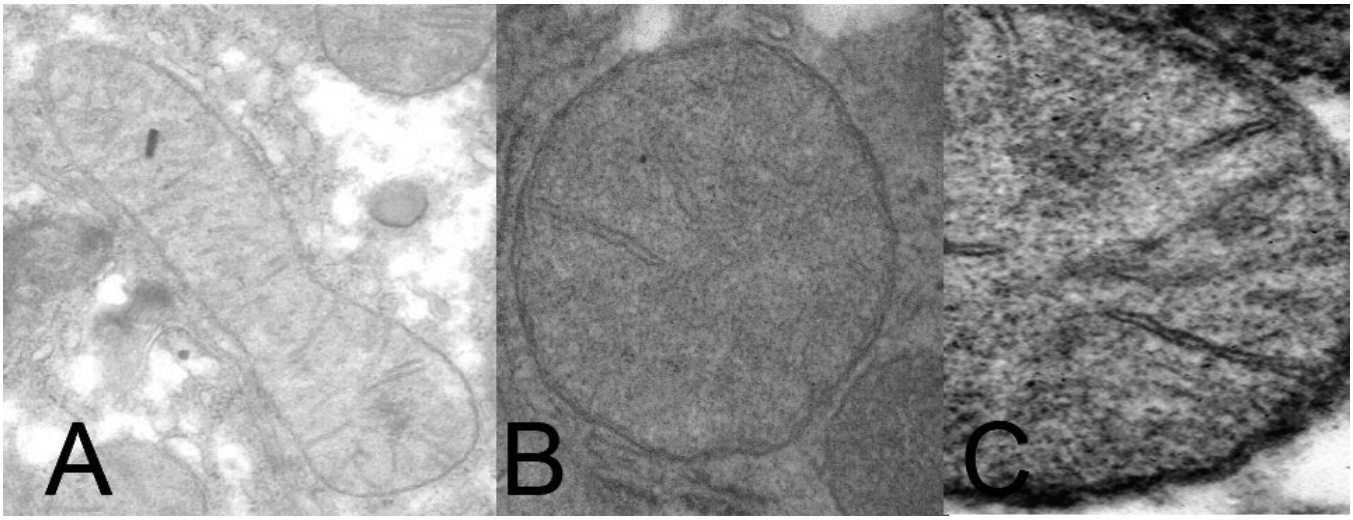

Fig. 1. Ultrathin sections (60 nm) from liver of control **(A)**, stressed **(B)** and stressed plus exercised **(C)** wistar rats stained with uranyl acetate and lead citrate and examined by transmission electron microscopy. Normal mitochondrial morphology in liver tissue **(A)**. Electron micrographs from stresed specimens **(B)** showed ultrastructural alterations predominantly mitochondrial swelling contrasting with minimal alterations exhibited by stressed plus exercised rats **(C)**. A, x 32,000; B, x 50,000; C, x 85,000

In control specimens, normal mitochondria were elongated and had numerous narrow pleomorphic cristae, evident as electron-transparent areas in a contiguous electron-dense matriz (Fig. 1). In emotionally stressed animals, several mitochondria showed ultrastructural abnormalities, predominantly mitochondrial swelling as well as cristae disarray and an important loss in number. Vacuolization was rarely observed (Fig. 1). In any case, these alterations in inner mitochondrial membrane structure were clearly attenuated quantitatively and qualitatively when specimens performed an 8-week training program (Fig. 1).

Discussion

In recent years there has been an increasing interest in assessing oxidative stress. In this respect, swimming is frequently preferred as an exercise model for small laboratory animals such as rats since it may be considered as part of their natural environment (Kramer et al., 1993). In addition, aversive stimulation used to promote running is not used in swimming (Mattson et al., 2000). This fact is of particular interest since these stimuli have been associated per se with oxidative stress and consequently may interfere with our results leading to mistaken conclusions.

Similarly, while biochemical changes in oxidative damaged tissues in general and liver tissue in particular have been extensively described, few studies have focused on the morphological changes under oxidative stress in order to identify potential early warning bioindicators (Caglar et al., 2003; Rosety et al., 2005).

Our study demonstrated that emotional stress induced by immobilization caused oxidative damage, since protein carbonyl content in liver homogenates was increased significantly when compared to control specimens. This result was in agreement with those previously published (Davydov et al., 2004; Torres et al., 2004; Fontella et al., 2005). Similar findings were reported by Domenicali et al. (2001) and Venditti et al. (2004) in rat liver exposed to ischemia-reperfusion injury and cold-oxidative stress respectively. In this respect it should be emphasized that our results were lower than those reported previously by Navarro and Boveris (2004). Determining whether this discrepancy is the result of tissue or age differences would be an interesting topic for future studies.

Furthermore, these alterations were accompanied by significant changes in the ultrastructure of the liver tissue. This fact is of particular interest, since these defects in the mitochondrial architecture would lead to damage of the mitochondrial metabolism and thus become a key contributor to intrinsic cell dysfunction and death (Kroemer and Reed, 2000). Ultrastructural changes associated with oxidative stress, such as increased vacuolization, was not so evident in our study as was reported in a previous study by Ault and Lawrence (2003).

The most striking feature of our study was that an 8-weeks moderate exercise program reduced oxidative damage in stressed specimens since it clearly attenuated changes in plasmatic total antioxidant status. In this way, we found significant differences between stressed rats (lot B) and stressed rats that performed our training program (lot C). Similar results were published by other authors in the liver (Kakarla et al., 2005) and brain (Radak et al., 2001) of exercised rats. In any case, it should be emphasized that our program was shorter than that designed by the latter authors. In addition, these antioxidant benefits were also evident in other tissues such as the heart (Asha-Devi et al., 2003). On the other hand, there is no reference in the literature regarding the improvement of mitochondria ultrastructural alterations induced by oxidative stress.

In any case, our results highlight the potential benefit of a moderate training program to attenuate oxidative damage induced by emotional stress. Further studies concerning other variables and tissues are required to increase the knowledge about the influence of moderate exercise in oxidative stress.

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