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.

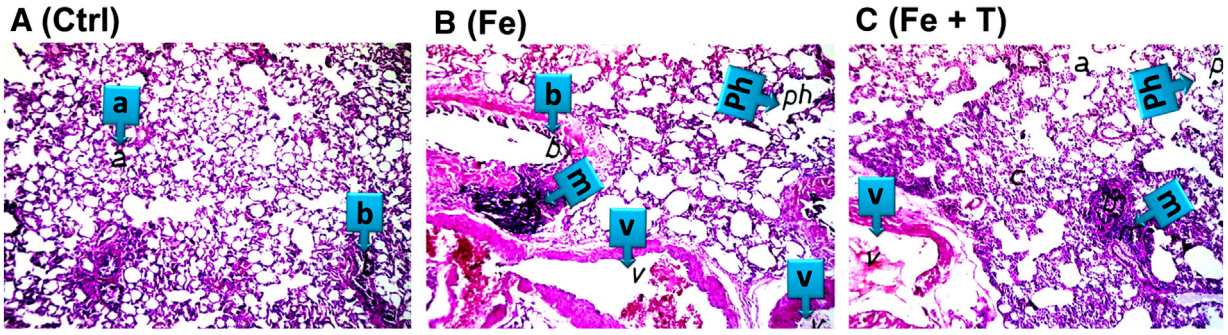

Fig. 1. Histopathological changes in lung tissues of rats that received iron at high altitudes (6000 ft) and the alleviating effect of trolox. (A) A representative photomicrograph from the control group (received distilled water orally once daily for 15 days) shows normal histological structure of air alveoli (indicated by a-labeled arrow) and bronchioles (indicated by b-labeled arrow). (B) A representative photomicrograph from iron only-treated group (received 2 mg elemental iron/kg, once daily for 15 days) shows severe congestion and dilatation of the blood vessels (indicated by v-labeled arrows), emphysema of the air alveoli (indicated by Ph-labeled arrows), and peribronchial inflammatory cell infiltration (indicated by m-labeled arrows). (C) A representative photomicrograph from iron and trolox-treated group (received 2 mg elemental iron/kg, once daily for 15 days and trolox 25 mg/kg, once daily for the last 7 days of iron supplementation) shows mitigated histopathological changes compared to iron only-treated group (B). Representative photomicrographs of sections taken from lung tissues one day after the last dose of iron/trolox supplementation and stained with hematoxylin and eosin, magnification, ×16.

Determination of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-6). The concentrations of IL-1β, IL-6, and TNF-α in lung tissue homogenates were determined using the commercially available ELISA kits (Ray Biotech, GA, USA). Briefly, 100 μl of the lung tissue homogenates and standards was incubated at room temperature for 2.5 h with the corresponding antibody pre-coated to wells of a microplate. Tissue homogenates were discarded and the plates were washed before incubation with biotinylated antibodies for 1 h at room temperature with gentle shaking. Antibody solution was discarded and the plates were washed again. Further incubation with streptavidin–HRP for 45 min with gentle shaking was done before detection with TMB (3,3′,5,5′-tetramethylbenzidine) solution. Finally, the reactions were stopped by adding 2 M H_2SO_4, and the absorbance was measured immediately at 450 nm. The concentrations of cytokines were calculated from the linear portion of the created standard curve.

Statistical analysis

Multiple comparisons among groups were analyzed for statistical significance by one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons posttest. Data were represented as mean ± standard deviation (SD). Differences were considered significant at $p < 0.05$. Graphs and statistical analysis were created using SigmaPlot 12 statistics software (Systat Software, Inc., San Jose, CA).

Results

Iron supplementation at high altitudes induces histopathological changes to lung tissues of rats

To explore the possible iron-induced histological alternation to lung tissues of rats kept at an altitude of 6000 ft above the sea level, lung tissue samples from the control and the treated groups were subjected to H&E staining followed by histopathological assessment. Representative histopathological photomicrograph of iron only-treated group (Fe) showed severe congestion, dilatation of the blood vessels, emphysema of the air alveoli, and peribronchial inflammatory cell infiltration (Fig. 1B) compared to the control group (Ctrl) that showed normal air alveoli and bronchioles (Fig. 1A). The iron-induced histopathological signs of inflammation and tissue injury were attenuated by co-administration of the antioxidant trolox (Fig. 1C), indicating that the iron-induced histopathological changes may be mediated through oxidant generation.

Iron supplementation at high altitudes alters oxidative stress markers in lung tissues of rats

Iron plays a central role in the redox reaction in biological systems and is involved in the generation of reactive oxygen species (ROS) (Armutcu et al., 2004; Valko et al., 2006). Because ROS have been implicated in acute lung injury (Lang et al., 2002) and may mediate the observed iron-induced histopathological changes to lung tissues of rats, the oxidative status of lung tissues of different studied groups was evaluated. Levels of lipid peroxidation expressed as malondialdehyde (MDA), protein oxidation marker (protein carbonyl content, PCC), reduced glutathione (GSH) and the total antioxidant capacity (TAC) in lung tissue homogenates were assessed. Iron only-treated group showed a significant increase in MDA and PCC (Fig. 2) and significant decrease in GSH and TAC (Fig. 3). Co-administration of trolox significantly attenuated the elevated levels of MDA and PCC but their levels were not returned back to the control levels (Fig. 2) and significantly restored the levels of GSH and TAC (Fig. 3), consistent with iron-induced oxidative lung tissue injury.

Iron supplementation at high altitudes upregulates IL-1, IL-6, and TNF-α levels in lung tissues of rats

It has been shown that oxidative tissue damage induces early response proinflammatory cytokines that contribute further to tissue injury (Bhandari and Elias, 2006; Chow et al., 2003). To evaluate the proinflammatory cytokines in the lung tissues of the control and the treated groups, levels of IL-1β, IL-6, and TNF-α in lung tissue homogenates were assessed. Iron supplementation significantly increased the levels of IL-1β, IL-6, and TNF-α compared to the control group (Fig. 4). Co-administration of trolox significantly reduced the iron-induced elevated levels of IL-1β, IL-6, and TNF-α but their levels were not returned back to the control levels (Fig. 4).

Discussion

High altitude-associated hypoxia induces physiological polycythemia (Faura et al., 1969; Huff et al., 1951; Reynafarje et al., 1959). Iron supplementation is, thus, recommended at high altitudes to decrease the incidence of the iron deficiency anemia that is prevalent at high altitudes (Abou-Zeid et al., 2006; Berger et al., 1997; Estrella et al., 1987). Iron and oxygen are intimately associated because iron is indispensible to oxygen transport. Under normal oxygen tension, iron absorption through the proximal intestine is strictly regulated to avoid iron overload (Chepelev and Willmore, 2011; Jomova and Valko, 2011). At high

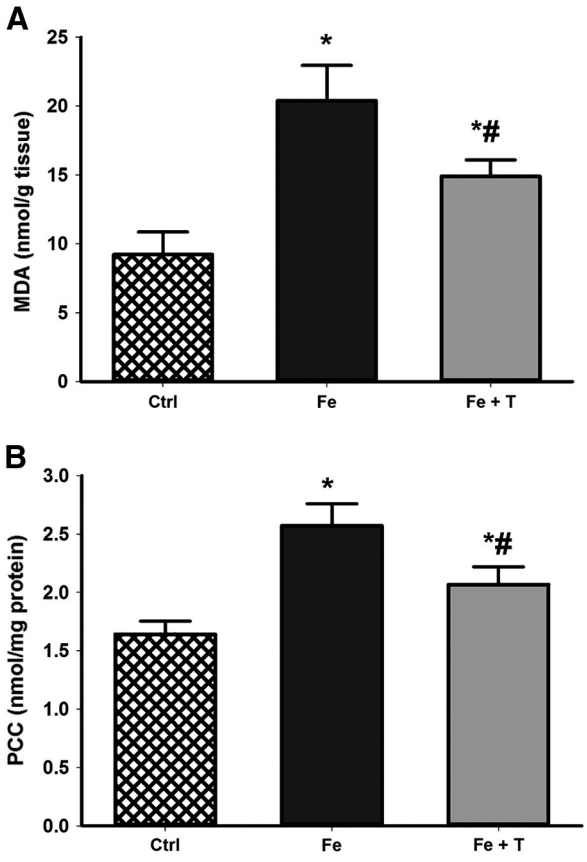

Fig. 2. Influence of iron supplementation at high altitudes (6000 ft) on lipid peroxidation and protein oxidation markers and the modifying effect of trolox. Lipid peroxidation marker expressed as malondialdehyde (MDA) and protein carbonyl content (PCC), were determined in lung tissue homogenates of three groups of rats. (Ctrl) Normal control group received distilled water orally, once daily for 15 days. (Fe) Iron only-treated group received 2 mg elemental iron/kg, once daily for 15 days. (Fe + T) Iron and trolox-treated group received 2 mg elemental iron/kg, once daily for 15 days and trolox 25 mg/kg, once daily for the last 7 days of iron supplementation period. Data are expressed as mean ± standard deviation. (*) Significant difference from the normal control group $p < 0.05$. (#) Significant difference from the iron only-treated group $p < 0.05$ (n = 8).

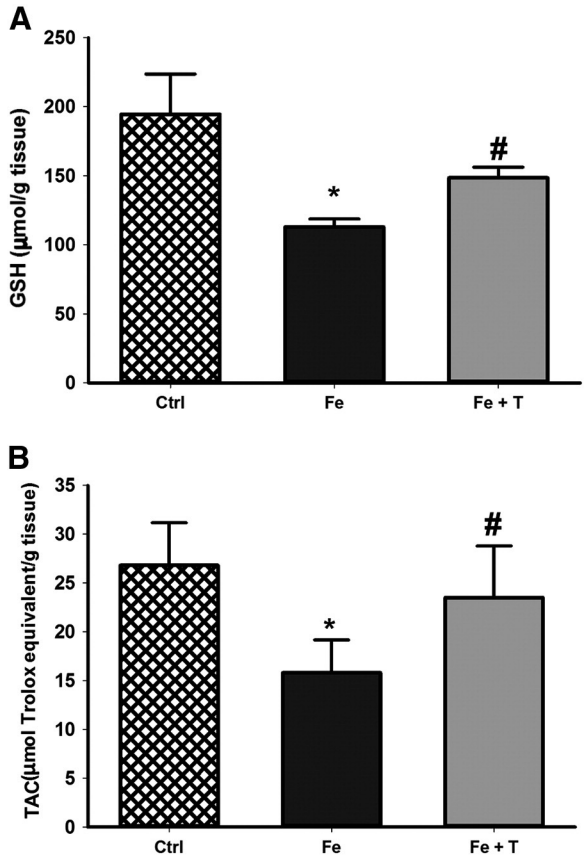

Fig. 3. Effect of iron supplementation at high altitudes (6000 ft) on antioxidant status of lung tissues of rats and the protective effect of trolox. Reduced glutathione (GSH) and total antioxidant capacity (TAC) were determined in lung tissue homogenates of different studied groups. (Ctrl) Normal control group received distilled water orally, once daily for 15 days. (Fe) Iron only-treated group received 2 mg elemental iron/kg, once daily for 15 days. (Fe + T) Iron and trolox-treated group received 2 mg elemental iron/kg, once daily for 15 days along with trolox 25 mg/kg, once daily for the last 7 days of iron supplementation period. Data are expressed as mean ± standard deviation. (*) Significant difference from normal control group $p < 0.05$. (#) Significant difference from iron only-treated group $p < 0.05$ (n = 8).

altitudes, however, hypoxia triggers hypoxia-inducible factor (HIF) signaling (Chepelev and Willmore, 2011; Peyssonnaux et al., 2007). HIFs increase intestinal iron uptake through induction of duodenal cytochrome B (DcytB) and divalent metal transporter-1 (DMT1) expression and repression of hepcidin hormone, an inhibitor of intestinal iron absorption (Peyssonnaux et al., 2007; Shah et al., 2009). HIF signaling, thus, increases the availability of iron to meet the demand for the physiological polycythemia that is required to improve oxygen uptake and delivery at high altitudes. Although iron is essential for numerous biochemical processes, it is a strong pro-oxidant (Lee et al., 2006). Ferrous iron reduces hydrogen peroxide to the powerful ROS, hydroxyl radical, in the Fenton reaction (Armutcu et al., 2004; Buijsse et al., 2007; Ganz, 2003; Valko et al., 2006). ROS induce tissue injury through interaction with essential biomolecules such as DNA, proteins, and lipids (Jomova and Valko, 2011; Mendes et al., 2009; Ryan and Aust, 1992). Increased availability of iron, thus, may exaggerate the possible ROS-induced tissue injury in oxidative stress vulnerable tissues.

Hypoxia prevalent at high altitudes has been shown to increase the rate of ROS formation (Klimova and Chandel, 2008). Up to 5% of the oxygen used by the ETC is reduced to superoxide anion radical due to incomplete reduction of molecular oxygen to water (Chepelev and Willmore, 2011). Complex III of the ETC is thought to be a major source of superoxide anion radicals (Chandel et al., 2000; Guzy et al., 2007). In addition, excessive ROS generation through xanthine oxidase system has been reported at high altitudes (Dosek et al., 2007). Moreover, enzymatic and non-enzymatic antioxidant systems are compromised at high altitudes (Chang et al., 1989; Dosek et al., 2007). Taking together, exposure to high altitudes is associated with vulnerability to oxidative stress. We investigated the possible iron-induced tissue injury in lung tissue because the lung is a highly susceptible organ to ROS due to its large surface area and continuous contact with air oxygen (Tkaczyk and Vizek, 2007). The current study showed, for the first time, that iron supplementation at high altitudes induced histopathological changes to lung tissues of rats. The histopathological changes include severe congestion, dilatation of the blood vessels, emphysema of the air alveoli, and peribronchial inflammatory cell infiltration (Fig. 1). Investigation of oxidative stress markers in lung tissues of iron only-treated rats showed that the levels of lipid peroxidation (MDA) and protein carbonyl content (PCC), a marker of protein oxidation, were significantly elevated compared to that of the control group (Fig. 2). In addition, the levels of reduced glutathione and total antioxidant capacity in lung tissues were significantly decreased (Fig. 3), consistent with iron-induced ROS-mediated lung tissue injury. The results are in line with the previously published work which showed that excessive production of ROS in lung tissues plays a central role in acute lung injury (Lang et al., 2002). In contrast to iron supplementation at high altitudes, a previous study has shown that iron supplementation at near sea level did not alter oxidative stress markers (Braekke et al., 2007). ROS causes additional damage through induction of inflammatory responses *via* activation of different transcription factors including activator protein-1

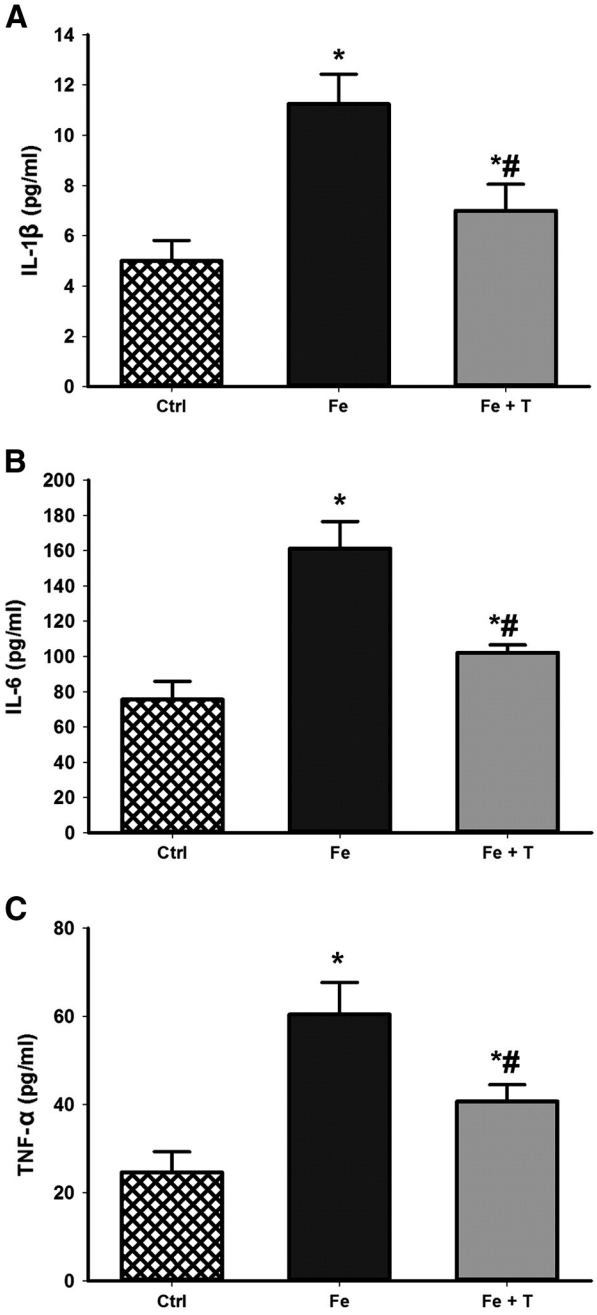

Fig. 4. Effect of iron supplementation on the levels of the proinflammatory cytokines (IL-1β, IL-6, and TNF-α) at high altitudes (6000 ft) and the mitigating effect of trolox. Levels of IL-1β, IL-6, and TNF-α were determined in lung tissue homogenates of different groups. (Ctrl) Normal control group received distilled water orally, once daily for 15 days. (Fe) Iron only-treated group received 2 mg elemental iron/kg, once daily for 15 days. (Fe + T) Iron and trolox-treated group received 2 mg elemental iron/kg, once daily for 15 days and trolox 25 mg/kg, once daily for the last 7 days of iron supplementation period. Data are expressed as mean ± standard deviation. (*) Significant difference from the normal control group at $p < 0.05$. (#) Significant difference from the iron only-treated group $p < 0.05$ (n = 8).

and nuclear factor-κB (Chow et al., 2003). Our results showed that the levels of the proinflammatory cytokines, IL-1β, IL-6, and TNF-α, are significantly elevated in the lung tissues in response to iron supplementation when compared to the normal control group (Fig. 4). It has been shown that ROS activates alveolar and interstitial macrophages to express the early response proinflammatory cytokines, IL-1 and TNF-α (Kunkel et al., 1997). Excessive release of early response proinflammatory cytokines triggers and intensifies the pulmonary inflammatory cascade (Sio et al., 2010). Early response proinflammatory cytokines can activate the lung endothelial cells, epithelial cells, and fibroblasts to produce chemokines which, in turn, attract inflammatory cells such as activated neutrophils (a major source of ROS), exacerbating tissue injury (Kunkel et al., 1997). Although proinflammatory cytokines intensify the inflammatory cascade and tissue injury, previously published data demonstrated that IL-1β, IL-6, and TNF-α promote hypoferremia, decreasing the availability of iron for participation in redox chemistry. This effect may diminish the iron-induced ROS-mediated tissue injury (Feelders et al., 1998; Goldblum et al., 1987; Weiss, 2005).

Co-administration of trolox, a water soluble vitamin E analog, alleviated the iron-induced histopathological changes to lung tissues of rats (Fig. 1C). In addition, trolox significantly decreased lipid peroxidation and protein carbonyl content (Fig. 2) and restored the levels of GSH and TAC (Fig. 3). It has been shown that trolox scavenges a variety of ROS including singlet oxygen, hydrogen peroxide, and hydroxyl radical (Hall et al., 2010). Because iron is a pro-oxidant and has been implicated in the generation of ROS that mediates the iron-induced oxidative tissue injury (Lee et al., 2006), trolox may protect against iron-induced oxidative stress through scavenging the iron generated ROS, reinforcing the oxidation as a possible underlining mechanism for iron-induced lung tissues injury. Trolox also significantly decreased levels of the proinflammatory cytokines, IL-1β, IL-6, and TNF-α (Fig. 4). It is worth noting that normal control rats (Fig. 1A) and rats treated only with trolox (data not shown) did not show any lung histopathological changes, indicating that 6000 ft altitude-associated oxidative stress is insufficient to induce significant lung tissue injury on its own. It is also worth mentioning that in iron and trolox treated group (Fe + T), trolox did not reduce the histopathological changes, the levels of the oxidative stress markers (except GSH and TAC), or the levels of the proinflammatory cytokines group to the control levels. A possible explanation is that, in our model, trolox was only taken for the last 7 days of iron supplementation period in a moderate dose, 25 mg/kg, once daily. Further study, thus, may be recommended to investigate the protective effect of longer treatment periods or higher doses of trolox.

Taken together, our results indicated that iron supplementation at high altitudes induced histopathological changes to lung tissues of rats that could be mediated through production of ROS and induction of inflammatory mediators. The current study highlights the possible limitations of iron supplementation at high altitudes and suggests trolox as a protective measure.

Conflict of interest statement

The authors have declared that there is no conflict of interest.

Acknowledgments

This work was supported by the High Altitude Research Center (HARC) at Taif University (8/M/1433 to SAS).

References

Abou-Zeid, A.H., Abdel-Fattah, M.M., Al-Shehri, A.S., Hifnawy, T.M., Al-Hassan, S.A., 2006. Anemia and nutritional status of schoolchildren living at Saudi high altitude area. Saudi Med. J. 27, 862–869.

Armutcu, F., Gurel, A., Aker, A., 2004. Serum iron concentrations, lipid peroxidation and superoxide dismutase activity in Turkish iron miners. Environ. Geochem. Health 26, 1–4.

Banchroft, J.D., Stevens, A., Turner, D.R. (Eds.), 1996. Theory and Practice of Histological Techniques. Churchill Livingstone New York, London, San Francisco, Tokyo.

Baron, V., Muriel, P., 1999. Role of glutathione, lipid peroxidation and antioxidants on acute bile-duct obstruction in the rat. Biochim. Biophys. Acta 1472, 173–180.

Berger, J., Aguayo, V.M., San Miguel, J.L., Lujan, C., Tellez, W., Traissac, P., 1997. Definition and prevalence of anemia in Bolivian women of childbearing age living at high altitudes: the effect of iron–folate supplementation. Nutr. Rev. 55, 247–256.

Bhandari, V., Elias, J.A., 2006. Cytokines in tolerance to hyperoxia-induced injury in the developing and adult lung. Free Radic. Biol. Med. 41, 4–18.

Braekke, K., Bechensteen, A.G., Halvorsen, B.L., Blomhoff, R., Haaland, K., Staff, A.C., 2007. Oxidative stress markers and antioxidant status after oral iron supplementation to very low birth weight infants. J. Pediatr. 151, 23–28.

Buege, J.A., Aust, S.D., 1978. Microsomal lipid peroxidation. Methods Enzymol. 52, 302–310.
Buijsse, B., Feskens, E.J., Moschandreas, J., Jansen, E.H., Jacobs Jr., D.R., Kafatos, A., Kok, F.J., Kromhout, D., 2007. Oxidative stress, and iron and antioxidant status in elderly men: differences between the Mediterranean south (Crete) and northern Europe (Zutphen). Eur. J. Cardiovasc. Prev. Rehabil. 14, 495–500.
Chandel, N.S., McClintock, D.S., Feliciano, C.E., Wood, T.M., Melendez, J.A., Rodriguez, A.M., Schumacker, P.T., 2000. Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1alpha during hypoxia: a mechanism of O_2 sensing. J. Biol. Chem. 275, 25130–25138.
Chang, S.W., Stelzner, T.J., Weil, J.V., Voelkel, N.F., 1989. Hypoxia increases plasma glutathione disulfide in rats. Lung 167, 269–276.
Chepelev, N.L., Willmore, W.G., 2011. Regulation of iron pathways in response to hypoxia. Free Radic. Biol. Med. 50, 645–666.
Chow, C.W., Herrera Abreu, M.T., Suzuki, T., Downey, G.P., 2003. Oxidative stress and acute lung injury. Am. J. Respir. Cell Mol. Biol. 29, 427–431.
Dosek, A., Ohno, H., Acs, Z., Taylor, A.W., Radak, Z., 2007. High altitude and oxidative stress. Respir. Physiol. Neurobiol. 158, 128–131.
Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82, 70–77.
Estrella, R., Hercberg, S., Maggy, G., Larreategui, J., Yepez, R., 1987. Evaluation of iron-deficiency anemia by an iron supplementation trial in children living at a 2,800-m altitude. Clin. Chim. Acta 164, 1–6.
Faura, J., Ramos, J., Reynafarje, C., English, E., Finne, P., Finch, C.A., 1969. Effect of altitude on erythropoiesis. Blood 33, 668–676.
Feelders, R.A., Vreugdenhil, G., Eggermont, A.M., Kuiper-Kramer, P.A., van Eijk, H.G., Swaak, A.J., 1998. Regulation of iron metabolism in the acute-phase response: interferon gamma and tumour necrosis factor alpha induce hypoferraemia, ferritin production and a decrease in circulating transferrin receptors in cancer patients. Eur. J. Clin. Invest. 28, 520–527.
Galaris, D., Pantopoulos, K., 2008. Oxidative stress and iron homeostasis: mechanistic and health aspects. Crit. Rev. Clin. Lab. Sci. 45, 1–23.
Galicia-Moreno, M., Rodriguez-Rivera, A., Reyes-Gordillo, K., Segovia, J., Shibayama, M., Tsutsumi, V., Vergara, P., Moreno, M.G., Fernandez-Martinez, E., Perez-Alvarez, V.M., Muriel, P., 2008. Trolox down-regulates transforming growth factor-beta and prevents experimental cirrhosis. Basic Clin. Pharmacol. Toxicol. 103, 476–481.
Ganz, T., 2003. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 102, 783–788.
Goldblum, S.E., Cohen, D.A., Jay, M., McClain, C.J., 1987. Interleukin 1-induced depression of iron and zinc: role of granulocytes and lactoferrin. Am. J. Physiol. 252, E27–E32.
Guzy, R.D., Mack, M.M., Schumacker, P.T., 2007. Mitochondrial complex III is required for hypoxia-induced ROS production and gene transcription in yeast. Antioxid. Redox Signal. 9, 1317–1328.
Hall, N.K., Chapman, T.M., Kim, H.J., Min, D.B., 2010. Antioxidant mechanisms of Trolox and ascorbic acid on the oxidation of riboflavin in milk under light. Food Chem. 118, 534–539.
Hawkins, C.L., Morgan, P.E., Davies, M.J., 2009. Quantification of protein modification by oxidants. Free Radic. Biol. Med. 46, 965–988.
Huff, R.L., Lawrence, J.H., Siri, W.E., Wasserman, L.R., Hennessy, T.G., 1951. Effects of changes in altitude on hematopoietic activity. Medicine 30, 197–217.
Jomova, K., Valko, M., 2011. Advances in metal-induced oxidative stress and human disease. Toxicology 283, 65–87.
Klimova, T., Chandel, N.S., 2008. Mitochondrial complex III regulates hypoxic activation of HIF. Cell Death Differ. 15, 660–666.
Kunkel, S.L., Lukacs, N.W., Chensue, S.W., Strieter, R.M., 1997. Chemokines and the inflammatory response. In: Remick, D.G., Friedland, J.S. (Eds.), Cytokines in Health and Disease. Dekker, New York, pp. 121–131.
Lang, J.D., McArdle, P.J., O'Reilly, P.J., Matalon, S., 2002. Oxidant–antioxidant balance in acute lung injury. Chest 122, 314S–320S.
Lee, D.W., Andersen, J.K., Kaur, D., 2006. Iron dysregulation and neurodegeneration: the molecular connection. Mol. Interv. 6, 89–97.
Maiti, P., Singh, S.B., Ilavazhagan, G., 2010. Nitric oxide system is involved in hypobaric hypoxia-induced oxidative stress in rat brain. Acta Histochem. 112, 222–232.
Mendes, J.F., Arruda, S.F., Siqueira, E.M., Ito, M.K., Silva, E.F., 2009. Iron status and oxidative stress biomarkers in adults: a preliminary study. Nutrition 25, 379–384.
Papanikolaou, G., Pantopoulos, K., 2005. Iron metabolism and toxicity. Toxicol. Appl. Pharmacol. 202, 199–211.
Peyssonnaux, C., Zinkernagel, A.S., Schuepbach, R.A., Rankin, E., Vaulont, S., Haase, V.H., Nizet, V., Johnson, R.S., 2007. Regulation of iron homeostasis by the hypoxia-inducible transcription factors (HIFs). J. Clin. Invest. 117, 1926–1932.
Reynafarje, C., Lozano, R., Valdivieso, J., 1959. The polycythemia of high altitudes: iron metabolism and related aspects. Blood 14, 433–455.
Ryan, T.P., Aust, S.D., 1992. The role of iron in oxygen-mediated toxicities. Crit. Rev. Toxicol. 22, 119–141.
Shah, Y.M., Matsubara, T., Ito, S., Yim, S.H., Gonzalez, F.J., 2009. Intestinal hypoxia-inducible transcription factors are essential for iron absorption following iron deficiency. Cell Metab. 9, 152–164.
Sinha, S., Dutta, A., Singh, S.N., Ray, U.S., 2010. Protein nitration, lipid peroxidation and DNA damage at high altitude in acclimatized lowlanders and native highlanders: relation with oxygen consumption. Respir. Physiol. Neurobiol. 171, 115–121.
Sio, S.W., Ang, S.F., Lu, J., Moochhala, S., Bhatia, M., 2010. Substance P upregulates cyclooxygenase-2 and prostaglandin E metabolite by activating ERK1/2 and NF-kappaB in a mouse model of burn-induced remote acute lung injury. J. Immunol. 185, 6265–6276.
Tkaczyk, J., Vizek, M., 2007. Oxidative stress in the lung tissue—sources of reactive oxygen species and antioxidant defence. Prague Med. Rep. 108, 105–114.
Valko, M., Rhodes, C.J., Moncol, J., Izakovic, M., Mazur, M., 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 160, 1–40.
Vij, A.G., Dutta, R., Satija, N.K., 2005. Acclimatization to oxidative stress at high altitude. High Alt. Med. Biol. 6, 301–310.
Weiss, G., 2005. Modification of iron regulation by the inflammatory response. Best Pract. Res. Clin. Haematol. 18, 183–201.
Welch, K.D., Davis, T.Z., Van Eden, M.E., Aust, S.D., 2002. Deleterious iron-mediated oxidation of biomolecules. Free Radic. Biol. Med. 32, 577–583.
West, J.B., 1995. Oxygen enrichment of room air to relieve the hypoxia of high altitude. Respir. Physiol. 99, 225–232.
West, J.B., 2004. The physiologic basis of high-altitude diseases. Ann. Intern. Med. 141, 789–800.