Greater free plasma VEGF and lower soluble VEGF receptor-1 in acute mountain sickness

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Vascular endothelial growth factor (VEGF) is a hypoxia-induced protein that produces vascular permeability, and limited evidence suggests a possible role for VEGF in the pathophysiology of acute mountain sickness (AMS) and/or high-altitude cerebral edema (HACE). Previous studies demonstrated that plasma VEGF alone does not correlate with AMS; however, soluble VEGF receptor (sFlt-1), not accounted for in previous studies, can bind VEGF in the circulation, reducing VEGF activity. In the present study, we hypothesized that free VEGF is greater and sFlt-1 less in subjects with AMS compared with well individuals at high altitude. Subjects were exposed to 4,300 m for 19–20 h (baseline 1,600 m). The incidence of AMS was determined by using a modified Lake Louise symptom score and the Environmental Symptoms Questionnaire for cerebral effects. Plasma was collected at low altitude and after 24 h at high altitude, or at time of illness, and then analyzed by ELISA for VEGF and for soluble VEGF receptor, sFlt-1. AMS subjects had lower sFlt-1 at both low and high altitude compared with well subjects and a significant rise in free plasma VEGF on ascent to altitude compared with well subjects. We conclude that increased free plasma VEGF on ascent to altitude is associated with AMS and may play a role in pathophysiology of AMS.

ACUTE EXPOSURE TO HIGH ALTITUDE can result in acute mountain sickness (AMS), the hallmark of which is headache (7). AMS can progress to high-altitude cerebral edema (HACE), a more severe and often deadly form of altitude illness thought to be due to leakage of the blood brain barrier (7). High-altitude hypoxia stimulates expression of vascular endothelial growth factor (VEGF) (5), which increases vascular permeability and produces brain edema in hypoxic animal models (3, 4, 10, 19). In addition, blocking VEGF prevents hypoxic brain edema (19). VEGF therefore deserves consideration as a mechanism to explain the vascular leak of AMS and HACE.

Previous studies investigating the role of VEGF in AMS found no significant correlation of plasma or serum VEGF with AMS (15, 22). However, recent work shows that hypoxia also stimulates expression of a circulating, soluble VEGF receptor, soluble fms-like tyrosine kinase receptor-1 (11). The soluble receptor, known as soluble Flt-1 (sFlt-1), binds circulating VEGF, thereby reducing VEGF-induced vascular leak and angiogenesis (11). Because the presence of sFlt-1 can have significant impact on VEGF-induced vascular leak, plasma sFlt-1 as well as VEGF concentration needs to be measured to assess the role of VEGF in AMS. We hypothesized that plasma sFlt-1 concentration would be less and free VEGF concentration therefore greater in subjects developing AMS compared with well subjects after acute exposure to high altitude.

Our approach was to expose subjects to acute hypobaric hypoxia by rapid ascent to 4,300 m (Pikes Peak, CO) from 1,600 m. We also measured plasma erythropoietin (EPO) concentrations because EPO is a biochemical marker of altitude stress (6).

MATERIALS AND METHODS

Study design. Approval from the Mesa State College Human Research Committee and the Colorado Multiple Institutional Review Board at the University of Colorado Health Sciences Center was obtained to perform this study. Informed consent was obtained from 20 subjects in accordance with National Institutes of Health guidelines. Subjects dropping out of the study were not replaced, and subjects could drop out at any time without penalty. All subjects were healthy adults who resided at an elevation of 1,370 to 1,645 m. Exclusion criteria included pregnancy and altitude exposure duration more than 24 h above 2,100 m within 2 wk of the study. None of the subjects had a prior history of AMS. Subjects were originally enrolled into a study that was designed to compare placebo to two drugs for AMS prevention. However, a loss of data and blood samples in the drug treatment groups led to them not being included in this study. The elevation of residence will be referred to as low altitude throughout this manuscript. Subjects were driven to 4,300 m (Pike’s Peak summit) over 2 h by car and stayed overnight. Plasma was collected from subjects at low (1,370 m or 1,645 m) and high (4,300 m) altitudes. High-altitude blood draws occurred after 19–20 h at high altitude or, if the subject was severely ill with AMS and treatment was required (intravenous dexamethasone and oxygen), a venous blood draw was performed just before treatment. Plasma was used for analyses of VEGF, sFlt-1, and EPO. Of the 20 subjects recruited, data from one subject could not be evaluated because of hemolysis during sample preparation at high altitude.

AMS. The Environmental Symptoms Questionnaire (ESQ-III short form) was completed before ascent and either after 19–20 h at altitude or on removal from the study as a result of severe AMS. Both an ESQ-III ≥0.7 and a Lake Louise Score of ≥3 with a headache present were required for diagnosis of AMS (13).

Plasma EPO, VEGF, sFlt-1. Whole blood was collected into Vacutainer tubes containing EDTA as an anticoagulant and centri-
fuged at 26°C for 30 min at 400 g. Plasma was withdrawn, aliquoted to 0.5 ml, flash frozen in liquid nitrogen, and stored at −80°C until use. ELISA kits were used for evaluation of plasma EPO, VEGF, and sFlt-1 (R&D Systems, catalog nos. DEP-00, DVE-00, DVR-100). When the coefficient of variance was less than 10% (n = 3 replicates per sample), data were considered reliable.

**Free VEGF.** The VEGF kit from R&D Systems has been shown to measure only VEGF not bound to sFlt-1 (1, 9, 16). Literature from R&D Systems in regard to VEGF ELISA kit DVE-00 reports no interference with sFlt-1 until greater than 1,250 pg/ml. Previous reports using this kit have referred to plasma VEGF measured using this method as “free VEGF” (12, 16); however, the method has never been tested to determine whether it identifies VEGF bound to another important plasma receptor, α2-macroglobulin (2). We have tested this VEGF ELISA kit for cross-reactivity with VEGF-bound α2-macroglobulin using methods identical to those used by R&D Systems to test for cross-reactivity with sFlt-1 and kinase domain receptor. Varying concentrations of α2-macroglobulin (10–250 ng/ml) were incubated with a midrange VEGF standard for 1 h at 37°C, then analyzed using the R&D Systems VEGF ELISA kit (DVE-00). There was a consistent 50% reduction in detectable VEGF in the presence of α2-macroglobulin at all concentrations tested. Circulating VEGF has been shown to bind noncovalently with native α2-macroglobulin, its binding affinity such that it remains in equilibrium with circulating VEGF (2), as demonstrated by our data.

VEGF measured decreased as α2-macroglobulin increased in concentration, suggesting that the ELISA is not measuring VEGF bound to α2-macroglobulin. Therefore, circulating VEGF measured by this ELISA kit is referred to as free plasma VEGF throughout this manuscript. The ELISA for sFlt-1 measures total plasma sFlt-1, that which is bound to VEGF and unbound.

**Statistics.** Data comparing low to high-altitude values were analyzed by use of paired Student’s t-tests. Independent Student’s t-tests were used to determine differences between well and AMS subjects at low and at high altitude. Correlations between VEGF, sFlt-1, EPO, and arterial oxygen saturation were also determined. Significance was set at $P < 0.05$ for all statistical analyses.

**RESULTS**

Of the 20 subjects in the study, sufficient plasma for all analyses was obtained at both altitudes in 19 subjects. The concentrations of plasma VEGF, sFlt-1, and EPO in well and AMS subjects are presented in Table 1. VEGF increased at high altitude in subjects who developed AMS but not in those who remained well. Although plasma sFlt-1 increased with ascent to high altitude in all subjects, concentrations were lower in the AMS compared with well subjects at both low and high altitude. Oxygen saturation was equivalent between well and AMS subjects at high altitude, 86.33 ± 1.33 vs. 88.78 ± 0.983, respectively. At 4,300 m, there was no correlation between oxygen saturation and plasma VEGF or sFlt-1; however, EPO positively correlated with arterial oxygen saturation, $R^2 = 0.31$, $P = 18$.

**DISCUSSION**

The main findings of this study are that subjects who developed AMS at high altitude had higher sFlt-1 levels at low and high altitude and increased free plasma VEGF compared with those who remained well. Because subjects who subsequently developed AMS had low plasma sFlt-1 at low altitude compared with those who did well, low-altitude sFlt-1 concentration may be a predictor of AMS.

VEGF is a hypoxia-induced protein that can acutely increase vascular permeability and may contribute to the development of AMS and HACE by increasing cerebral capillary permeability (17). In our study, AMS subjects had a greater increase on ascent to altitude in VEGF available to bind endothelium and cause vascular leak compared with well subjects. Previous reports indicating either no change in VEGF with ascent to altitude or a decrease in plasma VEGF that was not associated with AMS (15, 18) differed from the present study. Our study employed a more acute ascent profile than previous studies, and each subject was used as his or her own control, allowing paired data analysis. Also, sFlt-1 bound to VEGF was not measured in previous studies in subjects exposed to high altitude.

Previous studies have clearly shown that sFlt-1 plays a role in various pathologies and can inhibit activity of circulating VEGF (8, 12, 14, 21). Soluble VEGF receptor, sFlt-1, is an important factor when determining the pathophysiological role of VEGF because it binds plasma VEGF and effectively reduces the amount VEGF available to bind endothelium (4). Oral administration of sFlt-1 reduces VEGF-induced vascular permeability (17). The present study indicates that sFlt-1 is greater and free VEGF is less in those who remain well compared with those who develop AMS at high altitude. Furthermore, plasma sFlt-1 was greater in well subjects at low altitude, before ascent to altitude compared with those who developed AMS, suggesting that sFlt-1 plasma concentration at low altitude may be a predictor of AMS. Interestingly, elevated sFlt-1 concentration has recently been reported as a putative predictor of preeclampsia, before clinical onset (12). Before clinical onset, preeclamptic patients had greater plasma sFlt-1 and less free VEGF than healthy pregnancies at similar gestational time points. VEGF, and the similar placental-like growth

### Table 1. Concentrations of circulating plasma factors in well subjects compared to those with AMS

<table>
<thead>
<tr>
<th></th>
<th>Well (n = 10)</th>
<th>High altitude</th>
<th>AMS (n = 9)</th>
<th>High altitude</th>
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<tbody>
<tr>
<td>VEGF, pg/ml</td>
<td>13.7 ± 13.5 (0–81.5)</td>
<td>15.6 ± 69.9 (0–432.6)</td>
<td>20 ± 17.5 (0–141.8)</td>
<td>196.2 ± 771 (0–466.5)</td>
</tr>
<tr>
<td>sFlt-1, pg/ml</td>
<td>22.3 ± 4.9 (0–43.3)</td>
<td>37.6 ± 4.6* (25.5–50.6)</td>
<td>2.5 ± 1.6 (0–12.8)</td>
<td>17.1 ± 7.2* (0–59.4)</td>
</tr>
<tr>
<td>EPO, U/ml</td>
<td>6.8 ± 1.9 (0.6–13.4)</td>
<td>29.8 ± 8.1* (0–78.5)</td>
<td>6.85 ± 1.15 (2.3–11.5)</td>
<td>30.13 ± 3.75* (15.9–51.2)</td>
</tr>
<tr>
<td>ESQ-C</td>
<td>0.02 ± 0.02</td>
<td>0.26 ± 0.06</td>
<td>0.11 ± 0.06</td>
<td>1.42 ± 0.19</td>
</tr>
<tr>
<td>LL SQ</td>
<td>0.27 ± 0.19</td>
<td>3.5 ± 0.3</td>
<td>0.5 ± 0.22</td>
<td>6.5 ± 0.31</td>
</tr>
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</table>

Values are means ± SE, with ranges in parentheses. AMS, acute mountain sickness; sFlt-1, soluble VEGF receptor; EPO, erythropoietin; ESQ-C, Environmental Symptoms Questionnaire-Cerebral; LL SQ, Lake Louise Symptoms Questionnaire. *$P < 0.05$ well vs. AMS; †$P < 0.05$ high vs. low altitude.
Because ascent to high altitude. VEGF-endothelial interactions resulting well at altitude had greater plasma sFlt-1 before and after in subjects who developed AMS, whereas those who remained high altitude. concentrations of VEGF and sFlt-1 in humans ascending to oxygen saturation. Further research is needed to determine changes in oxygen content and delivery than to changes in hypoxia on VEGF and sFlt-1 may be more sensitive to altitude, as has been previously reported (6). The effect of hypoxia on VEGF and sFlt-1 may be more sensitive to changes in oxygen content and delivery than to changes in oxygen saturation. Further research is needed to determine the mechanisms by which hypoxia influences circulating concentrations of VEGF and sFlt-1 in humans ascending to high altitude.

Overall, free plasma VEGF increased with ascent to altitude in subjects who developed AMS, whereas those who remained well at altitude had greater plasma sFlt-1 before and after ascent to high altitude. VEGF-endothelial interactions resulting in vascular leak can be inhibited by administration of exogenous sFlt-1, and vascular leak has been strongly implicated in AMS and HACE (8, 19, 23). We conclude that free VEGF may play a role in the pathophysiology of AMS.

REFERENCES


