Oral antioxidant supplementation does not prevent acute mountain sickness: double blind, randomized placebo-controlled trial

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Summary

Background: Acute mountain sickness may be caused by cerebrovascular fluid leakage due to oxidative damage to the endothelium. This may be reduced by oral antioxidant supplementation.

Aim: To assess the effectiveness of antioxidant supplementation for the prevention of acute mountain sickness (AMS).

Design: A parallel-group double blind, randomized placebo-controlled trial.

Methods: The study was conducted in a university clinical research facility and a high altitude research laboratory. Eighty-three healthy lowland volunteers ascended to 5200 m on the Apex 2 high altitude research expedition. The treatment group received a daily dose of 1 g i-ascorbic acid, 400 IU of a-tocopherol acetate and 600 mg of a-lipoic acid (Cultech Ltd., Wales, UK) in four divided doses. Prevalence of AMS was measured using the Lake Louise Consensus score sheet (LLS). Secondary outcomes were AMS severity measured using a novel visual analogue scale, arterial oxygen saturation and pulmonary artery systolic pressure (PASP).

Results: Forty-one subjects were allocated to the antioxidant group, and 42 to the placebo group. There was no difference in AMS incidence or severity between the antioxidant and placebo groups using the LLS at any time at high altitude. At the pre-determined comparison point at Day 2 at 5200 m, 69% of the antioxidant group (25/36) and 66% of the placebo group (23/35) had AMS using the LLS criteria (P = 0.74). No differences were observed between the groups for PASP, oxygen saturation, presence of a pericardial effusion or AMS assessed by VAS.

Conclusion: This trial found no evidence of benefit from antioxidant supplementation at high altitude.

Trial registration number: NCT00664001

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Introduction

Acute mountain sickness (AMS), high altitude pulmonary oedema (HAPE) and high altitude cerebral oedema (HACE) are complications of ascent to high altitude. By definition, AMS is a benign condition, but it is likely that the same pathology underlies HACE.¹ In contrast, HAPE occurs in the context of pathologically elevated pulmonary artery pressures and uneven distribution of hypoxic pulmonary vasoconstriction across the pulmonary vascular bed.

Several features suggest that raised intracranial pressure (ICP) may be an important factor in the pathogenesis of AMS.²–⁴ Magnetic resonance imaging of HACE patients has demonstrated that the oedema in HACE is of the vasogenic type, rather than cytotoxic.⁵ Thus it is likely that altered cerebrovascular permeability has an important role in the development of AMS and HACE.

Reactive oxygen species (ROS) have been shown to alter the permeability of the blood–brain barrier in severe ischaemia, causing vasogenic cerebral oedema.⁶ Endogenous antioxidant systems may have some capacity to respond to oxidative stress in hypoxia. The plasma concentration of urate, a powerful endogenous antioxidant,⁷ rises on acute exposure to high altitude and may play a crucial antioxidant role in systemic hypoxia.⁸ This antioxidant prevents free-radical induced cerebral oedema in animal models.⁹

The pathogenesis of HAPE is understood to have two components: (i) increased pulmonary arterial pressures secondary to hypoxic pulmonary vasoconstriction¹⁰,¹¹ and (ii) an increase in endothelial permeability,¹² possibly due to stress rupture of pulmonary capillaries.¹³ There is much debate surrounding the cellular mechanisms of hypoxic pulmonary vasoconstriction, but it is likely that ROS have an important role.

There are numerous sources of ROS in hypoxia, including the mitochondrial electron transfer chain,¹⁴ haemoglobin (Hb) autoxidation¹⁵ and xanthine oxidase activity.¹⁶ There have been several reports of raised markers of oxidative stress in humans at moderate altitude (<3000 m).¹⁷–¹⁹

Oral antioxidant supplementation with preparations containing vitamins C and E in humans at altitude has been shown to decrease breath penticanes (a marker of oxidative stress),¹⁷ and improve erythrocyte filterability.²⁰,²¹ In a small randomized controlled trial, Bailey and Davies²² demonstrated a significant reduction in symptoms of AMS in subjects taking an oral antioxidant cocktail.

The antioxidants α-lipoic acid, vitamin C and vitamin E act synergistically to provide membrane protection from free radical damage, and may protect against hypoxia-induced vascular leakage.²³–²⁵ We hypothesized that this combination of antioxidants would reduce the severity of acute mountain sickness, and reduce pulmonary artery pressures, in healthy lowlanders acutely exposed to high altitude.

Methods

Apex 2: study population and sample size

Subjects participating in this study were recruited from the 103 members of the Apex 2 expedition to Bolivia. Of these, 83 participated in this trial. Expedition members were all healthy lowlanders who had not been >1500 m in the 3 months before the expedition. Informed consent was obtained from each subject after full written and verbal explanation of the study, which was approved by the Lothian Research Ethics Committee.

Subjects flew to La Paz, Bolivia (3800 m), where they acclimatized for 4 days before travelling by road to the Chacaltaya Research Laboratory (5200 m) where they stayed for 9 days. The expedition was split into five teams of 20–25 subjects who travelled to Bolivia in series, following an identical ascent profile. A team of eight scientists was stationed at Chacaltaya throughout the experiment. Blood samples and echocardiographic readings were taken on days 1, 3 and 7 at 5200 m. AMS questionnaires were completed daily throughout the expedition.

Oral antioxidant supplementation

The treatment group received a daily dose of 1 g L-ascorbic acid, 400 IU of α-tocopherol acetate and 600 mg of α-lipoic acid in sealed capsules. Placebo capsules were identical and made by the same supplier (Cultech Ltd., Wales, UK). Treatment commenced on the day of travel to high altitude, and continued for 14 days after ascent.

Randomization and double blinding

This trial was conducted in concert with a double-blind placebo-controlled randomized trial of sildenafil. A total of 103 research participants were entered into the randomization. A computer programme operated by an independent statistician (Dr Bill Adams, University of Edinburgh) was used to randomly assign volunteers to three groups: placebo/placebo (n = 42); antioxidant/placebo (n = 41); and placebo/sildenafil (n = 20). Antioxidant/placebo tablets were bottled by pharmacists experienced in
Outcomes

The primary outcome with respect to efficacy in AMS was the proportion of subjects with Lake Louise Consensus symptom score (LLS) > 3 on Day 2 at 5200 m. This is the time of peak AMS in previous studies following a similar ascent profile. Secondary outcomes were pulmonary artery pressure measured by echocardiography, oxygen saturation by pulse oximetry and the severity of AMS using a novel seven-question visual analogue scoring sheet (VAS). These tools provide a reliable means of assessing subjective symptoms. VAS scores are quoted in mm of symptom severity, with each symptom quantified at 0–100 mm.

Basic physiological measurements

Subjects’ resting blood pressure was measured daily using a mercury sphygmomanometer, and arterial oxygen saturation was measured using an infrared finger probe (Nellcor N-20PA, USA). Arterialized capillary blood gases were obtained by puncturing the ear lobe with a 19G needle (BD MicrolanceTM, BD Becton–Dickinson UK Ltd) following a 10-min application of a rubefacient (TransvasinTM cream, Seton Products, UK). Blood was drawn into 150-μl heparinized capillary tubes and analysed immediately using a calibrated blood gas autoanalyser (RapidlabTM 348, Bayer Diagnostics, UK). Measures of arterialized ear lobe PCO2 and pH show good agreement with arterial values.

Haematocrit

On each sample day at altitude, blood was drawn into a 2.7 ml EDTA tube using an 18G needle with participants in the supine position. Blood was transported, within 24 h, for analysis at a haematological laboratory in La Paz, Bolivia. At sea level, whole blood was analysed in the Western General Hospital, Edinburgh.

Echocardiography

Echocardiographic readings were obtained using a portable real-time, phased-array scanner with an integrated colour Doppler system (Acuson Cypress, Siemens Medical Solutions, UK). Subjects rested for 5 min before recordings were taken by experienced echocardiographers. Echocardiographs were stored for later quality control analysis. Systolic pulmonary arterial pressure was calculated from the pressure gradient between the right ventricle and the right atrium, using a modified Bernoulli equation. This method had an excellent correlation with invasive measurements in a high altitude field study.

Statistical analysis

Sample size was calculated a priori based on previous results. In order to achieve 80% power at a significance level of P < 0.05, we required a minimum of 32 subjects in each group to detect a 30% reduction in the prevalence of AMS on Day 2 at 5200 m. The difference in the proportion of AMS-positive individuals was assessed using the chi-squared test. AMS severity was compared using the Mann–Whitney U-test. Sequential parametric variables including PASP, SpO2, heart rate and full blood counts were compared between the drug and placebo groups using the Student’s t-test and a Bonferroni correction for multiple comparisons. VAS data were normalized using a square-root transformation. Statistical analysis was performed using manual calculations in Microsoft Excel 2007, and automated statistical tests in GraphPad Prism 3.0.

Ethical approval

The study was approved by the Lothian Research Ethics Committee.

Results

Exclusions

One subject developed HAPE before reaching the high altitude laboratory and was excluded from the trial. One subject (antioxidant group) was excluded from the AMS score analysis because of a gastrointestinal illness. Figure 1 shows the flow of participants through the trial. Seventeen subjects were evacuated from the high altitude laboratory and thereby withdrew from the trial because of...
severe symptoms of acute mountain sickness (10/41 antioxidant group, 7/42 placebo group). Data for these subjects are included until their evacuation. Anthropometric data are given in Table 1.

**Acute mountain sickness**

The overall incidence of AMS was comparable with previous studies in similar conditions. There was no difference in AMS incidence or severity between the treatment and placebo groups using the LLS scale at any time at high altitude. At the predetermined comparison point at Day 2 at 5200 m, 69% of the antioxidant group (25/36) and 66% of the placebo group (23/35) had AMS using the LLS criteria (P = 0.74). Likewise, there was no difference in symptom severity using the VAS scale (Table 2). A similar proportion of VAS scores was rejected due to a lack of agreement between repeated measures (placebo 20.5%; antioxidant 19.5%).

**Physiological observations and blood tests**

There was no difference between treatment groups in heart rate or arterial oxygen saturation. Haematocrit and capillary blood acid-base balance were also unaffected by treatment (Table 2).

**Pulmonary artery pressure**

Eight subjects were excluded from the PASP analysis by a blinded echocardiographer at the time of the examination (1/42 from the antioxidant group, 7/42 from the placebo group), as it was technically difficult to measure their PASP by transthoracic echocardiography. Sixteen further individual PASP readings were rejected as technically unsatisfactory following independent review after the expedition. There was no difference in PASP between antioxidant and placebo groups during acclimatization to high altitude (Figure 2).

**Discussion**

This trial demonstrates that the antioxidant mixture tested is not effective in reducing the incidence or severity of acute mountain sickness. Furthermore, there is no evidence of attenuation of altitude-induced pulmonary hypertension in subjects taking antioxidant supplements.

**Strengths and weaknesses**

Although there is wide variation, even within a single individual on successive days, in the severity of symptoms of acute mountain sickness, this trial was adequately powered to detect a substantial reduction the proportion of AMS-positive subjects such as that found by Bailey and Davies in a previous study of the same combination of

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**Table 1** Anthropometric characteristics of participants

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 42)</th>
<th>Antioxidant (n = 41)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.4 (±6.3)</td>
<td>21.2 (±2.3)</td>
<td>0.26</td>
</tr>
<tr>
<td>Male Sex (male/total)</td>
<td>62% (26/42)</td>
<td>44% (18/41)</td>
<td>0.10†</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.74 (±9.6)</td>
<td>1.73 (±9.7)</td>
<td>0.78</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.5 (±11.8)</td>
<td>67.2 (±10.1)</td>
<td>0.33</td>
</tr>
<tr>
<td>Body mass index</td>
<td>22.9 (±2.6)</td>
<td>22.5 (±2.6)</td>
<td>0.39</td>
</tr>
<tr>
<td>SpO2 (sea level)</td>
<td>98.3% (±1.3)</td>
<td>98.2% (±1.4)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Table shows mean ± SD. P-values are generated using unpaired Student’s t-test. †Chi-squared test.
antioxidants ($\alpha = 0.05$; $\beta = 0.8$, reduction in proportion of AMS-positive subjects: 25%). Furthermore, our controlled-ascent approach would be expected to increase the power of the study by eliminating variation in ascent profile within the study group. An incidental finding during this trial was the occurrence of pericardial effusions at high altitude, published elsewhere.\(^3\) Our trial was not powered to detect a difference between the treatment groups in prevalence of effusions.

Our result conflicts with the smaller study by Bailey and Davies, which found a 30% reduction in the mean AMS score over a 10-day period using the same antioxidant dose. The discrepancy with our result requires explanation. In that trial, treatment began 3 weeks before ascent, and ascent to high altitude was slower, taking 10 days and involved considerable physical effort. In contrast, we began treatment on the day of ascent, but prevented our subjects from taking unusual exercise to study the physiological effects of altitude in isolation. Although both studies were conducted at a similar altitude, our group had a more acute and consistent hypoxic exposure, ascending rapidly as a group by bus after only 4 days at an intermediate altitude. It is worth noting that the range of AMS symptom scores in the trial by Bailey and Davies was much smaller than in our study, indicating an unusually homogeneous population.

Table 2  Main outcomes

<table>
<thead>
<tr>
<th></th>
<th>n (placebo; antioxidant)</th>
<th>Placebo</th>
<th>Antioxidant</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLS (median)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1 (5200 m)</td>
<td>79 (40;39)</td>
<td>4 (IQR 2–5)</td>
<td>4 (IQR 2–7)</td>
<td>0.52*</td>
</tr>
<tr>
<td>Day 2 (5200 m)</td>
<td>71 (36;35)</td>
<td>4 (IQR 2–6)</td>
<td>5 (IQR 3–7)</td>
<td>0.35*</td>
</tr>
<tr>
<td>Day 3 (5200 m)</td>
<td>71 (36;35)</td>
<td>3 (IQR 1–5)</td>
<td>3 (IQR 1–6)</td>
<td>0.95*</td>
</tr>
<tr>
<td>Day 7 (5200 m)</td>
<td>58 (32;26)</td>
<td>1 (IQR 0–2)</td>
<td>0 (IQR 0–2)</td>
<td>0.54*</td>
</tr>
<tr>
<td>VAS (mm; min = 0; max = 700)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1 (5200 m)</td>
<td>71 (34;37)</td>
<td>195 (±113)</td>
<td>248 (±153)</td>
<td>0.23**</td>
</tr>
<tr>
<td>Day 2 (5200 m)</td>
<td>69 (34;35)</td>
<td>224 (±103)</td>
<td>243 (±116)</td>
<td>0.72**</td>
</tr>
<tr>
<td>Day 3 (5200 m)</td>
<td>61 (30;31)</td>
<td>223 (±129)</td>
<td>213 (±170)</td>
<td>0.53**</td>
</tr>
<tr>
<td>Day 7 (5200 m)</td>
<td>50 (27;23)</td>
<td>114 (±60)</td>
<td>125 (±94)</td>
<td>0.92**</td>
</tr>
<tr>
<td>SpO2 (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea level</td>
<td>69 (34;35)</td>
<td>98 (±1.3)</td>
<td>98 (±1.4)</td>
<td>0.77</td>
</tr>
<tr>
<td>Day 1 (5200 m)</td>
<td>74 (37;37)</td>
<td>77 (±8)</td>
<td>78 (±14)</td>
<td>0.88</td>
</tr>
<tr>
<td>Day 3 (5200 m)</td>
<td>55 (26;29)</td>
<td>75 (±5)</td>
<td>76 (±7)</td>
<td>0.45</td>
</tr>
<tr>
<td>Day 7 (5200 m)</td>
<td>37 (21;16)</td>
<td>77 (±7)</td>
<td>77 (±5)</td>
<td>0.84</td>
</tr>
<tr>
<td>PASP (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea level</td>
<td>67 (31;36)</td>
<td>17 (±5)</td>
<td>17 (±5)</td>
<td>0.92</td>
</tr>
<tr>
<td>Day 1 (5200 m)</td>
<td>72 (34;38)</td>
<td>34 (±10)</td>
<td>31 (±10)</td>
<td>0.57</td>
</tr>
<tr>
<td>Day 3 (5200 m)</td>
<td>62 (27;35)</td>
<td>32 (±8)</td>
<td>33 (±8)</td>
<td>0.37</td>
</tr>
<tr>
<td>Day 7 (5200 m)</td>
<td>53 (25;28)</td>
<td>33 (±7)</td>
<td>29 (±6)</td>
<td>0.24</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea level</td>
<td>78 (39;39)</td>
<td>44.4 (±2.7)</td>
<td>43.7 (±2.9)</td>
<td>0.27</td>
</tr>
<tr>
<td>Day 1 (5200 m)</td>
<td>73 (37;36)</td>
<td>47.5 (±5.2)</td>
<td>47.0 (±3.7)</td>
<td>0.58</td>
</tr>
<tr>
<td>Day 3 (5200 m)</td>
<td>68 (34;34)</td>
<td>49.9 (±5.0)</td>
<td>48.5 (±4.8)</td>
<td>0.23</td>
</tr>
<tr>
<td>Day 7 (5200 m)</td>
<td>63 (34;29)</td>
<td>49.6 (±4.5)</td>
<td>48.6 (±4.9)</td>
<td>0.45</td>
</tr>
<tr>
<td>PcCO2 (kPa)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea level</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Day 1 (5200 m)</td>
<td>42 (23;19)</td>
<td>3.5 (±0.3)</td>
<td>3.6 (±0.7)</td>
<td>0.41</td>
</tr>
<tr>
<td>Day 3 (5200 m)</td>
<td>38 (22;16)</td>
<td>3.3 (±0.3)</td>
<td>3.3 (±0.4)</td>
<td>0.90</td>
</tr>
<tr>
<td>Day 7 (5200 m)</td>
<td>20 (12;8)</td>
<td>3.2 (±0.4)</td>
<td>3.3 (±0.4)</td>
<td>0.72</td>
</tr>
<tr>
<td>H+ (µmol l$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sea level</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Day 1 (5200 m)</td>
<td>42 (23;19)</td>
<td>32.2 (±1.9)</td>
<td>30.4 (±6.8)</td>
<td>0.23</td>
</tr>
<tr>
<td>Day 3 (5200 m)</td>
<td>38 (22;16)</td>
<td>33.4 (±2.7)</td>
<td>33.7 (±3.8)</td>
<td>0.79</td>
</tr>
<tr>
<td>Day 7 (5200 m)</td>
<td>20 (12;8)</td>
<td>32.8 (±3.8)</td>
<td>33.3 (±3.4)</td>
<td>0.78</td>
</tr>
</tbody>
</table>

*Mann–Whitney U-test.

**Student's $t$-test on square-root transformed data.
One other study used small doses of an antioxidant mixture and found no effect of antioxidants on biochemical markers of oxidative stress, but with only nine subjects it was inadequately powered. A randomized trial of ginkgo biloba, which has antioxidant properties, found that it had no effect on symptoms of AMS.

The antioxidant mixture used is known to be biochemically and clinically effective in other disease states. Vitamin E has been shown to reduce pulmonary oxidative stress in patients with COPD. α-Lipoic acid prevents lipid peroxidation in humans with diabetic neuropathy and oral treatment reverses neuropathic symptoms. Trials of low dose vitamin C and E demonstrate a reduction in oxidative stress, although clinical results have been varied. An exogenous vitamin E-based antioxidant was neuroprotective in cerebral oedema, and an ascorbate-based intravenous infusion reduced intra-cranial pressure in a study of 80 patients with cerebral oedema of various aetiologies.

Secondary end points

Antioxidants also had no effect on pulmonary artery systolic pressure or arterial oxygen saturation. Although there is evidence that ROS are an important part of the mechanism of hypoxic pulmonary vasoconstriction, a fundamental problem has been elucidating whether an increase or a decrease in ROS leads to hypoxic vasoconstriction. Although much of the evidence for a role for ROS focuses on intracellular redox state, there is evidence that extracellular ROS have an important role, potentially due to a complex interaction with red blood cells. The failure of antioxidant supplementation to prevent pulmonary hypertension in this study cannot be taken as evidence against a role for ROS in hypoxic pulmonary vasoconstriction; it is, however, evidence against a clinical utility for antioxidants in this context. A larger number of subjects in the placebo group (seven subjects, compared with one in the treatment group) were difficult echo subjects and were excluded from the analysis because a reliable tricuspid regurgitation jet could not be found. Since there are no other significant differences between the groups, in particular in body mass index (Table 1), we conclude that this is most likely to be a chance variation.

The published evidence supports the hypothesis that exposure of healthy humans to hypobaric hypoxia leads to a global alteration of vascular permeability. Oedema of the lungs, brain, peripheral tissues and cornea has been previously described. This hypothesis is supported by the new finding of asymptomatic pericardial effusions in this study.

Conclusion

Overall, our data do not support the use of antioxidant supplementation in the prophylaxis or treatment of AMS. A variety of treatments with antioxidant properties are in widespread use among travellers to high altitude, often in preference to proven therapies such as acetazolamide. Our demonstration that a high-dose antioxidant triple-therapy does not reduce AMS suggests that antioxidant preparations are unlikely to be of benefit for the prophylaxis of AMS.

Conflict of interest

None declared.

Acknowledgements

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