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Review article

HIGH ALTITUDE AND FREE RADICALS

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ABSTRACT

High altitude exposure results in decreased oxygen pressure and an increased formation of reactive oxygen and nitrogen species (RONS), which is often associated with increases in oxidative damage to lipids, proteins and DNA. Exposure to high altitude appears to decrease the activity and effectiveness of antioxidant enzymes system. Moreover, during high altitude exposure several RONS generating source are activated, including mitochondrial electron transport chain, xanthine oxidase, and nitric oxide synthase (NO). Physical exercise at high altitude can further enhance the oxidative stress. The available information suggests that RONS are involved and are even a causative factor of acute mountain sickness. Supplementation of antioxidant seems to be a necessary step to prevent or decrease to high altitude exposure associated oxidative stress.

KEY WORDS: High altitude, reactive oxygen and nitrogen species, oxidative stress, oxidative damage, antioxidants, acute mountain sickness.

INTRODUCTION

Generation of reactive oxygen and nitrogen species (RONS) is a necessary consequence of aerobe metabolism. RONS are natural and physiological modulators of cellular redox milieu and thereby signaling, controlling factors of a wide range of known and unknown physiological, patho-physiological processes. Despite of the multi line antioxidant system, the level of RONS generation can exceed the capability of defense network, leading to oxidative stress (Askew, 2002). It is generally assumed that increases in aerobic metabolism or hyperoxia easily generates increased level of RONS and cause oxidative damage to lipids, proteins and DNA. Indeed, physical exercise, especially a single bout of exercise above a certain intensity or duration can result oxidative challenge and damage to different organs (Radak et al., 2001). However, it appears that the increased level of RONS production is not only due to the mitochondrial respiration, because anaerobic exercise also could cause oxidative damage (Radak et al., 1998). Moreover protection of endothelium by exogenous superoxide dismutase (SOD) prevented both the oxidative damage to lipids and xanthine oxidase activity, indicating that exercise-associated RONS production occurs by variety of sources and mechanism.

Similarly to anaerobic physical exercise exposure to high altitude often result in oxidative damage to macromolecules. Low oxygen pressure seems to be favorable to low RONS production, but it appears that high altitude exposure associated with increased oxidative damage, which could be the consequence of the increased activity of RONS generating and decreased activity of antioxidant systems. Moreover, according to our current understanding it cannot be ruled out the RONS are involved and maybe even play a causative role in the acute mountain sickness (AMS), high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE) (Bailey et al., 2001, Baumgartner et al., 2002, Chao et al., 1999). The present review will draw upon the available literature on high altitude, exercise and high altitude and oxidative stress.

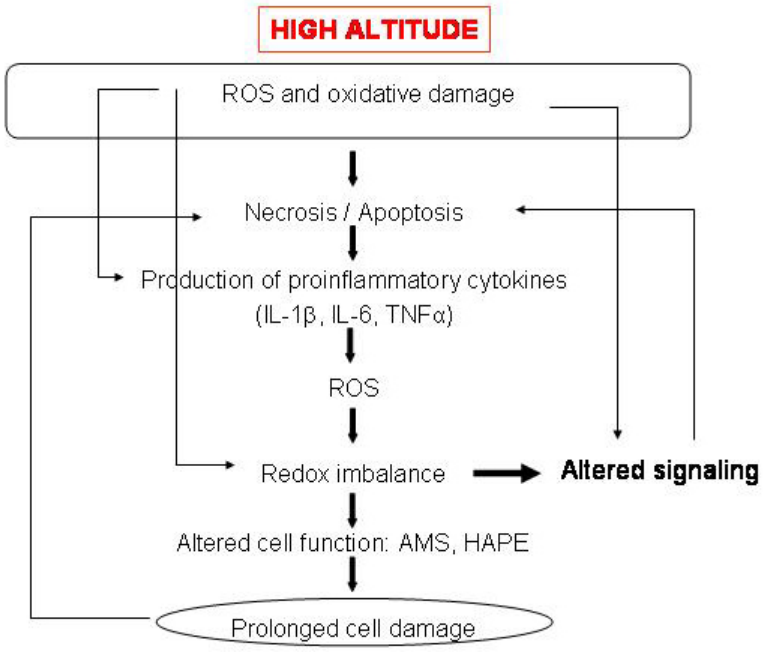

Figure 1. Possible mechanisms of HA induced inflammation.

High altitude and oxidative damage

In one of our study, we have used intermittent exposure (12 hr in every day) to an altitude of 4000m to study the muscle fiber type dependent changes in the activity and content of antioxidant enzymes and the level of lipid peroxidation (Radak et al., 1994). Our data revealed that the intermittent exposure to high altitude resulted in significant increase in lipid peroxidation in both, slow and fast type of muscle fibers of rats. Interestingly, when we applied 4 wk of continuous exposure to the same altitude, we did not measure increase lipid peroxidation, but the level of protein oxidation, measured by carbonyl derivatives was increased (Radak et al., 1997). Kumar et al. (1989) have found the short exposure (5 days) to an altitude of 7576 m caused increased lipid peroxidation level in plasma of rats. This result was confirmed by the same experimental protocol adding vitamin E supplanted groups (Ilavazhagan et al., 2001). Moreover, Nakanishi and co-workers (1995) reported that exposure to 5500m result in increased level of malondialdehyde in serum, lung, liver, heart and kidney.

Human studies revealed similar results. Moller et al., (2001) exposed twelve healthy subjects to an altitude of 4559 m, which caused a significant increase in DNA strand breaks, measured from urine. The damage was more prominent at the endonuclease-III sites. When humans were exposed simultaneously to high altitude (2700m) and cold exposure the level of urinary lipid peroxidation, DNA damage increased significantly (Schmidt et al., 2002). At the study of Operation Everest III the level of lipid peroxidation increased by 23% at 6000m, and by 79% at the altitude of 8848m indicating that the level of oxidative stress is parallel with the increase in altitude (Joanny et al., 2001). Thus, both human and animal studies are relatively consequently reporting that high altitude associated hypoxia is causing oxidative damage to lipids, proteins and DNA. This damage can be due to the increased level of ROS production and/or decreased level of antioxidant capacity.

The effect of high altitude on antioxidant systems

Aerobic cells developed enzymatic and non-enzymatic antioxidant system to regulate the effects of RONS. The enzymatic system contains mitochondrial (Mn-SOD), cytosolic (Cu,Zn-SOD, and extra-cellular SOD to convert reactive superoxide to less powerful hydrogen peroxide. Glutathione peroxidase (GPX) and catalase decompose hydrogen peroxide to water. Other enzymes, like thioredoxin and glutaredoxin systems are not discussed, since data are not available in relation to high altitude. The nonenzymatic system is very complex and many non-enzymatic antioxidants exist in cells. High altitude related studies measured the content glutathione, vitamin E, and vitamin C among the nonenzymatic antioxidant, therefore these agents are discussed in the present review.

There are only a few studies which examined the level of antioxidant enzyme capacity at high altitude. We have reported that 6 month of intermittent exposure to high altitude (4000m) resulted in decreased activity and protein content of mitochondrial SOD in skeletal muscle of rats (Radak

et al., 1994). This was confirmed by Nakanishi et al (1995), who have found that 5500m simulated altitude increased the level immunoreactive Mn-SOD in the serum and decreased it in liver and lung of the animals. The activity of glutathione peroxidase (GPX) also decreased in liver suggesting that liver might especially sensitive to high altitude induced oxidative stress (Nakanishi et al., 1995). In our other study we could not detect significant effect of 4 wk exposure to 4000m on the activities of antioxidant enzymes (Radak et al. 1997). Imai et al. (1995) compared the activity of GPX in serum of native highlanders (4000m) and subjects from sea level. They have found that people from high altitude had lower level of GPX activity. The activity and effectiveness of GPX is strongly dependent upon state of thiol system. Glutamyl-cysteinyl-glycine, is one of the main thiol/ antioxidant source of the cell, which continuously synthesized by glutamyl cycle. High altitude exposure decreases the level of reduced glutathione (GSH) and increase oxidized glutathione concentration (Ilvazhagan et al., 2001, Joanny et al., 2001).

Thus, it appears that the capacity of enzymatic and non-enzymatic antioxidant systems is somewhat decreasing at high altitude. There are trials to prevent the high altitude associated oxidative damage by supplementation of antioxidants. Schmidt et al., (2002) have applied an antioxidant mixture containing vitamin E, beta-carotene, ascorbic acid, selenium, alpha-lipoic acid, N-acetyl 1-cysteine, catechin, lutein, and lycopene to reduce oxidative stress caused by altitude. This mixture was effective and the level of oxidative damage was reduced.

Supplementation of vitamin E (40 mg per rat·day^{-1}) orally, 5 days prior to and during the period of hypoxic exposure of 7,576m to rats, significantly reduced the high altitude-induced increase in lipid peroxidation (Ilvazhagan et al., 2001). On the other hand, the antioxidant supplement mixture containing, 20,000 IU beta-carotene, 400 IU vitamin E, 500 mg vitamin C, 100 micrograms selenium, and 30 mg zinc, (in a daily base) did not prevented the oxidative damage of macromolecules (Pfeiffer et al., 1999).

A very short exposure to rats to an altitude of 8000 m resulted in increased melatonin level in the blood (Kaur et al., 2002). Melatonin besides a wide range of effects can act as an antioxidant. After the first 4 days following the exposure, the mitochondrial number and lipid droplets in the pinealocytes appeared to be reduced compared with those in control rats suggesting another source besides pinealocytes also produce melatonin.

It appears that exposure to high altitude decrease the activity and content of some antioxidant enzymes. Moreover, the effectiveness of thiol system is also reduced by high altitude. There are some indications that antioxidant supplementation reduces or prevents the high altitude induced oxidative damage to macromolecules.

RONS generating systems at high altitude

It is well demonstrated that massive oxygen supply results in increased formation in mitochondrial ROS production. However, it also appears that hypoxia can lead to reductive stress, which also results in increased ROS production by the mitochondrial electron transport system (Mohanraj et al., 1998). This is believed that ROS is generated at complex I and complex III of the electron transport chain. During hypoxia, less O_2 is available to be reduced to H_2O at cytochrome oxidase, causing accumulation of reducing equivalents within the mitochondrial respiratory sequence. This called as reductive stress, which leads to ROS formation by the auto-oxidation of one or more mitochondrial complexes such as the ubiquinone-ubiquinol redox couple. Khan and O Brien (1995) demonstrated increases in the cellular $NADH/NAD^+$ ratio during hypoxia associated reductive stress.

The xanthine dehydrogenase/oxidase system is a potent ROS generator during hypoxia/reperfusion conditions. Intermittent exposure to high altitude has similar characteristics than ischemia/reperfusion (Radak et al., 1994). On the other hand the changing pattern of ROS and nitric oxide (NO) is different during ischemia/reperfussion and exposure to high altitude. During ischemia/reperfusion the initial response is accompanied by a reversible increase in the generation of ROS and is blocked by antioxidants and by interventions that increase the tissue levels of NO. In contrast to ischemia/ reperfusion, ROS levels increase during hypoxia and return towards pre-hypoxic values after return to normoxia. Acclimatization involves up-regulation of inducible NO synthase (iNOS), suggesting that hypoxia leads to an alteration of the ROS/NO balance which is eventually restored during the acclimatization process (Gonzalez and Wood, 2001). This phenomenon may have relevance to the microcirculatory alterations associated with hypoxic exposure, including acute mountain sickness and high altitude pulmonary and cerebral edema. The findings of Serrano et al. (2002) indicates that the involvement of different type of NOS is different in NO production during high altitude, which can lead to increased formation of nitrotyrosine level in rat cerebellum after reoxygenation to sea level. It is well known that the UV radiation is significantly

increasing at high altitude, resulting in enhanced formation of RONS.

Accordingly to our current understanding it seems that high altitude associated increase in ROS generation is due to different sources, including mitochondrial respiratory chain, xanthine oxidase, and iNOS.

High altitude and exercise

High altitude training is often used by athletes to increase the number of red blood cells, which is believed to increase endurance performance. However, the oxidative stress related consequence of high altitude training is poorly known. It is well accepted that physical exercise increases the oxygen uptake and flux into the mitochondria and after a certain intensity and/or duration can lead to oxidative stress. It was also demonstrated that not only aerobic, but anaerobic exercise as well can lead to oxidative damage (reviewed by Radak et al., 2001). It is suggested that during anaerobic condition XO is one major source of ROS generation (Radak et al., 1995). The available data suggests both high altitude exposure and exercise alone could result in oxidative challenge and shift the redox state of cells. Therefore it is not surprising that the combined effects of high altitude and exercise could result in oxidative damage. We have demonstrated that training at altitude of 4000m resulted in increased carbonylation of certain muscular proteins, most probably including actin, which is major contractile protein (Radak et al., 1997). We have suggested that exercise escalates effects of altitude on ROS production and weakens the power of antioxidant system. This hypothesis was confirmed by human studies as well (Wozniak et al., 2001). Moller et al. (2001) concluded that hypoxia undermines the capacity of antioxidant system and reduce the body capacity to withstand oxidative stress produced by exhaustive exercise. Joanny et al. (2001) data further support this suggestion and points out the importance of antioxidant supplementation for individuals engaged with exercise at high altitude.

Increased physical activity at high altitude is increases the vulnerability of body to oxidative stress and can lead to oxidative damage. Therefore, antioxidant supplementation seems to be an important and natural tool to reduce the high altitude and exercise induced oxidative stress.

Acute mountain sickness (AMS) and RONS

Our current understanding about AMS is still far from being complete. The most common symptoms of AMS are headache, nausea, anorexia, insomnia, fatigue/lassitude, vomiting and dizziness. Many physiological events associated with the pathophysiology of AMS have been documented, included relative hypoventilation, impaired gas exchange (interstitial pulmonary edema) fluid retention and redistribution, and increased sympathetic drive (reviewed Hackett, 1999). In contrast, increased intracranial pressure and cerebral edema are documented in moderate to severe AMS, reflecting the continuum from AMS to HACE. In the development of HACE elevated cerebral capillary pressure occurs altering the function of brain blood barrier (BBB) producing brain edema. It appears that free radicals (e.g. oxygen and hydroxyl radicals), bradykinin, histamine, arachidonic acid and NO could be involved in the alteration of BBB (Schilling and Wahl, 1999).

Indeed there are some implications that RONS are involved and even are the causative factor of AMS (Bailey and Davies, 2001). HAPE, a potentially fatal clinical condition, represents a serious complication of AMS. HAPE is an increase in capillary permeability, which could occur as a result of an inflammatory reaction and/or free radical-mediated injury to the lung (Figure 1). Upon the findings of their study, Kleger et al. (1996) suggested that the inflammatory reaction, which was associated with HAPE, was rather a consequence than a causative factor of high-altitude pulmonary edema (Kleger et al., 1996). But, NO inhalation was used with a success to soften or curbed the symptoms of HAPE (Anand et al., 1998) and this observation suggests that NO play a causative role. The beneficial effects of NO inhalation was also nicely demonstrated on rat model, in which the mortality rate of control rats was 39.5% and just 6.2% in the NO treated group (Omura et al., 2000). Therefore it is hypothesized that susceptibility to HAPE may be related to decreased production of NO, an endogenous modulator of pulmonary vascular resistance, and that a decrease in exhaled NO could be detected during hypoxic exposure. Since, an exaggerated hypoxic pulmonary vasoconstriction is essential for development of HAPE.

Despite of our limited knowledge about AMS, the available information suggests that RONS are active players in the process, however it still not clear whether they are causative or associative agents.

CONCLUSIONS

Exposure to high altitude disrupts the efficiency of antioxidant system and due to the increased level of RONS production can lead to oxidative damage to macromolecules. Physical exercise can exacerbate

the effects of high altitude and further can increase the related oxidative stress. Antioxidant supplementation has been shown to have beneficial effects and can attenuate and/or prevent the oxidative damage associated with high altitude and exercise at altitude. It cannot be excluded that RONS are involved in the development of AMS, and especially NO seems to play an important role.

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KEY POINTS

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- High altitude-induced oxidative stress
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