Toxicology and Applied Pharmacology 274 (2014) 1–6

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Iron supplementation at high altitudes induces inflammation and oxidative injury to lung tissues in rats

Samir A. Salama [a,b,c,*], Hany A. Omar [d], Ibrahim A. Maghrabi [e], Mohammed S. AlSaeed [f], Adel E. EL-Tarras [a]

[a] High Altitude Research Center, Taif University, Al-Haweiah, Taif 21974, Saudi Arabia
[b] Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Cairo 11751, Egypt
[c] Department of Pharmacology and GTMR Unit, College of Clinical Pharmacy, Taif University, Al-Haweiah, Taif 21974, Saudi Arabia
[d] Department of Pharmacology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt
[e] Department of Clinical Pharmacy, College of Clinical Pharmacy, Taif University, Al-Haweiah, Taif 21974, Saudi Arabia
[f] Department of Surgery, College of Medicine, Taif University, Al-Haweiah, Taif 21974, Saudi Arabia

ARTICLE INFO

Article history:
Received 26 August 2013
Revised 22 October 2013
Accepted 30 October 2013
Available online 9 November 2013

Keywords:
High altitudes
Lung tissue injury
Iron supplementation
Trolox
Oxidative stress
Proinflammatory cytokines

ABSTRACT

Exposure to high altitudes is associated with hypoxia and increased vulnerability to oxidative stress. Polycythemia (increased number of circulating erythrocytes) develops to compensate the high altitude associated hypoxia. Iron supplementation is, thus, recommended to meet the demand for the physiological polycythemia. Iron is a major player in redox reactions and may exacerbate the high altitudes-associated oxidative stress. The aim of this study was to explore the potential iron-induced oxidative lung tissue injury in rats at high altitudes (6000 ft above the sea level). Iron supplementation (2 mg elemental iron/kg, once daily for 15 days) induced histopathological changes to lung tissues that include severe congestion, dilatation of the blood vessels, emphysema in the air alveoli, and peribronchial inflammatory cell infiltration. The levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), lipid peroxidation product and protein carbonyl content in lung tissues were significantly elevated. Moreover, the levels of reduced glutathione and total antioxidant capacity were significantly reduced. Co-administration of trolox, a water soluble vitamin E analog (25 mg/kg, once daily for the last 7 days of iron supplementation), alleviated the lung histological impairments, significantly decreased the pro-inflammatory cytokines, and restored the oxidative stress markers. Together, our findings indicate that iron supplementation at high altitudes induces lung tissue injury in rats. This injury could be mediated through excessive production of reactive oxygen species and induction of inflammatory responses. The study highlights the tissue injury induced by iron supplementation at high altitudes and suggests the co-administration of antioxidants such as trolox as protective measures.

Introduction

Reduced barometric pressure at high altitudes causes hypoxia that is proportional to the level of the altitude (West, 1995, 2004). Physiological polycythemia arises to compensate the high altitude associated hypoxia (Faura et al., 1969; Huff et al., 1951; Reynafarje et al., 1959). Iron supplementation is, thus, recommended to meet the polycythemic demand and to guard against iron deficiency anemia that is prevalent at high altitudes (Abou-Zeid et al., 2006; Berger et al., 1997; Estrella et al., 1987). Hypoxia inducible factors (HIFs) stimulate intestinal absorption of iron, increasing iron availability for erythropoiesis (Chepelev and Willmore, 2011; Peyssonnaux et al., 2007; Shah et al., 2009).

Although iron is essential for numerous biochemical processes, it is a strong pro-oxidant and is involved in the generation of reactive oxygen species (ROS) (Galaris and Pantopoulos, 2008; Mendes et al., 2009; Papanikolaou and Pantopoulos, 2005; Welch et al., 2002). Exposure to high altitudes is associated with oxidative stress that is developed within a month of exposure to the high altitude (Maiti et al., 2010; Sinha et al., 2010). The severity of the high altitude-associated oxidative stress is proportional to the degree of the altitude (Dosek et al., 2007). Acclimatization to high altitude-associated oxidative stress is a long term process that may require several months (Vij et al., 2005). At high altitudes, generation of ROS and reactive nitrogen species (RNS) is enhanced. ROS and RNS generating systems including electron transport chain (ETC), xanthine oxidase, and nitric oxide synthase are activated at high altitudes (Dosek et al., 2007). In addition, the enzymatic and non-enzymatic antioxidant systems are repressed (Chang et al., 1989; Dosek et al., 2007). Iron supplementation, thus, may boost the ROS

Abbreviations: DTNB, 5,5′-dithiobis (2-nitrobenzoic acid); DNPH, 2,4-dinitrophenyl hydrazine; ETC, electron transport chain; GSH, reduced glutathione; H&E, hematoxylin and eosin; HIFs, hypoxia inducible factors; IL-1β, interleukin-1β; IL-6, interleukin-6; MDA, malondialdehyde; PBS, phosphate buffered saline; RNS, reactive nitrogen species; ROS, reactive oxygen species; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; TCA, trichloroacetic acid; TNF-α, tumor necrosis factor-α.

* Corresponding author at: Division of Biochemistry, Department of Pharmacology, College of Clinical Pharmacy, Taif University, Al-Haweiah, Taif 21974, Saudi Arabia.
E-mail address: salama.3@buckeyemail.osu.edu (S.A. Salama).

0041-008X/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.taap.2013.10.034

generation at high altitudes with subsequent contribution to oxidative stress-induced tissue damage.

The lung is a highly susceptible organ to ROS because of its large surface area and continuous contact with air oxygen (Tkaczyk and Vizek, 2007). The current study aimed at exploring the potential lung tissue injury induced by iron supplementation at high altitudes (6000 ft above the sea level) in rats and the possible ameliorating effects of trolox, a water soluble vitamin E analog. Potential iron-induced lung tissue injury was evaluated by histological examination and determination of lipid and protein oxidation markers in rat lung tissues. In addition, the antioxidant status was assessed by evaluating the levels of reduced glutathione and total antioxidant capacity. Levels of early response proinflammatory cytokines (IL-1β, TNF-α, and IL-6) were also assessed.

Material and methods

Animals. Male Wistar rats weighing 150–200 g were obtained from the animal house of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were housed in polypropylene cages (four rats/cage) at Taif University animal facility (6000 ft above sea level) at controlled environment conditions (temperature 23 ± 2 °C, humidity 60 ± 10%, and a 12 h light/dark cycle) and were acclimatized for 45 days before starting the study. Standard commercial rat chow and water were allowed *ad libitum*. All procedures relating to animal care, treatments, and sampling were conducted in compliance with the guidelines of Taif University Research Ethical Committee.

Chemicals and kits. Ferrous sulfate heptahydrate (extra pure) was purchased from Loba Chemie (Colaba, Mumbai, India). Thiobarbituric acid, trichloroacetic acid, DTNB, and DNPH were purchased from Sigma Aldrich (St. Louis, MO, USA). All other chemicals were of high purity. Total antioxidant capacity (TAC) kit was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). IL-1β, TNF-α, and IL-6 kits were purchased from Ray Biotech (Norcross, GA, USA).

Treatment protocol. Twenty four rats were randomly distributed into three groups, eight animals in each group. Group I (normal control group, Ctrl), received standard rat chow; group II (iron only treated group, Fe), received ferrous sulfate heptahydrate (equivalent to elemental iron of 2 mg/kg, once daily, supplemented as iron fortified chow) for 15 consecutive days; group III (iron and trolox treated group, Fe + T), received ferrous sulfate heptahydrate (equivalent to elemental iron of 2 mg/kg once daily, supplemented as iron fortified chow) for 15 consecutive days and trolox (25 mg/kg once daily, orally in distilled water by gastric gavage) for the last 7 days of iron supplementation period. All animals were kept at Taif University animal facility (6000 ft above the sea level) during the acclimatization and the study periods. A trolox only treated group (8 additional rats) was proposed to test the protective effect of trolox on the possible lung tissue injury induced by the 6000 ft altitude-associated oxidative stress. Rats in this group were kept at 6000 ft altitude and received trolox (25 mg/kg once daily, orally in distilled water by gastric gavage) for the last 7 days of the experiment. Because the normal control group that was kept at 6000 ft altitude did not show any histopathological changes to lung tissues and there was no injury to protect from (Fig. 1A), results from trolox only treated group were excluded. The results from trolox only treated group were very similar to that of the normal control group. The selected doses of iron and trolox were consistent with previous literature (Baron and Muriel, 1999; Estrella et al., 1987; Galicia-Moreno et al., 2008). All animals were kept at Taif University animal facility (6000 ft above the sea level) during the acclimatization and the study periods.

Sample preparation. After one day of the last iron dose, animals were euthanized under deep ether anesthesia for the collection of lung tissues. The lung tissues were quickly removed, rinsed in ice cold saline, and divided for homogenization and histopathological examination. For homogenization, samples were weighed and homogenized (10% w/v) in phosphate buffered saline (PBS). Tissue homogenate was centrifuged at 10,000 ×*g* for 15 min and the supernatant was used for the determination of total protein and other biochemical parameters. Lung tissues designated for histopathological examination were processed as described in the following Histopathological examination section.

Histopathological examination. Autopsy samples were taken from the lung of rats in different groups and fixed in 10% formol saline for 24 h. Samples were then washed with tap water and dehydrated using serial dilutions of alcohols (methyl, ethyl and absolute ethyl). Specimens were cleared in xylene and embedded in paraffin at 56 °C in hot air oven for 24 h. Paraffin bees wax tissue blocks were prepared for sectioning at 4 μm thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin (H&E) stain for routine examination that was done using the light electric microscope (Banchroft et al., 1996).

Measurement of lipid peroxidation. Lipid peroxidation levels in lung tissue homogenates were measured by evaluating thiobarbituric acid reactive substances according to the method described by Buege and Aust (1978). Briefly, 1 ml of the lung tissue homogenate was mixed with an equal volume of 0.5% (w/v) thiobarbituric acid (TBA) prepared in 20% (w/v) trichloroacetic acid (TCA) and heated at 95 °C for 30 min. After stopping the reaction by placing the tubes on ice, samples were centrifuged at 10,000 ×*g* for 15 min and the absorbance of the colored supernatant was measured at 532 nm. TBARS concentrations were calculated using the extinction coefficient of 155 mM^{-1} cm^{-1} and the results were expressed as malondialdehyde (MDA) in nmol/g tissue.

Measurement of protein carbonyl content. Protein carbonyl content, a convenient index of protein oxidative modification, in lung tissue homogenates was measured using dinitrophenyl hydrazine (DNPH) according to the method described by Hawkins et al. (2009). Briefly, proteins were precipitated by the addition of 50% (w/v) solution of TCA after reaction with DNPH. Protein precipitates were then re-dissolved in 6 M guanidine–HCl and the absorbance was measured at 370 nm. Protein carbonyl content was calculated using the extinction coefficient of 22,000 M^{-1} cm^{-1} and expressed in nmol/mg protein.

Determination of low molecular weight thiols. Low molecular weight thiols (primarily composed of reduced glutathione, GSH) in lung tissue homogenates were measured spectrophotometrically using 5,5′-dithiobis 2-nitrobenzoic acid (DTNB) reagent based on the method described by Ellman (1959). Briefly, lung tissue homogenates were deprotonated with TCA solution (10% w/v) then centrifuged at 10,000 ×*g* for 10 min at 4 °C and the supernatant was separated. The absorbance of the color produced by the reaction of supernatant with 10 mM DTNB was measured at 412 nm and the concentration of GSH is expressed in μmol/g tissue.

Determination of total antioxidant capacity. Total antioxidant capacity (TAC) evaluates several antioxidants including macromolecules such as albumin, ceruloplasmin, and ferritin; and a variety of small molecules such as ascorbic acid, α-tocopherol, β-carotene, uric acid, and bilirubin. TAC was determined in lung tissue homogenates using the commercially available kit (Cayman total antioxidant assay) according to the manufacturer's instructions. The assay based on the ability of the antioxidants in the lung tissue samples to suppress the oxidation of 2,2-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS). The amount of oxidation product of ABTS that is inversely proportional to the TAC was measured spectrophotometrically at 405 nm and the TAC was expressed in μmol of trolox equivalent/g tissue using the created standard curve.