Research Article

Effects of Exercise on Oxidative Stress in Rats Induced by Ozone

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Oxidative stress (OS) induced by acute exercise is reduced by chronic exercise. Ozone (O₃) exposure produces OS. The aim of this study was to determine if aerobic exercise (AE) reduced OS produced by O₃. A pilot experiment was performed with male Wistar rats submitted to AE (trained to swim 90 min/day). Adaptation to exercise was demonstrated three weeks after training by means of changes in reduced nitrates (NOₓ) in plasma. Therefore, two-week training was chosen for the following experiments. Six of twelve trained rats were exposed to O₃ (0.5 ppm, 4 h/day, one hour before exercise). Two groups of sedentary animals (n = 6 each) were used as controls, one of which was exposed to O₃. At the end of the experiments NOₓ, 8-isoprostane (8-IP), malondialdehyde (MDA), superoxide dismutase (SOD) activity, and carbonyls (CBs) were measured in plasma. CBs did not change in any group. O₃-induced OS was manifested by reduced NOₓ and SOD activity, as well as increased 8-IP and MDA. Exercise significantly blocked O₃ effects although SOD was also decreased by exercise (a greater drop occurring in the O₃ group). It is concluded that AE protects against OS produced by O₃ and the effect is independent of SOD.

1. Introduction

Oxidative stress (OS) produced by acute exercise is characterized by an excess of free radicals. It was thought that mitochondria were the main source of free radicals in exercise; however, it is now known that even though mitochondria do contribute, other sources are the main contributors (xanthine oxidase, NADPH oxidase, and phospholipase A2) [1–3]. Chronic exercise reduces OS produced by acute exercise [1, 4, 5]. Adaptation to exercise is due, in part, to an increase in the endogenous antioxidant defense [1, 4–7]. The increase of nitric oxide (NO) availability takes part in the adaptation and the benefits produced by long-term exercise [8–10].

It is thought that the mechanism of adaptation to exercise includes activation of nuclear factor kappa B (NFkB) by free radicals, which upregulates the synthesis of endothelial NO synthase (eNOS) and antioxidant enzymes [8, 9, 11, 12]. Long-lasting moderate exercise has beneficial effects such as prevention of certain cancers, prolonged lifespan in rodents, reduction of cardiovascular effects of aging and menopause, better metabolic control and renal as well as cardiovascular protection in diabetes, and improvement of chronic heart failure [3, 5–7, 11, 13–16].

Ozone (O₃) is a common pollutant in urban areas. The effects of O₃ extend beyond the lung. O₃ exposure produces systemic OS [17]. O₃ exposure has been associated with premature mortality [18], cardiovascular mortality [19], myocardial infarction [20], and cerebrovascular diseases [21]. OS and endothelial dysfunction have been related to the cardiovascular toxic effects of O₃ [22].

The goal of this study was to determine if moderate aerobic exercise affected OS produced by O₃.
2. Material and Methods

2.1. Animals. Male Wistar rats, 10 weeks old (230–250 g), were supplied by Harlan Mexico. Animals were fed with Purina chow and water ad libitum and submitted to light/dark periods of 12/12 h. Animals were kept in a room fed with filtered air to maintain O₃ within normal concentrations (<0.05 ppm) according to the USA Environmental Protection Agency (http://www.epa.gov/air/ozonepollution/standards.html). The local Institutional Animal Committee approved all the procedures.

2.2. Training Protocol. Rats were trained to swim 90 min a day, 7 days a week. Animals swam in water at 35–37°C. Rats were trained to swim 90 min a day for 2 weeks, in order to have the same activity.

2.3. Ozone Exposure. O₃ exposure was made in groups of six animals in an OTC-1 chamber (In USA, Inc.). The chamber had a servomechanism to maintain O₃ concentrations at 0.5 ± 0.05 ppm. The chamber was programmed to destroy O₃ in such a way that it was impossible to open the chamber if O₃ concentration was above normal (≥0.05 ppm). Animals were exposed to O₃ 4 hours a day, every day (07:00–11:00 h).

2.4. Groups. Groups of 6 animals were formed as follows.

(1) Pilot groups: eight pilot groups were formed in order to analyze adaptation to exercise. Half of those groups were sedentary (kept in their cages, which allowed for free movement) and half were submitted to aerobic exercise, as mentioned above. One, two, four, or eight weeks after training, two groups (sedentary and trained) were anesthetized (sodium pentobarbital 45 mg/Kg, ip). The left carotid was cannulated with a PE50 catheter, and a blood sample (3 mL) was taken and treated with EDTA. The animals where then sacrificed by anesthesia overdose. Adaptation to exercise was evaluated by measuring reduced nitrates (NOₓ) in plasma using the Griess method (Cayman Chemical Co. kit).

(2) With the results of the pilot groups (see below), a two-week period (just before adaptation) was chosen for the following experiments.

(a) Sedentary group. This group remained sedentary and it was kept in the O₃ chamber at normal concentrations (<0.05 ppm), for 4 hours a day for 2 weeks, in order to have the same confinement stress as that the rats exposed to O₃.

(b) Sedentary group exposed to O₃: this group remained sedentary and was exposed to O₃ (0.5 ppm, 4 h a day) daily for 2 weeks.

(c) Trained group this was kept in the O₃ chamber at normal concentrations (see group (a)), and one hour later they were trained as explained.

(d) Trained group exposed to O₃ this group was exposed to O₃ (0.5, 4 h a day) daily for 2 weeks. One hour after O₃ exposure the animals swam as described.

At the end of the two-week experiment, all animals were anesthetized and 5 mL arterial blood samples were taken. The animals were then sacrificed by anesthesia overdose.

2.5. Oxidative Stress Evaluation. Arterial blood samples were heparinized and centrifuged at 1200×g, 15 min at 4°C. Plasma was separated and divided into 5 aliquots of 200 μL to measure

(a) reduced nitrates (NOₓ, modified Griess method, Cayman Chemical Co. Kit),
(b) 8-isoprostane (8-IP, Cayman Chemical Co. ELISA kit),
(c) Malondialdehyde (MDA, Cayman Chemical Co. TBARS kit),
(d) Protein carbonyls (Cayman Chemical Co. kit),
(e) Total activity of superoxide dismutase (SOD, Cayman Chemical Co. kit).

2.6. Statistical Analysis. Data are presented as mean ± standard error of the mean (SEM) of n experiments. Data were analyzed using the one way ANOVA test and Tukey’s multiple comparison test post hoc or the two-way ANOVA test and the Bonferroni test post hoc.

3. Results

3.1. Adaptation to Exercise. Results are shown in Figure 1. Adaptation to exercise, measured through NOₓ plasma concentration, was reached after two weeks of training. Therefore, a 2-week training was chosen for the experiments where rats were or were not submitted to O₃.

3.2. Oxidative Stress Measurement. Protein carbonyls were similar in all the groups (data not shown). O₃ exposure significantly decreased NOₓ levels (P < 0.05) (Figure 2), whereas it increased both 8-IP (Figure 3) and MDA levels (Figure 4) (P < 0.5). Exercise prevented those changes although the effect was partial on 8-IP. SOD activity (Figure 5) significantly decreased with O₃ and independently with exercise (P < 0.05). However, the combination of O₃ and exercise resulted significantly increased values of SOD activity.
The return to normal is considered adaptation to exercise. Data were analyzed using the two-way ANOVA test and the Bonferroni test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

**Figure 2:** Plasma NOx concentrations at week two. Ozone (O3) exposure (0.5 ppm 4 hours/day) significantly decreased whereas exercise (E, 90 min per day) significantly increased plasma NOx concentrations. The effect of O3 exposure was completely blocked by exercise. Data were analyzed using the one-way ANOVA test and Tukey’s multiple comparison test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

**Figure 3:** Plasma 8-isoprostane (8-IP) concentrations at week two. Ozone (O3, 0.5 ppm 4 hours/day) exposure significantly increased 8-IP levels. Even though exercise (90 min per day) did not change 8-IP, it partially but significantly blocked the O3 effect. Data were analyzed using the one-way ANOVA test and Tukey’s multiple comparison test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

**Figure 4:** Plasma malondialdehyde (MDA) concentrations at week two. Ozone (O3, 0.5 ppm 4 hours/day) exposure significantly increased MDA levels. Even though exercise (90 min per day) did not change MDA, it blocked completely O3 effect. Data were analyzed using the one-way ANOVA test and Tukey’s multiple comparison test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

**4. Discussion**

Acute exercise produces OS mainly through superoxide production [1, 2]. Chronic exercise reduces OS generated by acute exercise [1, 4, 5]. The mechanism of such adaptation seems to be through activation of NFkB by free radicals, which in turn increases the synthesis of antioxidant enzymes and NO synthases [4, 9, 12, 23]. Moreover, benefits produced by exercise seem to be given, at least in part, precisely by the induction of antioxidant enzymes and NO [1, 4, 9]. In the present study adaptation to exercise, evaluated through NO production, was reached after three weeks of training. Adaptation to exercise, measured through other biomarkers, was reported previously in the same period using a similar training model [24].

Since O3 exposure produces OS, we wanted to know if exercise could affect such OS just before adaptation to exercise was reached. Therefore, evaluation of OS in the presence or absence of O3 was made with or without two
weeks of training. We chose a two-week training period because it was the time when NO availability significantly increased, with no changes in concentrations thereafter.

Measurements were made in plasma in order to evaluate the systemic effects of exercise. Other authors report changes produced by exercise in skeletal muscle [24]. However, beneficial effects of exercise are probably systemic. OS produced by O3 was confirmed through the increase of 8-IP and MDA as well as the reduction of NO (group).

Exercise partially blocked O3 effects. Data were analyzed using the one-way ANOVA test and Tukey’s multiple comparison test post hoc. Data are shown as the mean ± standard error of mean (n = 6 per group).

It is concluded that AE protects against OS produced by O3, and the effect is independent of SOD.

Acknowledgments

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[3] M. Ristow and S. Schmeisser, “Extending life span by increased antioxidant defense is increased. Indeed, it has been reported that exercise increases SOD [11, 15]. However, this effect has been observed in studies with exercise training longer than 2 weeks. Measurements of the present study were done at the end of the second week of training, just one week before adaptation to exercise. In the present study, the protection of O3 effects produced by exercise (effects on 8-IP, MDA, NO2, and even SOD) could be attributable to changes in other endogenous antioxidants different from SOD (e.g., glutathione peroxidase and catalase).

SOD results are intriguing. O3 exposure, and independent exercise, reduced SOD activity, even though exercise with O3 exposure partially blocked O3 effects. It is known that exercise produces OS and, as a result, the endogenous antioxidant defense is increased. Indeed, it has been reported that exercise increases SOD [11, 15]. However, this effect has been observed in studies with exercise training longer than 2 weeks. Measurements of the present study were done at the end of the second week of training, just one week before adaptation to exercise. In the present study, the protection of O3 effects produced by exercise (effects on 8-IP, MDA, NO2, and even SOD) could be attributable to changes in other endogenous antioxidants different from SOD (e.g., glutathione peroxidase and catalase).

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