Effects of Exercise on Cardiac Oxidative Stress in Rats after Exposure to Cigarette Smoke

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ABSTRACT

Veloso CF, Costa CD, Rovani BT, Araldi ICC, Freitas RB, Cogo LA, Silveira AF, Bauermann LF, Silva AMV. Effects of Exercise on Cardiac Oxidative Stress in Rats after Exposure to Cigarette Smoke. \textit{JEPonline} 2013;16(5):21-27. The aim of this study was to evaluate the influence of physical exercise on the levels of oxidative stress in cardiac tissue after the inhalation of cigarette smoke in rats. Rats were divided into four groups: (a) non-smokers + non-exercised (NS+NE); (b) non-smokers + exercised (NS+E); (c) smokers + non-exercised (S+NE); and (d) smokers + exercised (S+E). The cigarette smoke was inhaled during 30 min, twice a day, and then extended to 10 cigarettes for 30 min twice a day. Physical exercise was performed on a treadmill. The initial velocity was 10 m·min\textsuperscript{-1} increasing up to the mark of 30 m·min\textsuperscript{-1}. Cardiac tissue lipoperoxidation (LPO) estimation was performed using the TBARS method. The values were expressed in levels of malondialdehyde (MDA), and enzymatic antioxidant defense was measured by catalase (CAT) activity. These findings indicate that the physical exercise induced lower levels of LPO and increased CAT activity, which attenuated the oxidative stress induced by exposure to cigarette smoke.

Key Words: Exercise, Heart, Oxidative Stress, Rats, Smoke
INTRODUCTION

Smoking is directly responsible for 5 million annual deaths around the world, which is expected to increase to over 8 million deaths per year related to tobacco for the next two decades. More than 80% of those deaths will occur in developing countries (19). Cigarette smoke is the most important risk factor for the development of atherosclerotic vascular diseases, including ischemic heart disease, stroke, and peripheral vascular disease (2,6,14).

Several recent studies have also demonstrated that chronic cigarette exposure can result in cardiomyopathy, characterized by the progressive and irreversible deterioration of cardiac function associated with interstitial fibrosis, cardiac myocyte vacuolization, arteriolar hyalinosis, and immune reaction in the heart (3,15,21). In addition, smoking is directly associated with decreased serum levels of antioxidants and, thus contributes to the occurrence of oxidative stress, increased products of lipid peroxidation, platelet aggregation, and atherogenesis (12).

The oxidative stress occurrence is due to an imbalance between oxidant and antioxidant defense, caused by an excessive generation of reactive oxygen species (ROS), which leads to the oxidation of biomolecules with consequent loss of its biological functions and/or homeostasis alterations (11). The interaction of free radicals with lipids is called lipid peroxidation (LPO). Membranes of mammalian cells contain large amounts of polyunsaturated fatty acids that can undergo peroxidative reaction chain, with subsequent breakdown of cell membranes and liposomes (20). A previous review showed that the levels of malondialdehyde (MDA), a LPO product, were higher in biological fluids in smokers compared with non-smokers (17).

The type, intensity, frequency, and duration of exercise are key factors in the response to oxidative stress in various tissues (25). For example, although intense physical exercise can elevate LPO and cause some problems such as inactivation enzymes present in the membrane cell (18), the effects of regular physical exercise improve the efficiency of antioxidant systems including the antioxidant enzyme catalase (CAT) and the non-enzymatic antioxidants that results in reactive oxygen species (ROS) underproduction (8,10,23). Therefore, the aim of this study was to evaluate the effect of physical exercise after smoke exposure on oxidative stress, evaluating the MDA levels and CAT activity on cardiac tissue of rats.

METHODS

Animals
The study group consisted of 40 male Wistar rats (age, ~60 days old; weight, 131 to 222 gm) from the Central Animal Facility of UFSM. They were kept in collective cages (5 rats per cage in the vivarium of the Department of Physiology and Pharmacology, Federal University of Santa Maria) on a light and dark cycle of 12 hrs with temperature between 20 to 25ºC, controlled humidity. The rats had free access to water and food. This study was registered and accepted by the Office of Research Support of the Center for Health Sciences under the responsibility of respecting the ethical principles for animal experimentation, which was adopted by the Brazilian College of Animal Experimentation. It was approved by the Committee on Care and Use of Experimental Animal Resources of Federal University of Santa Maria (UFSM), under registration Nº. 120/2010.

Procedures
The rats were divided into four groups: (a) non-smokers and non-exercised (NS+NE); (b) non-smokers and exercised (NS+E); (c) smokers and non-exercised (S+NE); and (d) smokers and
exercised (S+E). After completing the experimental protocol, the animals were euthanized by deep anesthesia induced by thiopental at 100 mg·kg\(^{-1}\) and, then, administered intraperitoneally for heart removal. The cardiac tissue was washed with saline solution (NaCl 0.9\%). One gm of tissue was homogenized in 5 volumes of potassium phosphate buffer (0.1 M, pH 7.4) using a Polytron mixer (Kinematica AG, Switzerland). Homogenate was centrifuged at 3000 g at 4ºC for 10 min to yield a low-speed supernatant that was used to measure levels of TBARS, a LPO marker, and the activity of the antioxidant enzyme catalase (CAT).

The cigarettes used were composed of 0.7 mg of nicotine, 9 mg of carbon monoxide, and 8 mg tar. Exposure to cigarette smoke was performed at the Department of Physiology and Pharmacology of UFSM. The exposure machine was a transparent chamber with dimensions of 40x45x30 cm, which was connected to a smoking device that removed the smoke and released the vacuum chamber. This model exposed the animals to the center smoke called "mainstream," which is equivalent to active smoking (24). This step was composed of groups S+E and S+NE. There was an initial release of smoke twice a day at a rate of 5 cigarettes for 30 min. The exposure was increased progressively to the rate of 10 cigarettes for 30 min twice a day. This rate was maintained until the end of the study, which corresponded to 2 months of exposure.

The exercise was conducted at the Laboratory of Biochemistry of Exercise of the Center of Physical Education and Sports. It was performed with a treadmill of individual lanes provided with holes for ventilation and individually covered by transparent acrylic movable lids. The rats in groups NS+E and S+E were subjected to treadmill sections at an initial speed of 10 m·min\(^{-1}\), progressing daily up to the mark of 30 m·min\(^{-1}\). The exercise corresponded to the second stage of the study (2nd month). During the first month, there was no procedure with the group NS+E. The S+E group had already been exposed to cigarette smoke at the first month, and went above the second month, concurrent with the physical exercise.

Cardiac tissue LPO estimation was performed using the TBARS assay as described by Buege and Aust (5) that quantifies the colorimetric reaction of the LPO product malondialdehyde (MDA) with thiobarbituric acid (TBA). After heating at 100ºC for reacting with TBA, samples tubes were cooled and centrifuged at 2000 g. The organic layer was collected and the absorbance was read at 535 nm using a spectrophotometer. CAT activity was determined from the rate of decomposition of H\(_2\)O\(_2\) monitored by decrease of 240 nm following the addition of tissue homogenate (1). The levels of MDA and CAT activity were normalized to the amount of heart protein content. The quantification of the protein was performed following the Lowry method, where the maximum absorbance for the solution of Folin ciocauteau due to its interaction to bovine serum albumin protein occurs at 625 nm (16).

**Statistical Analyses**

Statistical analysis was performed using the Statistical Package for Social Sciences, version 18.0. For the comparison among groups, the analysis of variance (ANOVA) was used followed by the Bonferroni post-hoc test. Results are presented as mean ± standard deviation (±SD). The differences were considered statistically significant when P<0.05.

**RESULTS**

The current results demonstrated a reduction of MDA levels in NS+NE (P=0.004), NS+E (P<0.001) and S+E (P<0.001) groups in comparison with rats in the smokers and non-exercised group (S+NE). Also, lower values were observed in the NS+E (P=0.001) and S+E (P=0.034) groups in comparison to the NS+NE group. There was no difference between NS+E and S+E (Figure 1 - A).
After exposure to cigarette smoke, a significant decrease in the catalase enzyme activity was observed in the smoking group (S+NE) compared to the NS+NE (P<0.001). The NS+E and NS+NE groups showed similar CAT activity. The catalase activity was higher in S+E compared to the S+NE (P<0.001). The NS+E group showed higher values when compared with S+NE (P=0.012). The results of the S+E group were similar to the NS+NE and the NS+E groups (Figure 1 - B).

**DISCUSSION**

The rats that exercised (NS+E and S+E) showed lower cardiac MDA levels in comparison to NS+NE, probably due to a protective effect generated by the practice of the exercise against oxidative species, exemplified by H₂O₂. A previous study demonstrated that aerobic training induces lower MDA formation in rat heart, which suggests the occurrence of compensatory adaptations in the antioxidant system tissue (26). Also, the increase in catalase activity in S+E may be related to the fact that the exercise protocol featured a physical training of moderate intensity, increasing the antioxidant defenses and tissue resistance to oxidative injuries (9,25).

Furthermore, it was observed that the interaction between smoking and physical exercise mitigates the ROS overproduction that resulted in decreased levels of MDA in cardiac tissue of animals in group S+E. Moreover, the level of MDA in group NS+NE was higher than measured in group S+E. Another interesting finding was the similar MDA levels between NS+E and S+E. This suggests a positive effect of physical exercise, regardless of exposure to cigarette smoke.

Baskaran et al. (4) also evaluated the effects of cigarette smoke on LPO and antioxidant enzymes in rats exposed to cigarette smoke twice daily for 30 days. The authors observed an increase in LPO in the liver, lungs, and kidneys of the exposed animals, but no change was found in brain and heart. However, in the present study, there was an elevated level of MDA in cardiac tissues of the S+NE animals. This finding is in agreement with Morrow et al. (22).

The enzyme catalase is considered the largest component of the primary antioxidant defense, acting in catalyzing the decomposition of hydrogen peroxide (H₂O₂) into water and oxygen, sharing it with glutathione peroxidase (GPx). In presence of low levels of H₂O₂, organic peroxides are preferably eliminated by GPx. At high concentrations of H₂O₂, the CAT is responsible for H₂O₂ detoxification.
The CAT may be a protective effect against certain tumors such as lung cancer (7). In the present study, the CAT activity was similar in NS+NE and NS+E groups. Therefore, this study suggests that physical exercise did not change the CAT functionality in non-smokers rats. However, the rats that smoked and exercised (S+E) had higher CAT activity versus the rats that smoked and did not exercise (S+NE).

By comparison, Zalata and colleagues (27) showed that children exposed to environmental tobacco smoke (ETS) had retarded growth, more chest problems, and gastroenteritis than the controls. A significant increase in mean comet tail length indicating DNA damage and a higher MDA level was observed in the ETS-exposed children (P<0.001) compared to the children with no exposure to ETS. Their results show that inhalation of ETS is associated with an increase in the level of oxidants and a simultaneous decrease in the level of antioxidants in the children’s blood. This status of oxidant-antioxidant imbalance (OS) may be one of the mechanisms that leads to DNA damage detected in lymphocytes of ETS-exposed children.

Chung-Man et al. (7) reported that in individuals with lung cancer CAT activity was significantly decreased in tumors compared with tumor-free lung tissues, which may lead to increased hydrogen peroxide intracellularly and create an intracellular environment favorable to DNA damage and the promotion of cancer. Their findings support the relationship between antioxidants and the reduction of tissue damage. Interestingly, while Kocygit and colleagues (13) found that exposure to cigarette smoke can alter the function of antioxidant enzymes, there was no change in CAT between smokers and non-smokers.

CONCLUSIONS

The current findings demonstrated mainly a greater level of LPO and a lower catalase activity in rats exposed to cigarette smoke and non-exercised. The physical exercise, independent to cigarette smoke inhalation, induced lower levels of LPO. In the groups exposed to cigarette smoke, the catalase activity was increased in rats exercised. All these observations may indicate high levels of oxidative stress in cardiac tissue when exposed to cigarette smoke and the efficiency of exercise to mitigate this phenomenon. Thus, this study suggests that moderate exercise provides a protective effect against oxidative stress induced by smoke.

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REFERENCES


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