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ORIGINAL ARTICLE

Effects of hyperoxia periodic training on free radicals production, biological antioxidants potential and lactate dehydrogenase activity in the lungs of rats, *Rattus norvigicus*

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Abstract Oxygen therapy has been widely used in lung injury (Li), adult respiraotory syndrome (ARDS) and inflammatory lung diseases as well as in mechanical ventilation in intensive care units. Exposure to hyperoxia is known to induct the production of reactive oxygen species (ROS) by mitochondria. Despite decades of research, the role of hyperoxia training in oxidative stress and ROS formation in the lungs is not known. The purpose of this study was to examine the effects of periodic-hyperoxia training on biological antioxidants (BAP) and lactate dehydrogenase (LDH) activities and free radicals (FR) production. Thirty adult male rats, matched with age and body weigh, were randomly assigned to three groups. The first group served as control (C) and the second (HP) was exposed to hyperoxia for 48. Animals in the third group (HP-T) were trained on hyperoxia for 1.5 h daily for three weeks. Following the exposure period for each group animals were sacrificed and lungs tissues were homogenized for BAP, LDH and FR determinations. LDH activity was determined by Randox protocol (Randox – UK). BAP and FR were determined using dROM method (H&D – Italy). Results showed that mean (±SD) BAP activity increased significantly ($p < 0.05$) from the baseline control of 7105.88 ± 2021.49 to 8611.20 ± 1245.26 (U/L) after hyperoxia training; then dropped to 6784.00 ± 1879.50 during hyperoxia exposure for 48 h. Whereas mean (±SD) FR production increased significantly ($p < 0.05$) from the baseline control of 262.50 ± 67.52 to 339.90 ± 64.84 during HP exposure for 48 h, then dropped to 211.13 ± 52.05 (Carr), during HP training. Similarly, LDH activity increased significantly ($p < 0.05$) from the

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baseline control of 210.31 ± 70.93 to 339.90 ± 64.84 during HP exposure for 48 h, then dropped to 159.30 ± 20.61(U/L), following HP-periodic training. Furthermore, the correlation ($r = 0.67\times$) of LDH on FR was significant ($p < 0.05$), implying that reduction in ROS generation induced by HP-periodic training is related to reduced rate of cell apoptosis caused oxidative stress. Based on the results of the present study HP-periodic training is recommended in order to resist oxidative damage in the lungs.

1. Introduction

Under normal physiological and metabolic conditions, single electrons sometimes escape from the transport chain which is located in the inner mitochondrial membrane and result in a single electron reduction of molecular oxygen forming a superoxide anion (O_2^-). Oxidative mitochondrial stress (OMS) occurs when O_2 tension is increased because the buildup of O_2^- can not be controlled. Thus superoxide production can be significantly enhanced during exposure to hyperoxia because the rate of electron transport is limited by the buildup of a large proton gradient in the inner mitochondrial membrane leading to variety of mitochondrial pathological changes such as swelling, concentrated cristae, dilution of the inner and outer membrane (Haffor, 2004; Haffor and Al-Johany, 2005). Clearly both OMS and O_2^- generation lead to the production of ROS such as hydrogen peroxide (H_2O_2) which in the presence of ferrous iron via the Fenton reaction, result in highly reactive hydroxyl radicals. Once mitochondrial and cellular enzymatic biological antioxidant potential (BAP) are overwhelmed by the buildup of ROS, oxidative damage and the subsequent cell death can occur. Therefore it can be expected that long duration exposure to hyperoxia can be a potential cause for numerous common lung diseases because it can triggers OMS, pneumocyte death as well as circulating neutrophils and alveolar macrophages defense responses. These defense responses can also contribute to magnification of ROS generation. It has been shown that exposure to hyperoxia beyond 24 h result in morphologic changes that are similar to pulmonary inflammation, atelectasis, oedema formation, irreversible loss of respiratory function and lung inflammation (Crapo, 1986; Jankov et al., 2003; Jafari et al., 2004).

Lactate dehydrogenase (LDH) catalyses the terminal step in anaerobic glycolytic pathway which is located in the cell cytoplasm. The elevated level of LDH reflects high compensatory anaerobic rate secondary to oxidative mitochondrial stress (OMS). Thus LDH activity can be reliable cytosolic early marker for OMS and the subsequent elevation in ROS formation.

The effects of hyperoxia and risk of bronchopulmonary dysplasia in infants or adult respiratory distress syndrome in adults begins with exposure period over 8 h (Arieli, 1998; Chavko et al., 1998; Demchenko et al., 2001). In healthy adult risk begins after 48 h (Comroe et al., 1945). Because symptoms caused by hyperoxia, requires long duration exposure, it can be speculated that short term exposure can cause adaptive protective changes related to OMS, ROS production and antioxidants activities. The role of periodic short duration exposure to hyperoxia on ROS production and enhancement of antioxidants resistance is not known nor have been examined in previous studies. The purpose of present study was to explore the effects of periodic-hyperoxia training (PHT) on free radicals (FR) production, lactate dehydrogenase (LDH) and biological antioxidant potential (BAP) activity.

2. Materials and methods

2.1. Experimental design

Twenty-four adult Wister Albino male rats, *Rattus norvigicus*, matched with age and body weigh, were randomly assigned to four groups, ten animals each. The first group served as control and the second, was exposed to hyperoxia for 48 h. Animals of the third group were engaged in periodic-hyperoxia-training (PHT) for 1.5 h daily for 3 weeks period. The control and experimental groups were sacrificed and the lungs were isolated and homogenized immediately in 0.9 saline solutions (4:1 ratio). All animals were treated according to standards described in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by National Institute of Health (NIH Publications 86–23 revised 1985).

2.2. Hyperoxia exposure

Animals of the first experimental group were placed in a closed box that has an inlet flow which was connected to 100% O_2 tank, medical grade, on which a regulator was connected to maintain flow at 5 l per minute (LPM). The out flow of the regulator passed through a humidifier in order to saturate the inspired air with H_2O. The outlet ventilation rate of the box was adjusted at 5 LPM to ensure that the concentration of oxygen in the box remains equal to 100% O_2 and maintain normal flow and maintain normal barometric pressure at 767 mm Hg. The temperature inside the box was adjusted at room temperature (22–24 °C).

2.3. Hyperoxia periodic training

Animals of the second experimental group were engaged in hyperoxia training program for three weeks period. Exposure to hyperoxia was conducted for thee intervals, for 30 min each, separated with 10 min breathing room air, normoxia.

2.4. Free radical determination

Free radicals production was measured, using the d-ROMs-4 test kits (Health & Diagnostic, Italy) according to the manufacturer's instructions. The test measures the levels of hydroperoxides (R-OOH) which are generated by peroxidation of biological compounds; lipid, amino acids, nucleic acids. This test is based on the principle of the ability of hydrogen peroxides to generate free radicals after reacting with some transitional metals (Fe_2^+/Fe_3^+), according to Fenton's reaction as follows:

$$H_2O_2 + Fe^{2+} = {}^{*}OH + OH^- + Fe^{3+}$$