ORIGINAL RESEARCH

Oxidative stress and erythropoietin response in altitude exposure

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Abstract

Purpose: Oxidative stress and erythropoietin (EPO) levels are increased following high altitude exposure. We hypothesized that the altitude-oxidative stress and EPO response would be associated with the presence or absence of acute mountain sickness (AMS) in subjects exposed at high altitude.

Methods: The study enrolled 29 healthy volunteers exposed at altitudes without strenuous physical exercise. Oxidative stress was determined by the spectrophotometric measurement of the colour occurring during the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA) on blood samples. Ferritin and EPO were also measured simultaneously.

Results: During a rise in altitude at 2000 and 3000 m, there were no changes in plasma ferritin level in either of the 2 groups with or without AMS. In contrast, EPO increased at an altitude of 3000 m and after returning to sea level (28.2 ± 2.7 , 26.9 ± 3.3 vs 12.2 ± 1.4 and 17.1 ± 1.6 , $P < 0.05$, in group without AMS; 29.3 ± 4.5 , 22.8 ± 2.7 vs 10.6 ± 1.0 and

16.1 ± 1.5 , # $P < 0.05$, in group with AMS; compared with the baseline level and at the height of 2000 meters). At a height of 3000 m, plasma MDA level was elevated compared with that at the altitude of baseline and 2000 m in both groups of subjects with and without AMS (3.77 ± 0.29 vs 1.14 ± 0.17 , and 1.64 ± 0.22 , $P < 0.001$, in subjects with AMS; 3.65 ± 0.39 vs 1.71 ± 0.21 , and 1.73 ± 0.21 , $P < 0.001$, in subjects without AMS). After returning to sea level, subjects without AMS had lower MDA oxidative stress compared with those with AMS (2.58 ± 0.26 vs 3.51 ± 0.24 , $P = 0.0223$). Along with a rise in altitude, the oxidative stress in these both groups was not correlated with the changes in EPO ($r^2 = 0.0728$, $P = 0.1096$).

Conclusion: High altitude-induced oxidative stress, detected by MDA assay, is not different between the two groups of subjects with and without AMS. Upon return to sea level, subjects without AMS had lower MDA oxidative stress burden and higher EPO level than those with AMS. Whether the subjects with altitude illness had delayed recovery from oxidative stress merits further investigation.

High-altitude illness, as a result of free-radical-mediated inflammatory injury, has an impact on the increase in capillary permeability.^{1,2} Oxidative stress in subjects exposed to high altitude would be associated with hypoxia and ischemia-reperfusion injury.³ This damage to the biological system influences immune function and enhances susceptibility to infection and/or delays recovery from altitude illness.⁴ High-altitude illness occurs in subjects who travel too high, too fast, and presents with headache, nausea, anorexia, fatigue, lassitude, and even death.⁵ Increased oxidative stress and reduced antioxidant defense were observed in animals, with exposure to a simulated milieu of high altitude.⁶ In addition, localized free radical generation caused by skeletal muscle damage may mediate vascular damage of the blood-brain barrier and contribute to the pathophysiology of acute mountain sickness (AMS).^{7,8} Furthermore, the erythropoietin (EPO) response to hypoxia was increased by altitude acclimatization.⁹ Thus, we hypothesized that the altitude-oxidative stress and EPO response would be associated with the presence or absence of AMS in subjects at high altitude. The present study was designed to compare nonspecific clinical symptoms at altitude with the changes in oxidative stress. To explore the dynamics of free radical generation, we included 29 healthy subjects exposed to altitude without strenuous physical exercise. The association of oxidative stress and the EPO response was also investigated in subjects with and without AMS.

Materials and methods

Subjects

The research was carried out according to the principles of the Declaration of Helsinki and was approved by the Human and Ethics Committee of Taipei Veteran General Hospital. Informed consent from each individual was obtained. We enrolled 29 healthy volunteers. None had received oral antioxidants or had clinical evidence of hepatitis B or C, systemic diseases, immunological disorder, or active infection.

The survey of acute mountain sickness was based on the Lake Louise Score (LLS) included the standardized Lake Louise Self-report Questionnaire.

Blood collection and plasma preparation

To avoid the effect of strenuous physical exercise on the generation of free radicals, subjects spent about 8 hours car-driving each day, during the experimental period of 3 days. Baseline blood samples were obtained using heparinized syringes. After arriving at an altitude of 2000 and 3000 m, they stayed at that altitude for two hours prior to testing. The blood, collected as previously described, was transferred to 2-mL Eppendorf tubes, and was separated by centrifugation at 4,500 rpm for 10 minutes. Plasma was collected and stored immediately at 4°C for subsequent biochemistry and malonaldehyde (MDA) assays.

Measurement of malonaldehyde

The principle of the method was based on the spectrophotometric measurement of the colour change during the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA); forming a MDA-TBA₂ complex that absorbs strongly at 532 nm. The concentration of TBA reactive substances was calculated by the absorbance coefficient of MDA-TBA complex and expressed in nmol/mL. In brief, 2,6-Di-tert-butyl-4-methylphenol (BHT) 0.25 g was prepared to a final 50 mL of ethanol. 1 mL plasma sample was added to 0.25 mL BHT solution, and was then mixed with 1 mL 20% trichloroacetic acid. The mixture was centrifuged at 13000 rpm for 5 min, 1.2 mL of the supernatant was transferred to another Eppendorf tube, followed by adding 0.5 mL of TBA. The mixture was heated in boiling water for 15 min, transferred to a disposable cuvette (Hellma 104-QS, German, 1400 µL) and left to stand at room temperature. MDA alone was used as the blank solution in each experiment. Absorbance, at a wavelength of 532 nm, was measured by spectrophotometry after the testing samples cooled.

TABLE. Characteristics of General and Biochemical Data in the Included Subjects

	Subjects without acute mountain sickness (n = 19)	Subjects with acute mountain sickness (n = 10)
Age (y)	49.3 ± 2.4	49.2 ± 3.1
Male/female	8/11	3/7
LLS	1.3±0.3	6.6 ± 0.5*
ΔEPO (μg/dL)	11.1±1.6	12.2±3.8
ΔFerritin (ng/mL) [§]	21.2± 7.8	10.9 ± 10.8

Values are expressed as mean ± SEM.

[§] Mean changes, from baseline, of ferritin level in response to altitude of 3000 m

LLS: Lake Louis Score; EPO, erythropoietin. * $P < 0.05$.

Statistical analysis

Experimental data are reported as mean ± SEM. $P < 0.05$ was considered statistically significant. The difference between the 2 groups was determined by Student's unpaired t test. Standard regression analysis and Pearson r correlation coefficients were used to determine the relationships between MDA and EPO/ferritin concentration.

Results

The table shows the general and biochemical data of subjects with and without acute mountain sickness. ΔEPO and ΔFerritin indicate the mean differences of EPO and ferritin levels detected between the baseline and at an altitude of 3000 m. Subjects fulfilling the criteria of AMS presented with headache, all at the altitude of 3000 m. There were no differences in ΔEPO and ΔFerritin between these 2 groups.

Changes in EPO and Ferritin during increase in altitude

During the study periods, plasma ferritin level was relatively stable without differences in all studied subjects (Fig 1A and 1B). Response to a rise in altitude at 3000 m, the change of mean plasma ferritin level showed insignificant increases in both 2 groups with and without AMS (Table). In contrast, plasma EPO

FIGURE 1. (A) Time course of mean plasma ferritin levels with and without AMS. (n=10, with AMS; n=19, without AMS). (B) Changes of ferritin from baseline to altitude of 3500 m. (n= 10, with AMS; n= 19 without AMS).

increased at an altitude of 3000 m and after returning to sea level (Fig 2; * $P < 0.05$, in group without AMS, 28.2 ± 2.7 , 26.9 ± 3.3 vs 12.2 ± 1.6 and 17.1 ± 1.6 ; # $P < 0.05$, in group with AMS, 29.3 ± 4.5 , 22.8 ± 2.7 vs 10.6 ± 1.0 and 16.1 ± 1.5 ; compared with the baseline level and at a height of 2000 m).

FIGURE 2. Time course of mean plasma EPO with and without AMS. (n=10, with AMS; n=19, without AMS).

FIGURE 3. Time course of plasma MDA with and without AMS. (n=10 with AMS; n=19 without AMS; $\tau P=0.0223$, compared with return to sea level; *,# $P<0.001$, with and without AMS respectively, compared with baseline and 2000 m).

Changes in MDA concentration during increase in altitude

Figure 3 illustrates the dynamic curve of plasma MDA level, expressed as the value of OD₅₃₂. At a height of 3000 m, plasma MDA level was elevated compared with that at baseline and 2000 m altitudes in both groups of subjects with and without AMS (3.77 ± 0.29 vs 1.14 ± 0.17 , and 1.64 ± 0.22 , $P<0.001$, in subjects with AMS; 3.65 ± 0.39 vs 1.71 ± 0.21 , and 1.73 ± 0.21 , $P<0.001$, in subjects without AMS). After returning

FIGURE 4. Correlation between the plasma MDA and EPO. ($r^2=0.0728$, $P=0.1096$) (n= 29).

to sea level, plasma MDA level decreased in subjects without AMS, but not in those with AMS in comparison with that at altitude of 3000 m (2.58 ± 0.26 vs 3.65 ± 0.39 , $P<0.05$, in subjects without AMS; 3.51 ± 0.24 vs 3.77 ± 0.29 , $P>0.05$, in subjects with MS). In addition, subjects without AMS had lower MDA oxidative stress compared with those with AMS (2.58 ± 0.26 vs 3.51 ± 0.24 , $P=0.0223$). Along with a rise in altitude, the MDA oxidative stress of the subjects was not correlated with changes in EPO ($r^2 = 0.0728$, $P = 0.1096$; Fig 4), and ferritin ($r = -0.008545$, $P = 0.9275$).

Discussion

High altitude oxidative stress, related to hypoxia, may link the development of the mountain sickness symptoms.^{5,10,11} In our study, the oxidative stress of plasma MDA, in groups with and without AMS, had similar increases during the time spent at altitude. In the experimental periods, there were no changes in hematocrit (data not shown) or ferritin level between the two groups. However, the EPO level had increased from baseline on the 2nd day after ascent to and staying at an altitude of 3000 m. The EPO responses to high altitude were not correlated with MDA levels. After returning to sea level, MDA decreased towards

baseline in subjects without AMS, but remained steady in subjects with AMS.

In response to high-altitude hypoxia, endogenous antioxidants and F2-isoprostanes level (products of lipid peroxidation) were elevated.¹¹ Other studies have demonstrated that subjects with AMS have a marked increase in inflammatory cytokine, but no differences in oxidative stress of F2-isoprostanes level compared with those without AMS.^{12,13} Our study showed similar results that oxidative stress in plasma MDA had increased in both groups with and without AMS, during exposure to high altitude. Hypoxia increases the production of EPO. We have characterized the time course of the EPO response in our study. Severe hypoxia provides a sufficient stimulus to increase EPO production within 4 h.¹⁴ On ascent to 2000 m, the EPO response to hypoxia was modified and increased within 48 hours.^{9,15} EPO attenuates apoptosis and has protective effects against ischemia-reperfusion injury via a free radical scavenger's modulator and anti-inflammatory mediator.¹⁶⁻¹⁸ Our study has shown the EPO level is increased at the 2nd day at 3000 m. After returning to sea level, subjects without AMS had relatively lower MDA and higher EPO levels than those with AMS. This indicates that EPO may protect subjects from altitude illness. In response to hypoxic-ischemic preconditioning, EPO effect could stable free radical metabolism, enhance aerobic performance and attenuate the oxidative stress.¹⁹⁻²¹

At high altitude, hypoxia might induce an inflammatory response with moderate systemic increases of these inflammatory markers.^{13,22} Inflammation and exposure to oxidative stress are believed to increase ferritin synthesis.²³ Ferritin, an iron-storage and acute-phase reactive protein, could also contribute to promote an increase in oxygen radical intermediates through Fenton complex.^{24,25} In our study, no changes of ferritin levels were noted despite the increase in MDA at altitude. There was no correlation between headache prevalence of AMS and ferritin, consistent with the previous report.²⁶ Therefore, analysis of ferritin could not account for altitude-

related oxidative stress. Without any change of iron intake at altitude, a dramatic decrease in ferritin level has been reported.^{9,27} The different duration of exposure to high altitude in these previous studies may cause such discrepancy.

High altitude may promote free radical generation. Our results showed that high altitude-induced oxidative stress, detected by MDA assay, is not different between groups with and without AMS. On return to sea level, the oxidative stress burden of MDA decreased to the baseline level in subjects without AMS, but remained nearly steady in subjects with AMS. However, EPO level had the converse change with MDA. Whether the subjects with altitude illness exhibit delayed recovery from oxidative stress merits further investigation.

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