Regular physical exercises decrease the oxidant pulmonary stress in rats after acute exposure to mineral coal

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ABSTRACT

Several studies have pointed the regular low to moderate intensity physical exercise as an important agent to combat the oxidant stress. The purpose of this study was to investigate the effect of the physical exercise in the pulmonary oxidant response after inhaling mineral coal dust. Twenty-four male Wistar rats (200-250 g) were randomly divided in two groups with their respective controls (trained, n = 6; non-trained, n = 6). All animals received mineral coal dust via tracheal instillation (saline 3 mg/0.5 ml, 3 days/week for 3 weeks), or 0.5 ml of 0.9% saline solution. Forty-eight hours after the last instillation, the trained group was submitted to a progressive exercise program on treadmill for twelve weeks (up to 17 m.min⁻¹, 50 min/day⁻¹, 10% inclination). Forty-eight hours after the last training, every animal was killed by decapitation, and their lungs and soleus were surgically removed for later biochemical analysis. The citrate synthase activity was determined in the soleus muscle, and the damages on the lipids and proteins were assessed into the lungs by means of the TBARS concentration and by determining the carbonyl groups, respectively. The results show that the regular practice of physical exercises reduces significantly the present TBARS levels in trained rats, decreasing the oxidation levels in proteins of both groups compared to their respective controls. The results lead us to suggest that the regular physical exercise on treadmill is an agent capable to soften the pulmonary oxidant damages caused by inhaling mineral coal particles.

INTRODUCTION

Inhaling occupational and atmospheric pollutants, such as mineral coal, has contributed significantly to the appearance and progression of several respiratory disorders, such as infection, inflammations, pneumoconiosis, chronic bronchitis, among others.

Recent studies have shown that the pulmonary change after inhaling industrial particles is mediated mainly by the formation of reactive Oxygen specimens (ROS). According to Tao and co-workers, such biochemical response leads to the macrophage activation and to the recruitment of polymorphonuclear cells that induce the increase in the inflammatory mediators.

The ROS are normally produced along the cellular metabolism, mainly at the mitochondrial level, and they are fundamental to several physiological actions, have significantly contributed to decrease the probability of the disease. But it seems quite possible that the long-term physical exercise can improve the defense system against the ROS action.

The routine therapeutic interventions, especially the pharmacological actions, have significantly contributed to decrease the problem severity, but it presents a few limitations. It is believed that the regular physical exercise exert a positive effect on the cardio respiratory capability and on the pulmonary biochemical response.

Several studies have described the metabolic ways in the ROS production while exercising, but little is yet known about the influence of the regular physical exercises on the ROS releasing after inhaling mineral particles. The information contained in the literature is only presuppositions that demand further scientific investigations.

Thus, the purpose of this paper was to investigate the effect of the regular physical exercise in the pulmonary oxidant response after inhaling mineral coal dust. It is believed that the disclosure of this aspect is extremely relevant in order to achieve a better understanding on the biochemical phenomenon of the pulmonary oxidant damages as well as its relationship to the physical exercise.

MATERIALS AND METHODS

Every procedure was performed according to the “Guiding Principles in the Care and uses of Animals”, and it was duly approved by the Ethics Committee of the Extremo Sul Catarinense University.

Coal preparation: 1 kg of rough mineral coal collected at the Carbonifera Cooperminas located in the city of Criciúma, Santa Catarina State, Brazil. A 300 g sampling was grinded in a sphere-crusher for 3 hours at a 25 Hz frequency. The coal was analyzed at the Ground and Fertilizer Analysis Laboratory of the Extremo Sul Catarinense University, Criciúma, Santa Catarina State, Brazil, and it presented the following mineralogical characteristics: copper (0.003%), iron (2.5%), zinc (0.003%), and silica (27.3%). The coal particles used in this trial had diameter of up to 15 µm.

Coal instillation: Twenty-four male Wistar rats (200-250 g) were used in the trial protocol. The animals were gathered in specific cages at controlled 22°C room temperature, 12:12 clare-obscure cycle, and with free access to food and water. To the coal instillation, the animals were ketamine (80 mg/kg of the body mass, i.d.), and xylazine (20 mg/kg of the body mass, i.d.) anesthetized, and they received direct administration by tracheal instillation of 3 mg/0.5 ml of saline or 0.5 ml of 0.9% saline solution once every three days for nine days, in a procedure adapted from the model described by Pinho and co-workers.

Keywords: Physical training. Oxidant damages. Lungs. Free radicals.
The animals were randomly divided in two groups with their respective controls: Trained (TR, n = 6), and Non-trained (NTR, n = 6).

Training protocol: Forty-eight hours after the last coal instillation, every animal was adapted on the fitted treadmill (10 m/min, no inclination, 10 min/day). The trained groups (coal and saline) were submitted to a progressive training program for 12 weeks with velocity up to 17 m/min, time of up to 50 min/day, and up to 10% inclination gradually increased. Forty-eight hours after the last training session, every animal was killed by decapitation, and their lungs and soleum were surgically removed and immediately stored at -80°C for later analysis.

Citrate synthase (CS): The citrate synthase activity was determined from the HS-group using 5, 5′-dithiobis (2-nitrobenzoic acid – DTNB)40. 100 mg of muscle (soleum) were PGS-homogenized and centrifuged for 10 minutes, 1000Xg, 4°C. To the reaction, 100 µl of 1 mM-DTNB were used dissolved in 10 ml of 1 M-Tris/HCl (8.1 pH), 30 µl of 10 mM-Acetyl-CoA dissolved in Milli Q water, 20 µl of the sampling, 800 µl of the solution (1 mM-EDTA/Triton X100 0.05%, pH 7.4, 25°C). The enzymatic activity was measured at 412 nm absorbance for 3 minutes, with 7 minutes incubation. The process was repeated, and the CS activity initiated when 50 µl oxaloacetate dissolved was added to 0.1 M-Tris/HCL. The 13.600 M absorption coefficient was used.

Reactive specimens to the thiobarbiturate (TBARS): As peroxidation index of the lipids, the reactive substances formation was verified upon the warming of the thiobarbituric acid spectrophotometrically measured (532 nm), and expressed as the malondialdehyde (MDA) (nmol/mg protein)13.

Protein carbonylation: The oxidizing damages of proteins were measured through the determination of the carbonyl grouping based on the reaction with the dinitrophenylhydrazine16.

The carbonyl content was spectrophotometrically determined in 370 nm using a 22000 M coefficient.

Protein determination: The total quantity of protein was determined using the Lowry technique17.

Statistical analysis: The data was expressed in mean and standard deviation, and they were statistically analyzed using the t Student test to analyze the citrate synthase (CS) activity, as well as the one-way variance analysis (ANOVA) followed by the Tukey’s post hoc test to the oxidizing damage assays. The significance level set for the statistical tests was p < 0.05. The SPSS (Statistical Package for the Social Sciences) version 10.0 was used as statistical package.

Reagents: Thiobarbituric acid, DTNB, acetyl-CoA, oxaloactate, dinitrophenylhydrazine, adrenaline, and Hydrogen peroxide were acquired from Sigma Chemical (St. Louis, MO).

RESULTS

Citrate synthase (CS): The activity of the CS was used as indicator for the activities of the physical exercises. The results (table 1) indicate that the training program performed on the treadmill was sufficient to increase the oxidant capability of the skeletal muscle (TR = 0.58 ± 0.01 U/mg protein, NTR = 0.32 ± 0.04 U/mg protein, p < 0.05).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± EPM (nmol/mg protein)</th>
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<tbody>
<tr>
<td>Trained (TR)</td>
<td>0.589 ± 0.010</td>
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<tr>
<td>Non-trained (NTR)</td>
<td>0.327 ± 0.042</td>
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</table>

Lipoperoxidation: As the level of the oxidant damage in the membrane’s lipids, the TBARS formation was assessed after exposure to mineral coal. As is seen in figure 1, the results indicate a significant increase in the lipoperoxidation in rats exposed to the coal (coal = 0.123 ± 0.025 nmol/mg protein; Control = 0.082 ± 0.014 nmol/mg proteins, p < 0.05).

The results also show that the regular practice of physical exercises reduces significantly the TBARS formation in trained rats compared to non-trained rats (TR = 0.053 ± 0.009 nmol/mg protein, and NTR = 0.123 ± 0.025 nmol/mg protein, p < 0.05).

Protein carbonylation: In order to check the oxidant damages to proteins, the carbonyl groups were assessed based on the reaction to the dinitrophenylhydrazine. According to figure 2, the results do not show any significant difference in the protein damage in rats after exposure to mineral coal compared to the Control group (coal = 0.407 ± 0.075 nmol/mg protein, and Control = 0.249 ± 0.027 nmol/mg protein). Also, it was verified that the regular physical exercise decreases the oxidant response caused by the exposition to the mineral coal (TR = 0.136 ± 0.010 nmol/mg protein, and NTR = 0.407 ± 0.075 nmol/mg protein, p < 0.05).

DISCUSSION

The citrate synthase is a regulatory enzyme in the production of the aerobic power that catalyzes the oxaloacetate and CoA acetyl condensation for the citrate in the tricarboxylic acid cycle18. Several studies have been using the mitochondrial enzyme activity to confirm or not the influence of the physical exercise in the oxidant adaptation of the skeletal muscle in rats10,19,19. But few studies have demonstrated the CS response after the endurance training on treadmill.
It is observed on table 1 a significant increase in the CS activity in the soleum muscle of rats after a training program. Likewise, Alessio and Goldfarb have shown that the training on treadmill in young rats increases the CS activity in the gastrocnemius twice more than in the control group.

In another study, Radáč and co-workers have shown an important increase in the CS activity in different skeletal muscles in young and middle age rats after a nine week training in water. Although our study confirms these results, other studies mentioned by Pereira and co-workers has presented opposite responses. Probably such difference between these studies is due to the different types of muscular fibers used or the different training protocols or in the methods used to determine the enzymatic activity or even to the differentiated molecular adaptations (translational and post-translational modifications) in the CS adaptation during and post-physical training.

Recent studies show that the pulmonary response by occupational and atmospheric pollutants such as the coal, causes a inflammatory chain-response mediated by the macrophage activation, and the recruitment of polymorphonuclear cells, cytokine, chymosin, and reactive Oxygen specimens (ROS). After inhaling these pollutants, the ROS production can be significantly increased, causing damages in the cellular constituents.

The oxidant response to the physical exercise is determined by the type, frequency, duration and intensity, that by one side can generate the ROS formation which is able to cause cellular damages and inflammation, and on the other hand, the regular physical endurance exercise can be the most efficient antioxidant defense system, establishing a balance between the damages induced by the ROS and the antioxidant repairing systems.

Therefore, due to both end points observed in the oxidant response to the physical exercise, the results attained through the physical training are not yet conclusive. In this study, we raised the hypothesis that the regular and continuing physical aerobic training are not yet conclusive. In this study, we raised the hypothesis that the regular and continuing physical aerobic exercises can be an agent capable to soften the protein and lipid damage in the membrane caused by the exposure to mineral coal.

The data on the lipids initiated from their oxidation result from the free radical reaction with polyunsaturated fatty acids present in the lipoproteins of the membranes. The changes in the lipoperoxidation markers are more frequent evidences observed into the pulmonary tissue after exposure to atmospheric pollutants, and after inhaling crystalline silica in a rat model: time course of pulmonary reactions. Mol Cell Biochem 2002;234:177-84.


All the authors declared there is not any potential conflict of interests regarding this article.

REFERENCES


