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ORIGINAL ARTICLE

Attenuating systemic inflammatory markers in simulated high-altitude exposure by heat shock protein 70-mediated hypobaric hypoxia preconditioning in rats

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multiple organ dysfunction syndrome

Background/Purpose: The primary goal of this study was to test whether high-altitude exposure (HAE: 0.9% O_2 at 0.47 ATA for 24 hours) was capable of increasing the systemic inflammatory markers as well as the toxic organ injury indicators in rats, with a secondary goal to test whether preinduction of heat shock protein (HSP) 70 by hypobaric hypoxia preconditioning (HHP: 18.3% O_2 at 0.66 ATA for 5 h/day on 5 days consecutively for 2 weeks) attenuated the proposed increased serum levels of both the systemic inflammatory markers and the toxic organ injury indicators.

Methods: Rats were assigned to: (1) non-HHP (21% O_2 at 1.0 ATA)+non-HAE (21% O_2 at 1.0 ATA) group; (2) non-HHP+HAE group; (3) HHP+non-HAE group; (4) HHP+HAE group; and (5) HHP+HSP70 antibodies (Ab)+HAE group. For the HSP70Ab group, a neutralizing HSP70Ab was injected intravenously at 24 hours prior to HAE. All the physiological and biochemical parameters were obtained at the end of HAE or the equivalent time period of non-HAE. Blood samples were obtained for determination of both the systemic inflammatory markers (e.g., serum tumor necrosis factor-α, interleukin-1β, E-selectin, intercellular adhesion molecule-1, and liver myeloperoxidase activity) and the toxic organ injury indicators (e.g., nitric oxide metabolites, 2,3-dihydroxybenzoic acid, and lactate dehydrogenase).

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Results: HHP, in addition to inducing overexpression of tissue HSP70, significantly attenuated the HAE-induced hypotension, bradycardia, hypoxia, acidosis, and increased tissue levels of both the systemic inflammatory markers and the toxic organ injury indicators. The beneficial effects of HHP in inducing tissue overexpression of HSP70 as well as in preventing the HAE-induced increased levels of the systemic inflammatory markers and the toxic organ injury indicators could be significantly reduced by HSP70Ab preconditioning.
Conclusion: These results suggest that HHP may downgrade both the systemic inflammatory markers and the toxic organ injury indicators in HAE by upregulating tissue HSP70.

Introduction

Hypoxia encountered at high-altitude exposure (HAE) is associated with acute mountain sickness, high-altitude pulmonary edema (HAPE), and high-altitude cerebral edema.[1] People who travel to high altitude are commonly confronted with problems such as mental dysfunction and memory deficit,[2] insomnia, dizziness, nausea,[3] hypophagia[4] and motor impairment.[5] The HAE-induced acute pulmonary edema is thought to be related to increased sympathetic tone, exaggerated hypoxic pulmonary vasoconstriction, decreased hypoxic ventilator drive, increased pulmonary capillary pressure, failure of pulmonary capillaries, and alveolar fluid leak across capillary endothelium.[6] In addition, high-altitude pulmonary hypertension is associated with a free radical-mediated reduction in pulmonary nitric oxide bioavailability.[7] In addition to the hydrostatic stress, increased alveolar—capillary permeability caused by inflammation may be necessary or causal. For example, Schoene and colleagues[8,9] found that neutrophils and elevated concentrations of plasma proteins, thromboxane metabolites, and proinflammatory cytokines in bronchoalveolar lavage fluid were observed in patients with well-established HAE. In animals, severe hypoxia (0—3% oxygen) stimulates vascular endothelial cells, leukocytes and macrophages *in vitro* to release proinflammatory cytokines.[10—13] In rats, a simulated HAE (0.9% O_2 at 0.47 ATA for 24 hours) caused pulmonary edema, inflammation, and hemorrhage[14,15] as well as brain edema, hippocampal oxidative stress, and cognitive dysfunction.[16]

It has been well documented that the pathogenesis of multiple organ dysfunction syndrome (MODS) in septic shock is related to tissue production and release of the systemic inflammatory markers including proinflammatory cytokines interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and myeloperoxidase and the toxic organ injury indicators nitric oxide metabolites (NO_x^-), dihydroxybenzoic acid (DHBA), and lactate dehydrogenase (LDH) in rats.[17,18] It is not known whether HAE, like toxic septic shock, is able to cause elevation of both the systemic inflammatory markers and the organ injury indicators.

Hypobaric hypoxia preconditing (HHP) is known to increase blood hemoglobin and tissue oxygen delivery[19,20] and to increase the neuronal resistance to subsequent severe hypoxia.[21,22] More recently, we observed that HHP [18.3% O_2 at 0.66 ATA (atmosphere absolute) for 5 h/day on 5 days consecutively for 2 weeks] in rats, in addition to inducing lung overexpression of HSP70, significantly attenuated the HAE-induced pulmonary edema, inflammation, and ischemic and oxidative damage in rats.[15] Again, it is unknown whether the proposed HAE-induced increased systemic inflammatory markers and the toxic organ injury indicator can be affected by HSP70-mediated HHP.

Therefore, the present study was first to assess the changes of the tissue levels of the systemic inflammatory markers, the organ injury indicators, the cardiovascular parameters, and the blood gas and acid—base parameters during HAE in rats without or with HHP. Then, the secondary aim was to determine whether the proposed beneficial effect of HHP in reducing the systemic inflammatory markers and the toxic organ injury indicators during HAE is caused by the preinduction of HSP70 prior to the onset of HAE.

Materials and methods

Animals

Adult Sprague—Dawley rats (weight 254 $\pm$ 12 g) were obtained from the Animal Resource Center of the National Science Council of the Republic of China (Taipei, Taiwan). The animals were housed four in a group at an ambient temperature of 22 $\pm$ 1°C, with a 12-hour light/dark cycle. Pellet rat chow and tap water were available *ad libitum*. All protocols were approved by the Animals Ethics Committee of the Chi Mei Medical Center (Tainan, Taiwan) in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act (IACUC Approval No: 100052305). At the end of the experiments, control rats and any rats that had survived HAE were killed with an overdose of sodium pentobarbital.

Experimental groups and hypobaric hypoxia preconditioning (HHP)

Rats were randomly assigned to one of the following five groups: (a) the (non-HHP+normobaric air (NBA)) group: animals were treated with non-HHP or NBA (21% O_2 at 1.0 ATA) for 2 weeks plus NBA; (b) the (non-HHP+HAE) group: animals were treated with non-HHP or NBA for 2 weeks plus HAE (9.7% O_2 at 0.47 ATA); (c) the (HHP+NBA) group: animals were treated with HHP [13.9% O_2 at 0.66 ATA (atmosphere absolute) for 5 h/day on 5 days consecutively for 2 weeks] plus NBA; (d) the (HHP+HAE) group: animals were treated with HHP plus HAE; and (e) the