Change in plasma vascular endothelial growth factor during onset and recovery from acute mountain sickness

David A. Dorward\textsuperscript{a},*, A.A. Roger Thompson\textsuperscript{a}, J. Kenneth Baillie\textsuperscript{a}, Margaret MacDougall\textsuperscript{b}, Nikhil Hirani\textsuperscript{c}

\textsuperscript{a}APEX (Altitude Physiology Expeditions), c/o Dr. F. Kristmundsdottir, College Office, College of Medicine and Veterinary Medicine, The University of Edinburgh, The Chancellor’s Building, 49 Little France Crescent, Edinburgh EH16 4SB, UK
\textsuperscript{b}Public Health Sciences, University of Edinburgh Medical School Teviot Place, Edinburgh EH8 9AG, UK
\textsuperscript{c}Department of Respiratory Medicine, Royal Infirmary of Edinburgh, 52 Little France Crescent, Edinburgh EH16 4SA, UK

Received 6 October 2005; accepted 14 June 2006

\textbf{Keywords}
Vascular endothelial growth factor; Acute mountain sickness; High altitude; Acclimatisation; Hypoxia

\textbf{Summary}
There is an increasing body of evidence suggesting that altered vascular permeability may be an important component of the pathogenesis of acute mountain sickness (AMS). Vascular endothelial growth factor (VEGF) is a potent permeability factor subject to hypoxic regulation but its role in the pathogenesis of AMS is yet to be defined. We examined the relationship between plasma VEGF and AMS on ascent to high altitude and subsequent acclimatisation.

Thirty-eight healthy lowlanders (median age 21, range 18–31) flew to La Paz, Bolivia (3650 m) on the Apex 2 research expedition. After 4–5 days acclimatisation, they ascended by vehicle over 90 min to the Chacaltaya laboratory (5200 m). We measured plasma VEGF in venous blood at sea level and at 6 h and 3 and 7 days at 5200 m. AMS was scored using the Lake Louise consensus system.

Using serial measurement of plasma VEGF at 5200 m, following partial acclimatisation at 3650 m, we demonstrated a highly significant change in VEGF levels \((P<0.0005)\) with a rise in VEGF in approximately 80\% of subjects by day 7 at 5200 m. We found no evidence of an association between AMS and change in VEGF levels on ascent to either 3650 or 5200 m. We provide novel data of change in plasma VEGF levels during acclimatisation to high altitude, but our results do not support the hypothesis that circulating unbound VEGF is an important component of the pathogenesis of AMS.

\textcopyright 2006 Elsevier Ltd. All rights reserved.

\*Corresponding author. Tel.: +44 131 229 5481; fax: +44 131 242 9301.
E-mail address: D Dorward@doctors.org.uk (D.A. Dorward).
Introduction

Despite an increasing number of visitors to high altitudes, much is still unknown regarding the pathophysiology and treatment of acute mountain sickness (AMS) and the potentially fatal conditions high-altitude pulmonary oedema (HAPE) and high-altitude cerebral oedema (HACE). AMS is a common condition that follows rapid ascent to high altitude (greater than 2500 m) and is characterised by a range of symptoms including headache, insomnia, dizziness and gastrointestinal upset. It has been proposed that AMS and HACE form part of the spectrum of illness caused by vasogenic cerebral oedema and raised intracranial pressure. Likely underlying mechanisms of cerebral oedema at high altitude include elevated cerebral capillary pressure, impaired cerebral autoregulation, free radical-mediated damage to the blood–brain barrier and alteration in blood–brain barrier permeability through various hypoxia-regulated mediators.

One such mediator is vascular endothelial growth factor (VEGF), shown to be significantly upregulated in the presence of hypoxia in rat lungs and tumour cell lines through transcription factor hypoxia inducible factor-1 (HIF-1). As well as a powerful angiogenic and endothelial-cell-specific mitogen, VEGF is a potent permeability factor expressed in most tissues including the lung and brain. Alternative splicing of exons six and seven of the VEGF mRNA produces six isoforms, and are all soluble products whereas VEGF and are all soluble products whereas VEGF and 165 are all soluble products whereas VEGF and 189 and 206 are cell-associated. Hypoxia-induced cerebral oedema in a mouse model can be attenuated by administration of a VEGF-neutralising antibody and it has therefore been proposed that VEGF alters blood–brain barrier permeability, thus contributing to the development of brain swelling and oedema and thus symptomatic AMS and HACE.

Three studies have previously investigated the relationship between VEGF and AMS, but no consistent relationship has been found. However, meaningful comparison is difficult given the differences in research methods and the definitions of AMS used. For example, Maloney et al. did not use a standardised ascent profile and defined AMS by means of a Lake Louise self-assessment score-sheet. As per Lake Louise consensus scoring, AMS was defined as a total score of 2; in contrast, Walter et al. defined AMS as Lake Louise score of 5. These studies also generated conflicting data regarding the response of VEGF to high altitude hypoxia. Data from Walter et al. suggest that there is an increase in plasma VEGF on arrival at high altitude while Tissot van Patot et al. found a significant increase only in subjects diagnosed with AMS. None of these studies made serial measurements at altitude.

Several studies have investigated the effect of hypoxia-induced VEGF expression over time. Kuo et al. described an increase in rat brain VEGF mRNA and protein levels on exposure to hypoxia. Interestingly, although VEGF mRNA and protein expression increased during days 1 and 2, mRNA levels were reduced by day 4 and had returned to baseline levels by day 7. In contrast, tissue protein levels exhibited a biphasic response falling on day 4 but rising again on day 7.

Given the above experimental findings we sought to gather novel data on the response of plasma VEGF expression to prolonged hypoxia by taking serial VEGF samples in a cohort of healthy lowlanders. We also aimed to study the relationship between plasma VEGF concentration and oxygen saturation (SpO2), pulmonary artery systolic pressure (PASP) and AMS following controlled ascent to high altitude (5200 m).

Materials and methods

Subjects and study design

Forty-two healthy lowland subjects participating in the Apex 2 expedition (Altitude Physiology Expeditions) and residing at an altitude less than 50 m were enrolled. The study was approved by the Lothian Research Ethics Committee. All subjects were in the placebo arm of a study investigating drug treatments at high altitude. One subject was excluded with HAPE and samples could not be obtained for 3 others. Of the 38 remaining (24 males, median age 22, range 18 to 25 and 14 females, median age 20, range 19–28), 6 were evacuated with symptoms of AMS at various points from day 1 onwards at 5200 m. Data were included up to the time of evacuation.

Subjects were flown from Britain to La Paz, Bolivia (3650 m) and after 4 or 5 days acclimatisation they ascended by road, over a period of 90 min, to the Chacaltaya laboratory (5200 m), where they stayed for 8 days (Fig. 1). Venous blood samples were taken in EDTA at five time-points: at sea-level prior to departure, at 5200 m on day 1 (within 6 h of arrival), day 3 and 7, and again at sea-level at least 6 weeks after return to the UK. Venous blood was immediately centrifuged at 3000g for 10 min (Labofuge 200, Kendro Laboratory Services, Herts, UK). Plasma aliquots were transported on dry ice by specialist international courier and stored at −80 °C prior to analysis.

Assessment of AMS score, SpO2 and pulmonary artery systolic pressure

On each evening of the expedition subjects completed a Lake Louise AMS self-assessment score-sheet. As per Lake Louise consensus scoring, AMS was defined as a total score of 3 with the presence of headache. At the same time, peripheral oxygen saturation (SpO2) was measured using digital pulse oximetry (Nellcor, N-20PA, USA). Echocardiography was performed on day 1, 3 and 7 at 5200 m within 3 h of venous blood sampling using a portable real-time phased-array echocardiographic scanner (Acuson Cypress, Siemens Medical Solutions, UK) operated by an experienced cardiologist. PASP was estimated by adding the clinically determined jugular venous pressure to the right ventricular to right atrial pressure gradient. The gradient was calculated using the modified Bernoulli equation.

ELISA

Concentrations of the predominant VEGF isoforms, VEGF121 and VEGF165, in plasma obtained from the subjects were measured by sandwich ELISA (Quantikine® Human VEGF Immunoassay, DVE00, R&D systems, MN, USA). In brief,
100 µl of undiluted plasma were incubated for 1 h at room temperature in a 96-well plate coated with monoclonal VEGF antibody. Following three washes polyclonal detection antibody was added with substrate solution subsequently used. Absorbance was measured at 750 nm in a microplate reader. For standardisation, serial dilutions of recombinant hVEGF (121 and 165) were assayed simultaneously. Levels below the lower limit of detection of this assay (3.9 pmol/l) were classed as undetectable and ascribed a plasma VEGF concentration of 0 pmol/l. Two individuals did not have detectable VEGF in any of their samples and a further 9 individuals had one or more samples in which VEGF was undetectable. Measurements were performed in triplicate and results were excluded as unreliable where an upper limit of 20% in the coefficient of variation was exceeded. Nine measurements were excluded in this way.

Statistics

A significance level of 5% was assumed throughout for all statistical analyses.

The variation in % change from sea-level baseline of VEGF, SpO₂ and PASP over the four time-points (encompassing ascent and acclimatisation to high altitude, and return to sea level) was assessed using the Friedman test. In turn, the Wilcoxon matched pairs signed ranks test (with a correction for multiple comparisons using the Simes method) was used in performing the corresponding pairwise comparisons over stages of the expedition for each of the above variables. To identify possible discrepancies across gender, similar tests were performed with VEGF levels for males and females separately.

The Mann–Whitney U-test was used to compare percentage change from sea-level baseline VEGF levels between those who did and did not develop AMS on day 2 at 3650 m or day 2 at 5200 m. The percentage change in VEGF level from pre-expedition sea level to day 1 at 5200 m was calculated and based on careful examination of the distributions for these data for positive and negative AMS cases separately, subjects were classified into the following four categories according to the magnitude of percentage change: ‘−200 < % change < 0’, % change = 0’, ‘0 < % change < 200’, % change > 300’. No subjects had a % change in VEGF of between 200 and 300. Fisher’s exact test was used to assess the relationship between these categories and AMS status on day 2 at 5200 m and on day 2 at 3650 m. These time points were chosen because, consistent with previous work, peak prevalence of AMS occurred on day 2 following ascent. For completeness, and on account of the small sample size, change in VEGF levels from sea level to day 1 at 5200 m was also examined by classifying the VEGF percentage change into the broader categories ‘% change < 0’ and ‘% change > 0’. The resultant data were examined using the chi-squared test of association and, where appropriate, Fisher’s Exact test.

Relationships between continuous variables were examined by means of individual scatter-plots and where these graphs were suggestive of at least a very weak linear trend, Pearson correlation coefficients (PCCs) were calculated as a measure of the strength of the corresponding linear trends. The relationships examined at the univariate level by these methods included:

1. AMS score for day 2 at 5200 m against age and percentage changes in VEGF, PASP and SpO₂ level from pre-expedition sea level to day 1 at 5200 m.
2. AMS score for day 2 at 3650 m against age and percentage changes in VEGF, PASP and SpO₂ level from pre-expedition sea level to day 1 at 5200 m.
3. AMS score for day 1 at 5200 m against age and percentage changes in VEGF, PASP and SpO₂ level from pre-expedition sea level to day 1 at 5200 m.
4. As for (3) but with all measurements, including AMS scores, taken at day 3 rather than at day 1.
5. As for (3) but with all measurements, including AMS scores, taken at day 7 rather than at day 1.

During this exploratory process, two criteria for identification of a potential factor for inclusion in a future multiple regression analysis were that the value of the
PCC was at least 0.3 and that this measure of a linear association was consistent with the behaviour exhibited in the corresponding scatter-plot. Further, it was required that on entry to the linear regression model the factor in question made a significant contribution in explaining the variability in AMS scores for the corresponding regression analysis at the univariate level (as demonstrated by the related $F$-test). According to these non-stringent criteria, at most one continuous factor was retained per dependent variable. In order to assess the effect of possible confounding caused by gender differences, hierarchical multiple regression analyses were performed for those variables which survived the screening process. Where the corresponding $F$-test revealed that the inclusion of gender made a significant improvement to the predictive value of the model, Pearson’s partial correlation coefficient (controlling for gender) was assumed as the more accurate measure of the strength of the linear relationship between the continuous factor in the model and the corresponding response variable for VEGF.

Results

Distribution of sea-level VEGF within sample group

Baseline VEGF expression ranged from 0 to 408 pmol/l (median = 30.3 pmol/l, $n = 36$). Nineteen individuals expressed a VEGF level less than 50 pmol/l at sea level and 8 of these had undetectable baseline VEGF concentrations (Fig. 2).

Variation in plasma VEGF levels

The non-parametric tests to examine overall changes in VEGF over ascent and acclimatisation revealed a highly significant association between VEGF and stage of expedition (ascent, acclimatisation and return to sea level) ($P < 0.0005$). VEGF levels are available for 29 subjects on both the pre-expedition sea-level sample and day 7 at 5200 m; of these, 24 subjects demonstrated a rise in VEGF at high altitude. Likewise, all subjects had lower VEGF levels on return to sea level, than on day 7 at 5200 m (Fig. 2). Medians and corresponding ranges for VEGF at each stage of the expedition are provided in Table 1. The corresponding results for the pairwise comparisons are summarised in Table 2, showing a highly significant difference between both sea-level samples and day 7 at 5200 m. Separate analysis of males and females showed a significant association overall between stage of expedition and VEGF levels for both sexes, but the association was stronger in females ($P < 0.0005$) than males ($P = 0.02$).

Plasma VEGF and AMS

Plasma VEGF level at sea level before the expedition did not predict susceptibility to AMS on ascent to high altitude (Fig. 3). Furthermore, there was no evidence of...
an association between AMS status and VEGF category on ascent to high altitude. This was true regardless of whether the reference point for AMS status was day 2 at 5200 m or day 2 at 3650 m (Table 3). Similar analyses performed defining AMS as a Lake Louise score \( \geq 4 \) had a negligible effect on the test results.

The comprehensive multivariate analysis described above did not show any evidence of an association between plasma VEGF and AMS scores. The scatter-plots and linear regression analyses demonstrated no evidence for any association between AMS score and percentage change in VEGF level from baseline. In the main, scatter-plots exhibited random behaviour.

### Table 1 Summary statistics for plasma VEGF, oxygen saturation (\( \text{SpO}_2 \)), pulmonary artery systolic pressure (PASP) and prevalence of acute mountain sickness (AMS) for different stages of the expedition.

<table>
<thead>
<tr>
<th>Category</th>
<th>Sea level(^{a})</th>
<th>Day 1 5200 m</th>
<th>Day 3 5200 m</th>
<th>Day 7 5200 m</th>
<th>Sea level(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (pmol/l)</td>
<td>30.3</td>
<td>43.0</td>
<td>45.3</td>
<td>39.0</td>
<td>42.2</td>
</tr>
<tr>
<td>Range (min, max)</td>
<td>(0.0, 408.3)</td>
<td>(0.0, 409.7)</td>
<td>(0.0, 420.4)</td>
<td>(0.0, 450.9)</td>
<td>(0.0, 439.4)</td>
</tr>
<tr>
<td>( n ) number</td>
<td>36</td>
<td>37</td>
<td>32</td>
<td>31</td>
<td>31</td>
</tr>
<tr>
<td>( \text{SpO}_2 (%) )</td>
<td>99.0</td>
<td>76.5</td>
<td>74.5</td>
<td>78.5</td>
<td>( )</td>
</tr>
<tr>
<td>Range (min, max)</td>
<td>(95.0, 100.0)</td>
<td>(60.0, 90.0)</td>
<td>(63.0, 81.0)</td>
<td>(59.0, 86.0)</td>
<td>( )</td>
</tr>
<tr>
<td>( n ) number</td>
<td>32</td>
<td>34</td>
<td>24</td>
<td>26</td>
<td>( )</td>
</tr>
<tr>
<td>PASP (mmHg)</td>
<td>17.0</td>
<td>31.5</td>
<td>31.0</td>
<td>33.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Range (min, max)</td>
<td>(8.0, 27.5)</td>
<td>(21.0, 67.0)</td>
<td>(22.0, 55.0)</td>
<td>(22.0, 43.0)</td>
<td>(17.0, 30.0)</td>
</tr>
<tr>
<td>( n ) number</td>
<td>30</td>
<td>32</td>
<td>25</td>
<td>26</td>
<td>( )</td>
</tr>
<tr>
<td>Prevalence of AMS (%)</td>
<td>0</td>
<td>59.5</td>
<td>51.5</td>
<td>12.5</td>
<td>( )</td>
</tr>
<tr>
<td>(( \text{SE} ))</td>
<td>(0.0)</td>
<td>(8.1)</td>
<td>(8.7)</td>
<td>(5.8)</td>
<td>( )</td>
</tr>
<tr>
<td>( n ) number</td>
<td>38</td>
<td>37</td>
<td>33</td>
<td>32</td>
<td>( )</td>
</tr>
</tbody>
</table>

Six of the 38 subjects were evacuated with symptoms of AMS at various points from day 1 onwards at 5200 m. Data were included up to the time of evacuation.

\(^{a}\)Pre-expedition sea level.

\(^{b}\)Post-expedition sea level.

\(^{\dagger}\)Data not available.

### Table 2 Comparisons of plasma VEGF levels between all sample points using paired data.

<table>
<thead>
<tr>
<th>Categories compared</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sea level(^{a}) vs. Day 1</td>
<td>0.767</td>
</tr>
<tr>
<td>Sea level(^{a}) vs. Day 3</td>
<td>0.194</td>
</tr>
<tr>
<td>Sea level(^{a}) vs. Day 7</td>
<td>0.003</td>
</tr>
<tr>
<td>Sea level(^{a}) vs. Sea level(^{b})</td>
<td>0.005</td>
</tr>
<tr>
<td>Day 1 vs. Day 3</td>
<td>0.032</td>
</tr>
<tr>
<td>Day 1 vs. Day 7</td>
<td>(&lt; 0.0005 )</td>
</tr>
<tr>
<td>Day 1 vs. Sea level(^{b})</td>
<td>0.003</td>
</tr>
<tr>
<td>Day 3 vs. Day 7</td>
<td>0.017</td>
</tr>
<tr>
<td>Day 3 vs. Sea level(^{b})</td>
<td>0.005</td>
</tr>
<tr>
<td>Day 7 vs. Sea level(^{b})</td>
<td>(&lt; 0.0005 )</td>
</tr>
</tbody>
</table>

The pairwise comparisons represented were performed using the Wilcoxon matched pairs signed ranks test and the \( P \)-values obtained on application of the Simes method\(^{23} \) to correct for multiple comparisons.

\(^{\dagger}\)Day 1, Day 3 and Day 7 denote time spent at 5200 m.

\(^{\ast}\)Pre-expedition sea level.

\(^{\dagger}\)Post-expedition sea level.

![Figure 3](image-url) Baseline (sea level) plasma VEGF levels in AMS positive and AMS negative subjects. AMS positive individuals are those with a Lake Louise score \( \geq 3 \) on day 2 at 5200 m. AMS is most likely to develop on the day following arrival at altitude and therefore we chose to use AMS data from day 2 at 5200 m to examine the relationship between sea-level VEGF expression and AMS. Data were available for 34 subjects; VEGF levels for 2 subjects were excluded through the quality control and AMS data were not available for 2 subjects who had been evacuated by day 2 at 5200 m.
Variation in oxygen saturation and pulmonary artery systolic pressure

Consistent with previous results, the median $\text{SpO}_2$ level fell following ascent and PASP increased significantly. Exploratory analyses using scatter plots were not indicative of any association between VEGF level and $\text{SpO}_2$ or PASP.

Discussion

In this study we have examined the relationship between plasma VEGF and AMS on ascent to high altitude and subsequent acclimatisation.

Baseline VEGF levels

Among the 38 individuals studied, we found significant variation in baseline plasma VEGF levels (0–408 pmol/l). The upper limit of our range is 10-fold higher than that reported in healthy controls by Thickett et al., but others have reported plasma VEGF ranges similar to ours. It is unlikely that the high upper limit of baseline VEGF expression in our study is due to platelet-derived VEGF since plasma was separated at a high centrifugal force (3000g).

VEGF levels on ascent to altitude

VEGF levels changed significantly over the course of ascent and acclimatisation to high altitude, and a large majority (80%) of subjects demonstrated an increase in VEGF levels during acclimatisation to high altitude.

Hypoxia-induced upregulation of VEGF, both at a transcriptional and protein level in vitro, and in the circulation in vivo, in conditions characterised by systemic hypoxia such as acute respiratory distress syndrome (ARDS) is well documented. However, serum VEGF did not increase in a study of HAPE-susceptible individuals following 24 h exposure to hypobaric conditions equivalent to 4000 m. Previous studies described an increase in VEGF levels immediately following ascent but we demonstrate that in the majority of subjects there was a rise in VEGF levels by day 7 at 5200 m. It is possible that an immediate increase in VEGF levels following ascent to 5200 m was missed because we allowed subjects to partially acclimatise to 3650 m. At the post-expedition sea-level sample point, the failure of VEGF levels to return to pre-expedition values was unexpected. This suggests that exposure to high altitude may have caused some more prolonged alteration in the regulation or production of VEGF.

VEGF and AMS

Accumulation of in vitro data and animal studies suggested a role for the HIF-1 regulated cytokine VEGF in the pathogenesis of AMS and HACE. This hypothesis is supported by the observation that dexamethasone, which is effective both in the prophylaxis and treatment of AMS, blocks hypoxic induction of VEGF mRNA. Previous publications found no significant difference in plasma VEGF between individuals with AMS and those without. One controlled study involving 5 subjects suggested that VEGF change on ascent to altitude may be predictive of the onset of AMS. Our results do not support this hypothesis (Table 3), or the hypothesis that baseline VEGF levels predict AMS susceptibility (Fig. 3).

Recently Tissot van Patot et al. described lower levels of soluble VEGF receptor Flt-1 (sFlt-1) combined with a significant increase in plasma VEGF in individuals with AMS. The apparent discrepancy between this study and our work may be attributable to small sample size, individual variability and different ascent profile, including the altitude at which measurements were made. It should also be noted that subjects in their study did not reside at sea level but at altitudes of between 1370 and 1645 m. As a soluble factor under hypoxic regulation, sFlt-1 acts to sequester unbound VEGF from the circulation, therefore preventing its interaction with the endothelium-bound receptors that mediate its effects upon endothelial function and vascular permeability. A hypoxic-mediated increase in sFlt-1 could therefore counteract an increase in VEGF production preventing it from mediating an effect. In those individuals with AMS a lower level of sFlt-1 may allow

### Table 3 Tests for association between AMS category and percentage change in VEGF.

<table>
<thead>
<tr>
<th>Stage of expedition for diagnosis of AMS</th>
<th>Classification assumed for % change in VEGF</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 2 at 5200 m</td>
<td>Classification 1</td>
<td>0.207*</td>
</tr>
<tr>
<td>Day 2 at 5200 m</td>
<td>Classification 2</td>
<td>0.765</td>
</tr>
<tr>
<td>Day 2 at 3650 m</td>
<td>Classification 1</td>
<td>0.840*</td>
</tr>
<tr>
<td>Day 2 at 3650 m</td>
<td>Classification 2</td>
<td>1.000*</td>
</tr>
</tbody>
</table>

Analyses were performed comparing pre-expedition sea level to day 1 at 5200 m using the VEGF categories: 0, <200 <% change <0', '% change = 0' and 0 < % change <200', '% change >300' (classification 1) and using the VEGF categories % change <0' and % change >0' (classification 2). AMS is most likely to develop on the day following arrival at altitude and therefore we chose to use AMS data from day 2 at 3650 m and day 2 at 5200 m to examine the relationship between sea level VEGF expression and AMS category. Data were available for 35 subjects at 3650 m and 33 subjects at 5200 m; % change in VEGF was not available for 3 subjects and AMS data were not available for 2 subjects who had been evacuated by day 2 at 5200 m.

*Indicates those instances where Fisher’s exact test was used.
unbound VEGF to affect blood brain barrier permeability and contribute to the development of AMS. The importance of the cytokine in the development of high-altitude illness is therefore uncertain. Furthermore, recent data from T2 weighted magnetic resonance imaging (MRI) studies suggests that AMS may not be caused by vasogenic oedema as previously thought.31,32

The role of inflammatory mediators in the pathogenesis of high-altitude illness is currently under scrutiny. Swenson et al. proposed early HAPE to be due to non-inflammatory breach of the alveolar-capillary barrier33 given that initiation of HAPE is associated with an increase in pulmonary capillary pressure.34 Previous studies have proposed a role for VEGF in the pathogenesis of hypoxic pulmonary hypertension.4,35 However, our findings show no clear relationship between systemic VEGF and PASP on days 1, 3 and 7 at 5200 m.

Compartmentalisation of VEGF expression

Whilst there is no apparent relationship between plasma VEGF and AMS, the findings of this study as well as previous research do not preclude the involvement of VEGF in the pathogenesis of high-altitude illness. In a murine model of normobaric hypoxia-induced cerebral oedema, systemically delivered anti-VEGF neutralising antibody attenuated vascular leak. Brain tissue expression, but not plasma levels of VEGF were increased following the hypoxic insult.12 Hypoxia-induced VEGF production by brain-derived microvascular endothelial cells (BMEC) has been shown to act in an autocrine fashion in the regulation of blood–brain barrier permeability36 and it is likely that local tissue or compartmentalised expression of VEGF is critical in mediating oedema.12,37 A similar concept applies to the lung in which there is significant compartmentalisation of VEGF to the alveolar surface relative to the vascular compartment. This difference in distribution is attributable to both the production of VEGF in alveolar type II epithelial cells and a tight epithelial barrier preventing diffusion to the endothelial surface.38 Interestingly, VEGF levels in bronchoalveolar lavage fluid (BALF) of patients with HAPE have been shown to be significantly reduced relative to healthy control subjects.39 In ARDS a reduction in BALF VEGF is associated with a significant increase in circulating plasma VEGF, potentially due to loss of alveolar/epithelial barrier integrity.24,40

Limitations of the study

Our study had several limitations. Firstly, it was not possible to gather blood samples from subjects on day 2 at 5200 m or whilst at the intermediate altitude of 3650 m. We may therefore have missed an early rise in plasma VEGF on initial ascent to 3650 m or a change on day 2 at 5200 m, the day on which there was the highest prevalence of AMS.

By spending 4 days at 3650 m, subjects were able to partially acclimatise at this altitude and this may have reduced the impact of further ascent on VEGF expression. However, our study may more closely simulate the behaviour of many climbers because of the inclusion of a period of pre-acclimatisation before ascent to a summit. We believe that our ascent profile provides useful information on the role of VEGF in AMS, as despite this pre-acclimatisation ascent to 5200 m caused a sizeable fall in inspired oxygen fraction and provoked a high incidence of AMS.

Secondly, it has been suggested that measurement of absolute VEGF levels ought to quantify not only free VEGF levels but also its complexes with sFlt-1, the soluble VEGF receptor sKDR, and placenta growth factor (PLGF).41 At present, assays of these VEGF-complexes are technically demanding and not commercially available. However, as described previously, sFlt-1 levels may prove to be associated with AMS susceptibility.

Thirdly, on account of the size of our study cohort and the number of exclusions, it is not possible to exclude a relationship between unbound VEGF and AMS.

Finally, meaningful comparison with previous studies is hindered by different ascent profiles, final altitude of acclimatisation, the definition of AMS and subject demographics. Although our cohort was younger than in previous studies of VEGF at altitude, our study contributes novel data to this area by performing serial VEGF measurements following non-exertional ascent to very high altitude.

Conclusion

We have demonstrated a significant change in VEGF levels during acclimatisation to high altitude. Despite the popular hypothesis that hypoxic induction of VEGF resulting in alterations to blood–brain barrier permeability may lead to AMS and HACE, we found no evidence of a relationship between changes in unbound plasma VEGF levels and AMS. Nevertheless the possibility remains that at a cellular level, VEGF contributes to the pathogenesis of high-altitude illness through autocrine and paracrine effects at the blood–brain barrier.

Acknowledgements

We thank the volunteers and researchers who took part in the Apex 2 expedition; the Wellcome Trust Clinical Research Facility, Edinburgh; MRC Centre for Inflammation Research, Edinburgh; the Instituto de Investigaciones Fisicas, Universidad Mayor de San Andres; and the Instituto Boliviano de Biología de Altura, La Paz, Bolivia. We are very grateful to Marken, our international couriers.

References


