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 Holzgraefe, Peter E. Ballmer, and Peter Bärtsch. 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 contributory 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 subcohort 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, subcohort 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 sarcolemmal membrane permeability and inflammatory response, either as a cause or epiphenomenon of AMS and/or high-altitude pulmonary edema, remains to be elucidated.

free radicals; skeletal tissue damage; inflammation; neurological symptoms

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 neuromaging 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

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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 $\gamma$-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)-1$\beta$, IL-2, IL-6, IL-8, and TNF-$\alpha$ were determined by using commercial assays (Quantikine, 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 (herein 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

<table>
<thead>
<tr>
<th>Table 1. Lake Louise AMS scores at low and high altitude</th>
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<tbody>
<tr>
<td>Condition</td>
</tr>
<tr>
<td>Healthy</td>
</tr>
<tr>
<td>AMS</td>
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</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 ($P < 0.05$).
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, brain membranes, and the blood-brain barrier (11). The enzyme is normally present in the blood in low levels, but its levels increase in several neurodegenerative disorders (11). The observed increase in NSE levels at high altitude suggests that oxidative damage may contribute to neuronal injury, as previously suggested by several studies (34, 49). Clinical AMS was defined as a Lake Louise score of >5 points. SL, sea-level control value; HA-1d, measurement taken within 2–3 h after reaching high altitude; HA-2d, measurement taken the morning of the day 2 at high altitude; HA-3d, measurement taken on the morning of day 3 at high altitude.

**Fig. 1.** Changes (means ± SD) in total creatine phosphokinase (CPK) activity at 4,559 m in subjects with and without acute mountain sickness (AMS) (n = 49). Clinical AMS was defined as a Lake Louise score of >5 points. SL, sea-level control value; HA-1d, measurement taken within 2–3 h after reaching high altitude; HA-2d, measurement taken the morning of the day 2 at high altitude; HA-3d, measurement taken on the morning of day 3 at high altitude.

*Significantly different compared with SL as a function of group (P < 0.05).
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, considered a useful clinical adjunct, superior in some instances to other neurochemical markers such as S100B (28), for the prediction of neurological outcome (34).

Isoprostanes are exclusive products of free radical-mediated peroxidation to arachidonyl radicals and are generally considered reliable biomarkers for the assessment of lipid peroxidation in vivo (30) associated, but not causally linked, to a variety of neurological diseases such as degenerative dementias (20), Down’s syndrome, spinal cord injury (10), and posttraumatic neuronal/vascular degeneration (29). However, there are no published studies to our knowledge that have simultaneously examined changes in the peripheral concentration of F2-isoprostanes and NSE in any human models of neurological disease, thus making comparison with the present findings difficult. The stability of F2-isoprostanes and NSE in the present study suggests that free radical-mediated molecular damage, at least to neurons, is unlikely to be a significant mechanism operant in AMS.

In conjunction with hypoxia, the possibility of ischemic damage subsequent to intense hypocapnic cerebral vasoconstriction and tissue alkalosis has also been suggested to account for the residual neuropsychological dysfunction recorded among mountaineers after ascent to albeit higher altitudes (18, 19). Although psychometric testing was not employed in the present study, we failed to demonstrate any association between NSE and arterial PCO2 or pH. Thus the possibility of focal cerebral ischemia would appear unlikely, despite marked hyperventilation and alkalosis.

However, our findings need to be considered with tentative caution; although the sample size employed in the present study was consistent with previous investigations, subcohort analyses were, to an extent, constrained by limited statistical power. Furthermore, the apparent disassociation recently shown to exist between oxidative stress biomarkers in the jugular compared with the peripheral venous circulation after traumatic brain injury (29) highlights the interpretive significance of sampling site.

The possibility that damage to the BBB and/or other predilection areas particularly susceptible to hypoxic brain damage, such as the hippocampal CA1 sector, the striatum, and the thalamic reticular nucleus (17), could occur with vascular leak into the brain interstitial fluid independently of neuronal damage also warrants consideration. The incorporation of a single peroxidation/neuronal damage biomarker confined to the venous circulation in the present study should therefore be considered a limitation that deserves more thorough investigation in future studies. Functional neuroimaging during invasive localized venoarterial and cerebrospinal fluid sampling of a more comprehensive selection of brain proteins (22) specific to “redox-sensitive” cerebral compartments (BBB, neuron, glia, myelin) combined with electron paramagnetic resonance spectroscopy, the only molecular technique sine qua non for the direct detection and characterization of free radical species (4), is also recommended. Direct application of these techniques during the critical phase before the onset of established illness would allow for a more definitive examination of the brain’s potential contributions to AMS.

In contrast, the pathophysiological significance of increased sarcolemmal membrane permeability warrants further investigation. Despite the interpretive limitations associated with CPK as a quantitative marker of tissue damage (23), the increase in the present study has confirmed previous observations (3) and displayed a remarkably similar temporal sequence to symptoms of AMS. Although it has been suggested that damaged tissue peroxidizes more rapidly than healthy, structurally intact tissue (15), conflicting studies have reported damage and inflammation in animal and human exercise myopathy in the absence of lipid peroxidation (9, 31).

Although recent studies have suggested that inflammation is merely an epiphenomenon rather than an initiating cause of HAPE (21, 35), the situation with AMS may be potentially more complex and especially difficult to resolve if, as in many

Table 3. Effects of high altitude on biomarkers of lipid peroxidation, tissue damage, and proinflammatory mediators

<table>
<thead>
<tr>
<th>Marker</th>
<th>Condition</th>
<th>Healthy (n = 10)</th>
<th>HA-1d</th>
<th>HA-2d</th>
<th>HA-3d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid peroxidation</td>
<td>F2-isoprostanes, pg/ml</td>
<td>Healthy (n = 10)</td>
<td>10.9±3.7</td>
<td>15.8±7.0</td>
<td>16.0±10.6</td>
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<tr>
<td></td>
<td></td>
<td>AMS (n = 14)</td>
<td>13.5±6.6</td>
<td>13.3±5.7</td>
<td>13.2±4.2</td>
</tr>
<tr>
<td>Tissue damage</td>
<td>Total CPK, μM</td>
<td>Healthy (n = 10)</td>
<td>54.3±24.9</td>
<td>62.1±47.6</td>
<td>47.6±24.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMS (n = 14)</td>
<td>55.1±26.3</td>
<td>69.3±29.9</td>
<td>52.9±23.1</td>
</tr>
<tr>
<td></td>
<td>NSE, μg/l</td>
<td>Healthy (n = 10)</td>
<td>7.1±3.6</td>
<td>7.9±2.7</td>
<td>5.2±3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMS (n = 14)</td>
<td>8.2±4.5</td>
<td>6.1±2.0</td>
<td>7.9±5.6</td>
</tr>
<tr>
<td>Proinflammatory mediators</td>
<td>TNF-α, pg/ml</td>
<td>Healthy (n = 9)</td>
<td>5.3±2.5</td>
<td>8.1±8.5</td>
<td>5.3±4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMS (n = 14)</td>
<td>3.6±1.6</td>
<td>4.3±2.6</td>
<td>3.1±2.0</td>
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<tr>
<td></td>
<td>IL-1β, pg/ml</td>
<td>Healthy (n = 10)</td>
<td>40±63</td>
<td>46±76</td>
<td>43±68</td>
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<td></td>
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<td>AMS (n = 14)</td>
<td>24±76</td>
<td>23±75</td>
<td>26±82</td>
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<td></td>
<td>IL-6, pg/ml</td>
<td>Healthy (n = 9)</td>
<td>3±3</td>
<td>7±5</td>
<td>4±5</td>
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<tr>
<td></td>
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<td>AMS (n = 14)</td>
<td>2±4</td>
<td>5±5*</td>
<td>18±18*</td>
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<tr>
<td></td>
<td>CRP, mg/l</td>
<td>Healthy (n = 10)</td>
<td>2.8±2.4</td>
<td>4.0±3.7</td>
<td>4.2±3.9</td>
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<tr>
<td></td>
<td></td>
<td>AMS (n = 14)</td>
<td>1.7±1.0</td>
<td>2.4±1.7</td>
<td>4.6±3.2*</td>
</tr>
</tbody>
</table>

Values are means ± SD. CPK, creatine phosphokinase; NSE, neuron-specific enolase; CRP, C-reactive protein. *Significantly different compared with preascent control value as a function of condition (P < 0.05). †Significantly different between conditions as a function of time (P < 0.05). Note that one subject presented with abnormally high concentrations of TNF-α and IL-6 at pre-ascent and was subsequently excluded from group analyses.
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 CRP. 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 sarcosomal 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