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

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Tissot van Patot, Martha C., Guy Leadbetter, Linda E. Keyes, Jamie Bendrick-Peart, Virginia E. Beckey, Uwe Christians, and Peter Hackett. Greater free plasma VEGF and lower soluble VEGF receptor-1 in acute mountain sickness. J Appl Physiol 98: 1626–1629, 2005. First published January 13, 2005; doi:10.1152/japplphysiol.00589.2004.—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.

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VEGF ELISA kit for cross-reactivity with VEGF-bound 2-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 2-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 (DYE-00). There was a consistent 50% reduction in detectable VEGF in the presence of 2-macroglobulin at all concentrations tested. Circulating VEGF has been shown to bind noncovalently with native 2-macroglobulin, its binding affinity such that it remains in equilibrium with circulating VEGF (2), as demonstrated by our data.

VEGF measured decreased as 2-macroglobulin increased in concentration, suggesting that the ELISA is not measuring VEGF bound to 2-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 $P \leq 0.05$ 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. EPO increased at high altitude in all subjects. Oxygen saturation was equivalent between well and AMS subjects at high altitude, $86.33 \pm 1.33$ vs. $88.78 \pm 0.983$, 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>Plasma Factor</th>
<th>Low Altitude</th>
<th>High Altitude</th>
<th>AMS (n = 9)</th>
<th>High Altitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF, pg/ml</td>
<td>13.7±13.5 (0–81.5)</td>
<td>135.6±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*† (25.5–50.68)</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>LLSQ</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>
</tbody>
</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; LLSQ, Lake Louise Symptoms Questionnaire. *P < 0.05 well vs. AMS; †P < 0.05 high vs. low altitude.
factor, are crucial for normal pregnancy. Inducing transcription of sFlt-1 in rats produced preeclamptic-like disease, and subsequent exogenous administration of VEGF and placent al-like growth factor rescued the animals from disease. These data indicate that circulating VEGF has significant impact on endothelial function that can be inhibited by binding to sFlt-1. Further studies are needed to clarify the correlation between plasma sFlt-1 and free VEGF at low altitude and at high altitude.

One limitation of this study is that variation between samples was relatively large and potential differences between VEGF at low and high altitude in well subjects may not have been evident in this small sample size. Because whole blood was processed to plasma by the same personnel at both altitudes and equipment used to process blood at low altitude was transported for use at high altitude, it is unlikely that experimental error was imposed by processing techniques at low vs. high altitude. Vacutainers were used to withdraw venous blood samples at both altitudes; however, the lower barometric pressure at 4,300 m reduced the efficiency of the Vacutainers. For example, a 10-ml Vacutainer only collected 4–6 ml blood at high altitude. Increased handling of the blood led to a large degree of hemolysis. Interestingly, the hemolysis occurred primarily in the drug treatment groups, with only one placebo sample hemolyzing at altitude. Subjects in this study were treated with a placebo because they were initially part of a larger study designed to investigate prevention of AMS by drug treatment. It is unlikely that the placebo had an effect on circulating VEGF, sFlt-1, or EPO concentrations.

Circulating VEGF has been shown to bind noncovalently with native α2-macroglobulin, and its binding affinity is such that it remains in equilibrium with circulating VEGF and probably does not alter VEGF function (2). There is no evidence to suggest that α2-macroglobulin is altered by hypoxia. Because α2-macroglobulin reduces VEGF detected by the ELISA kit used in this study (see MATERIALS AND METHODS), it is unlikely that α2-macroglobulin is responsible for the increased VEGF at altitude. Interestingly, in this study, oxygen saturation was not lower in subjects with AMS compared with those who remained well at high altitude. Furthermore, although VEGF and sFlt-1 are hypoxia-sensitive proteins, circulating concentrations of neither protein were correlated with oxygen saturation. However, plasma concentrations of EPO, also a hypoxia-sensitive protein, were negatively correlated with oxygen saturations at high 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


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