Pathophysiological significance of peroxidative stress, neuronal damage, and membrane permeability in acute mountain sickness

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Bailey, Damian M., Gian-Reto Kleger, Manfred Holzgrafe, Peter E. Ballmer, and Peter Bärsch. Pathophysiological significance of peroxidative stress, neuronal damage, and membrane permeability in acute mountain sickness. J Appl Physiol 96: 1459–1463, 2004. First published October 31, 2003; 10.1152/japplphysiol.00704.2003.—Free radical-mediated changes in vascular permeability and subsequent inflammatory response may be a contributor pathogenetic cofactor responsible for the development of neurological sequelae associated with acute mountain sickness (AMS). To investigate this, 49 subjects were examined at sea level and serially after rapid ascent to 4,559 m. Although the venous concentration of total creatine phosphokinase activity was measured in all subjects, a complementary examination of lipid peroxidation (F2-isoprostanes), inflammatory (TNF-α, IL-1β, IL-2, IL-6, IL-8, C-reactive protein), and cerebrovascular tissue damage (neuron-specific enolase) biomarkers was confined to a subgroup of 24 subjects. A selective increase (P < 0.05) in total creatine phosphokinase was observed in subjects diagnosed with AMS at high altitude (n = 25) compared with apparently healthy controls (n = 24). However, despite a marked increase in IL-6 and C-reactive protein attributable primarily to subjects developing high-altitude pulmonary edema, subgroup analyses demonstrated no selective differences in F2-isoprostanes, neuron-specific enolase, or remaining proinflammatory cytokines due to AMS (n = 14). The present findings are the first to demonstrate that free radical-mediated neuronal damage of sufficient degree to be detected in the peripheral circulation does not occur and is, therefore, unlikely to be an important, initiating event that is critical for the development of AMS. The pathophysiological significance of increased sarcosomal membrane permeability and inflammatory response, either as a cause or epiphenomenon of AMS and/or high-altitude pulmonary edema, remains to be elucidated.

Acute mountain sickness (AMS) is a cerebral syndrome characterized by nonspecific symptoms that can develop in otherwise healthy individuals within 6–10 h of rapid ascent to altitudes above 2,500 m (13). Despite its prevalence and associated morbidity, a complete understanding of related mechanisms remains elusive due to the lack of prospective studies examining the illness early in its evolution, technical difficulties encountered when attempting to differentiate between hemodynamic and permeability phenomena, and underlying complexity associated with the illness (13). Recent neuroimaging studies implicate extracellular vasogenic cerebral edema as the predominant mechanism operant in high-altitude cerebral edema and arguably moderate to severe AMS (14), although the precise mechanisms responsible for disruption of the blood-brain barrier (BBB) remain largely unresolved.

Although much attention has focused on hemodynamic factors, emerging evidence also suggests a pathogenic role for free radicals. AMS appears to be associated with a peripheral increase in peroxidation footprints and skeletal tissue damage (3) with clear neuroprotective benefits conferred by antioxidant prophylaxis (1). It is conceivable, therefore, to suggest that indiscriminate, systemic peroxidation could result in global neuronal injury, potentially representative of more localized damage to the BBB. In concert with intracranial inflammation, these events could initiate vascular leakage into the interstitial compartment and raise intracranial pressure, thus accounting for at least the neurological sequelae associated with AMS. However, there are no studies, to our knowledge, that have examined the temporal relationship between molecular markers of oxidative stress and tissue damage, specifically to the cerebral vasculature, in established AMS.

To extend these preliminary findings, serial examinations were conducted in two cohorts of subjects after rapid ascent to 4,559 m to examine changes in molecular markers of skeletal and cerebrovascular tissue damage in relation to previously reported changes in lipid peroxidation and inflammation, albeit in high-altitude pulmonary edema (HAPE) “susceptibles” (21). However, because AMS typically precedes HAPE and is confined to neurological as opposed to respiratory symptoms, we considered it appropriate to distinguish between the metabolic features of AMS and non-AMS independently of HAPE in the present study. We hypothesized that subjects diagnosed with AMS would present with neurobiochemical evidence of neuronal damage subsequent to enhanced oxidative stress.

METHODS

Subjects and Design

Forty-nine apparently healthy subjects provided written, informed consent following approval by the ethical committee of the University of Bern. They had participated in two separate studies that adopted identical treatment protocols (7, 21). Measurements were conducted at baseline 3–4 wk before ascent to high altitude (pre-ascent), within 2–3 h of arrival at the Capanna Regina Margherita mountain hut (HA-1d), and during the following mornings after the first (HA-2d) and second night (HA-3d). The ascent to high altitude involved a passive ascent...
to 3,200 m and, from there, active ascent to 3,611 m. After an overnight stay at this altitude, subjects continued their ascent on foot to 4,559 m, where they remained for 40 h.

Total creatine phosphokinase (CPK) activity was measured in all subjects, whereas molecular markers of lipid peroxidation, inflammation, and cerebral tissue injury were examined in a subcohort of 24 subjects.

Metabolic Measurements

Lipid peroxidation. Plasma F$_2$-isoprostanes were measured by stable-isotope dilution mass spectrometric assay (26) with a precision of ±6% and accuracy of 96%.

Skeletal and neuronal damage. Serum total CPK activity and neuron-specific enolase (NSE) were incorporated as markers of skeletal and neuronal damage, respectively. Total CPK was determined by using a diagnostic kit measured on a Vitros 750 analyzer (Ortho-Clinical Diagnostics). A standard monoclonal radioimmunoassay was incorporated for the measurement of NSE. This test incorporates a monoclonal two-site, single-incubation immunoradiometric assay. The monoclonal antibodies bind to the γ-subunit of the enzyme. There were no cases of hemolysis, which may have influenced data interpretation in light of the high concentration of NSE found in red cells and platelets (8, 24).

Proinflammatory mediators. Plasma concentrations of selected proinflammatory cytokines, specifically interleukin (IL)-1β, IL-2, IL-6, IL-8, and TNF-α were determined by using commercial assays (Quintikine, RD Research and Diagnostics System, Minneapolis, MN). C-reactive protein (CRP) was assessed by using turbidimetry as previously described (21).

Blood gases. Radial arterial samples were collected by using a microsampler (Biomedical, Graz, Austria) and analyzed for blood gases (Ciba Corning Diagnostics, Dietlikon, Switzerland).

Altitude Illness

AMS. Subjects were examined for AMS according to established methods (6). Briefly, physical symptoms were scored on a 0–3 scale (indicating nil, mild, moderate, or severe, respectively) for headache, gastrointestinal upset, fatigue and/or weakness, lassitude, and insomnia (self-assessment score). Changes in mental status (0–4), ataxia determined during a standardized heel-to-toe test (0–4), and peripheral edema (0–2) were also quantified (clinical score). The cumulative AMS score (self assessment + clinical score) was calculated for each respective time point at sea level and at high altitude, and a positive diagnosis was ascribed when the total cumulative score was more than five points in the presence of a headache (6).

HAPE. Chest radiography was performed at preascent and HA-3d by using a mobile X-ray unit (TRS, Siemens, Stockholm, Sweden), and the subsequent diagnosis of HAPE was confirmed as previously described (36).

Statistical Analyses

A Shapiro-Wilks test was applied to each dependent variable to assess distribution normality. Parametric and nonparametric equivalents (incorporating appropriate a posteriori comparisons) of a two-way mixed ANOVA with one between (condition: healthy vs. AMS) and one within (time: preascent vs. HA-1d vs. HA-2d vs. HA-3d) subjects factor were incorporated to examine the effects of condition and time on selected physiological parameters. After a simple main effect and interaction, Bonferroni-corrected paired-sample t-tests were employed to make a posteriori comparisons of time at each level of condition. Bonferroni-corrected Wilcoxon’s matched-pairs signed rank tests served as the nonparametric equivalents. Between-group comparisons were assessed by using a one-way ANOVA with an a posteriori Tukey’s honestly significant difference test. The nonparametric equivalents included a Kruskal-Wallis test and Bonferroni-corrected Mann-Whitney U-tests applied to each level of the time factor. The relationship between selected dependent variables was assessed by using a Pearson product moment correlation or Spearman’s rank correlation. Significance for all two-tailed tests was established at an alpha level of $P < 0.05$, and data are expressed as means ± SD.

RESULTS

Altitude Illness

In the large cohort ($n = 49$), 25 subjects were diagnosed with AMS, and, of these, 10 developed HAPE. Of the 24 subjects who constituted the subcohort, for whom a full set of metabolic data were available, 14 were diagnosed with AMS and 4 developed HAPE. In the latter, the clinical diagnosis of HAPE was confirmed on HA-2d in one subject and on HA-3d for the remaining three. Table 1 summarizes the corresponding Lake Louise AMS scores for the latter. HAPE was preceded by AMS in all cases; thus both conditions were pooled to improve statistical power and facilitate between-group comparisons (hereon-in denoted as AMS).

Arterial Blood Gases

Subjects with AMS were clearly more hypoxemic at high altitude compared with subjects who remained apparently healthy (Table 2). Differences in arterial PCO$_2$ and alveolararterial O$_2$ difference were unremarkable; the latter was a likely consequence of insufficient power (0.32).

Skeletal Tissue and Neuronal Damage

Compared with sea-level control values, an increase in total CPK was observed by the first day at high altitude in subjects diagnosed with AMS, whereas, in contrast, a decrease was noted by the third day in subjects who remained apparently healthy ($n = 49$; Fig. 1). Metabolic changes associated with AMS or HAPE were identical, which justified the pooled analyses (AMS + HAPE) to improve statistical power (Table 3). However, examination of the subcohort indicated that, although a general increase in CPK was observed at high altitude, between-group differences were not apparent, due in part to insufficient power (power = 0.05). Likewise, between-group differences in the plasma concentration of NSE (Table 3) were also unremarkable (power = 0.17).

Lipid Peroxidation and Proinflammatory Mediators

AMS did not influence the plasma concentration of F$_2$-isoprostanes despite the general increase (pooled data for healthy + AMS subjects) observed at high altitude (Table 3). One healthy subject presented with abnormal concentrations of

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Table 1. Lake Louise AMS scores at low and high altitude

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>Preascent</th>
<th>HA-1d</th>
<th>HA-2d</th>
<th>HA-3d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>10</td>
<td>0±0</td>
<td>3±2</td>
<td>3±1</td>
<td>2±2</td>
</tr>
<tr>
<td>AMS</td>
<td>14</td>
<td>0±0</td>
<td>6±3*</td>
<td>7±3*</td>
<td>5±3*</td>
</tr>
</tbody>
</table>

Values are means ± SD. AMS, acute mountain sickness; HA-1d, 2–3 h at high altitude; HA-2d, morning of day 2 at high altitude; HA-3d, morning of day 3 at high altitude. *Significant between-group difference as a function of time ($P < 0.05$).
TNF-α and IL-6 at sea level and was subsequently excluded from group analyses. The plasma concentration of IL-2 and IL-8 were below detection limits, and changes in TNF-α and IL-1β, as a function of either condition or time, were also unremarkable. In contrast, a comparatively greater increase in IL-6 and CRP was observed by days 1 and 2, respectively, at high altitude in subjects presenting with both AMS and HAPE. However, these changes were not apparent when subjects with HAPE (n = 4) were excluded from the overall analyses.

**Correlational Analyses**

No relationships were observed between pooled changes (calculated as the difference for each high-altitude time point minus preascend values for both subgroups) among F2-isoprostanes, markers of skeletal and neuronal damage, or proinflammatory cytokines. Furthermore, metabolic changes were unrelated to changes in AMS at high altitude with the exception of a moderate association between the increase in IL-6 on HA-1d to HA-2d in subjects diagnosed with clinical AMS (r = 0.57, P < 0.05). The increase in IL-6 in this subgroup was also related to the decrease in arterial O2 saturation (r = −0.51, P < 0.05) and arterial PO2 (r = −0.23, P < 0.05) and increase in alveolar-arterial O2 difference (r = 0.33, P < 0.05) on HA-1d to HA-2d. Changes in NSE were unrelated to changes in Pco2 or pH.

**DISCUSSION**

Rapid ascent to the Capanna Regina Margherita is an established paradigm for the induction of AMS in 30–60% of mountaineers, depending on individual susceptibility (33). By defining the temporal association between molecular markers of oxidative stress, skeletal tissue and neuronal damage, inflammation, and clinical symptomatology, the present investigation has revealed several important findings. The data based on the large cohort (n = 49) suggested a selective increase in skeletal tissue damage in subjects who developed AMS. However, this observation could not be confirmed when a complementary examination of related metabolic parameters was confined to a smaller subcohort of 24 subjects, partly due to the consequence of limited statistical power. The transient increase in IL-6 and CRP was likely a consequence of HAPE and, although equivocal, considered unlikely to be initiating factors associated with AMS per se. Finally, there was no molecular evidence for lipid peroxidation or neuronal damage in AMS. In conclusion, the present findings are the first to exclude free radical-mediated neuronal damage and inflammation as initiating events critical for the development of AMS. However, the pathophysiological significance of increased sarcolemmal membrane permeability deserves future consideration.

Although hemodynamic factors, such as impaired cerebral autoregulation, increased capillary pressure, ischemia, and neurogenic factors, may all act in concert to increase BBB permeability (13), the possibility of localized free radical-mediated neuronal and global cerebrovascular tissue damage at high altitude has not been investigated to date. This is surprising in light of the synergistic effects of inspiratory hypoxia and physical exercise (2, 3) that compound free radical generation coupled with the prevalence of neurovascular disorders and subsequent neurobehavioural dysfunction documented at extreme high altitude (18, 19). Furthermore, modest antioxidant defenses, high mitochondrial density, abundance of transition metals, auto-oxidizable neurotransmitters, and neuronal membrane lipids rich in polyunsaturated fatty acid side chains exposed to a high mass-specific O2 flux render the cerebrovascular endothelium particularly susceptible to damaging redox reactions (16). Clinical studies have subsequently shown a decrease in cerebral edema and improved neurological outcome after antioxidant supplementation in patients suffering from neurodegenerative disease (12), acute ischemic stroke and traumatic brain injury (11).

The dimeric enzyme NSE is the neuronal form of the glycolytic enzyme enolase (2-phospho-D-glycerate hydrolase) and is located almost exclusively in neuronal cell bodies,
axons, and neuroendocrine cells (32). After acute insult to the CNS, the structural integrity of the BBB is altered and subsequent astrogial disintegration can result in NSE leakage into the peripheral circulation. The appearance of NSE is, therefore, diagnostic of parenchymal cerebral injury, directly correlated with lesion volume and, as such, considered a useful clinical diagnostic of parenchymal cerebral injury, directly correlated with lesion volume and, as such, considered a useful clinical diagnostic of parenchymal cerebral injury, directly correlated with lesion volume and, as such, considered a useful clinical diagnostic of parenchymal cerebral injury, directly correlated with lesion volume and, as such, considered a useful clinical diagnostic of parenchymal cerebral injury, directly correlated with lesion volume and, as such, considered a useful clinical diagnostic of parenchymal cerebral injury, directly correlated with lesion volume and, as such, considered a useful clinical diagnostic of parenchymal cerebral injury, directly correlated with lesion volume and, as such, considered a useful clinical diagnostic of parenchymal cerebral injury, directly correlated with lesion volume and, 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cases, it is followed by HAPE, whose pathophysiology is likely different. The selective increase in IL-6 and CRP in the present study appeared to be dominated by subjects with HAPE. However, although it is possible that the transient expression of CRP reflected a delayed acute phase reaction consequent to HAPE, the immediate rise in IL-6 on HA-1d, before subjects had already developed HAPE, may suggest at least a contributory role for AMS. The metabolic significance of this response awaits clarification since factors other than tissue damage, most notably α-adrenergic activation, are equally capable of modulating the IL-6 response to high altitude (25), possibly to promote angiogenesis (27). Clearly, future research is required to establish cause from effect and confirm the functional significance of our findings in the context of AMS.

In conclusion, the present findings suggest that AMS is associated with a selective increase in sarcolemmal membrane permeability and IL-6. Whether the metabolic sequelae are a cause or epiphenomenon of AMS remains to be elucidated. More significantly, we believe this to be the first study to tentatively suggest that free radical-mediated neuronal damage is not an initiating event and critical for the development of AMS. However, a more direct and specific analytical approach is warranted before we can unequivocally exclude free radical-mediated cerebrovascular damage as an important pathophysiological mechanism.

REFERENCES