Exaggerated Endothelin Release in High-Altitude Pulmonary Edema

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Background—Exaggerated pulmonary hypertension is thought to play an important part in the pathogenesis of high-altitude pulmonary edema (HAPE). Endothelin-1 is a potent pulmonary vasoconstrictor peptide that also augments microvascular permeability.

Methods and Results—We measured endothelin-1 plasma levels and pulmonary artery pressure in 16 mountaineers prone to HAPE and in 16 mountaineers resistant to this condition at low (580 m) and high (4559 m) altitudes. At high altitude, in mountaineers prone to HAPE, mean (±SE) endothelin-1 plasma levels were ≈33% higher than in HAPE-resistant mountaineers (22.2±1.1 versus 16.8±1.1 pg/mL, P<0.01). There was a direct relationship between the changes from low to high altitude in endothelin-1 plasma levels and systolic pulmonary artery pressure (r=0.82, P<0.01) and between endothelin-1 plasma levels and pulmonary artery pressure measured at high altitude (r=0.35, P=0.05).

Conclusions—These findings suggest that in HAPE-susceptible mountaineers, an augmented release of the potent pulmonary vasoconstrictor peptide endothelin-1 and/or its reduced pulmonary clearance could represent one of the mechanisms contributing to exaggerated pulmonary hypertension at high altitude. (Circulation. 1999;99:2665-2668.)

Key Words: endothelin • hypertension, pulmonary • altitude • edema • hypoxia

High-altitude pulmonary edema (HAPE) is a life-threatening form of noncardiogenic edema characterized by exaggerated pulmonary hypertension. Pulmonary hypertension enhances the pressure that forces liquid across the endothelial barrier into the alveolar space and is thought to play an important part in the pathogenesis of HAPE. Consistent with this concept, prevention of the exaggerated pulmonary vasoconstriction by nifedipine administration markedly reduces the incidence of pulmonary edema in HAPE-susceptible subjects. The exact underlying mechanisms predisposing to exaggerated altitude-induced pulmonary vasoconstriction remain unknown.

Endothelin-1, an endothelium-derived peptide, is a potent and long-lasting vasoconstrictor thought to play an important role in the regulation of pulmonary vascular tone. In humans, endothelin-1 plasma concentrations are closely associated with the severity of chronic pulmonary hypertension.

Endothelin-1 infusion increases pulmonary artery pressure in rats, cats, and humans, whereas the endothelin receptor antagonist bosentan attenuates hypoxia-induced pulmonary vasoconstriction in rats and pigs.

We therefore examined effects of high-altitude exposure (4559 m) on endothelin-1 plasma concentration and pulmonary artery pressure in mountaineers susceptible to HAPE and mountaineers resistant to such edema.

Methods

Subjects and Study Design

We studied 16 mountaineers (3 women and 13 men; age, 41±11 years [mean±SD]) who had had radiographically documented HAPE within the previous 4 years. Another 16 mountaineers (5 women and 11 men; mean age, 41±10 years) with a history of repeated alpine-style climbing to peaks ≥4000 m and no symptoms of HAPE or acute mountain sickness served as control subjects. One to 4 weeks after a baseline examination at low altitude (580 m; barometric pressure, 710 mm Hg), the subjects ascended in groups of 2 to 4 from 1130 to 4559 m (barometric pressure, 440 mm Hg) within 22 hours. The ascent consisted of a transport by cable car to an altitude of 3200 m; a 1 1/2-hour climb to an altitude of 3611 m, where the subjects stayed overnight; and on the next day, a 4 1/2-hour climb to the high-altitude research laboratory at Capanna Regina Margherita. The subjects then spent 2 days and 2 nights in this hut. The experimental protocol was approved by the institutional review board on human investigation, and all subjects provided written informed consent.

General Procedures

Subjects were studied at low altitude and 18 to 24 hours after arrival at the hut. After the subjects rested quietly in the supine position for
Arterial oxygen saturation (%)  
Endothelin-1 (pg/ml)  
Pulmonary artery pressure (mmHg)  

Figure 1. Mean ± SE values at high altitude (4559 m) for arterial oxygen saturation (Sao2), venous endothelin-1 plasma concentration and systolic pulmonary artery pressure in 16 mountaineers prone to HAPE (open bars) and 16 control subjects resistant to this condition (filled bars). *P < 0.05, patients vs control subjects.

Doppler Echocardiography
To measure systolic pulmonary artery pressure, echocardiographic recordings were obtained with a real-time, phased-array sector scanner (model 2500, Hewlett-Packard) with an integrated color Doppler system and a transducer containing crystal sets for imaging (2.5 MHz) and continuous-wave Doppler recording (1.9 MHz). The recordings were stored on VHS videotape for analysis by an investigator who was unaware of the subject’s clinical history. All reported values represent the mean of ≥3 measurements. Systolic pulmonary artery pressure was calculated from the pressure gradient between the right ventricle and the right atrium with continuous-wave Doppler echocardiography and the clinically determined mean jugular venous pressure. Color Doppler echocardiography was used to locate the tricuspid regurgitation jet. Maximal velocity was then determined by careful application of the continuous-wave sampler on the regurgitation jet. To calculate the transtricuspid pressure gradient, a modified Bernoulli equation was used, in which transtricuspid pressure equals 4 times the square of the tricuspid jet velocity.5 At this high-altitude laboratory, systolic pulmonary artery pressure measurements in 17 subjects obtained by echocardiography and pulmonary artery catheterization were found to be closely correlated (r = 0.87, P < 0.001), and the mean (±SD) difference between echocardiographic and invasive pulmonary artery pressure measurements was 0.5 ± 5.6 mm Hg.21

Radiography
Each morning, posteroanterior chest radiographs were obtained with a mobile unit (TRS, Siemens) with a fixed target-to-film distance of 140 cm at 133 kV and 4 to 6 mA · sec⁻¹. In subjects in whom clinical evidence of HAPE developed, additional radiographs were obtained when symptoms first appeared. The radiographs were analyzed according to previously described criteria by a radiologist who was unaware of the subject’s clinical history.5

Endothelin Measurement
Blood samples were drawn from subjects in the supine position from an antecubital vein and immediately put on ice. The samples were then centrifuged at 3000 rpm for 10 minutes, and the plasma was snap-frozen in liquid nitrogen. Endothelin-1 plasma levels were measured by radioimmunoassay (RIA) with SepPak C18 cartridges (Millipore-Waters) as described previously.22 Extraction recoveries were ≥90%. For the radioimmunoassay, an antiserum against endothelin-1 from Peninsula Laboratories was used. After a preincubation of the samples with antiserum for 24 hours, ¹²⁵I-labeled endothelin-1 (Anawa) was added, and the incubation was continued for another 24 hours. Bound and free endothelin-1 was separated by use of a second antibody system (Amerlux-M, Amersham). The sensitivity of the test was 0.5 pg/mL.

Statistical Analysis
Statistical analysis (JMP statistical software, SAS Institute) was performed by use of 2-tailed paired and unpaired t tests for comparisons between and within groups, respectively. Correlation coefficients were calculated according to the method of least squares. A value of P < 0.05 was considered statistically significant. Unless otherwise indicated, data are expressed as mean ± SE.

Results
After 18 to 36 hours at 4559 m, 8 of the 16 HAPE-prone subjects but none of the HAPE-resistant subjects developed radiographic evidence of pulmonary edema (radiographic score ranged from 2 to 16; mean, 9.6 ± 1.4).

Figure 1 shows that at high altitude, HAPE-prone subjects had more severe hypoxemia and pulmonary hypertension than HAPE-resistant subjects. In HAPE-prone subjects, the exaggerated pulmonary vasoconstriction was accompanied by significantly higher endothelin-1 plasma concentration (Figure 1). The endothelin-1 plasma level and systolic pulmonary artery pressure were comparable in HAPE-prone subjects who did (23.6 ± 1.4 pg/mL, 66 ± 3 mm Hg) and who did not (21.0 ± 1.6 pg/mL, 66 ± 5 mm Hg) develop pulmonary edema. Figure 2 shows that there was a direct correlation between the altitude-induced increase in endothelin-1 plasma concentration and systolic pulmonary artery pressure. At high altitude, there was also a direct relationship between endothelin-1 plasma levels and systolic pulmonary artery pressure (r = 0.35, P = 0.05), whereas endothelin-1 plasma concentration was inversely related to arterial oxygen saturation (r = −0.53, P < 0.005). In the 8 HAPE-prone subjects with radiological evidence of pulmonary edema, there was no significant relationship between radiological score and arterial oxygen saturation (r = −0.44, P = 0.27), whereas arterial oxygen saturation and endothelin-1 plasma levels were inversely related (r = −0.73, P = 0.04). Systemic arterial pressure at high altitude was comparable in both groups (mean arterial pressure, 99 ± 3 and 99 ± 2 mm Hg in HAPE-prone...
and HAPE-resistant subjects, respectively), and there was no significant relationship between systemic blood pressure and endothelin-1 plasma concentration.

At low altitude, systemic pulmonary artery pressure (25±1 versus 23±2 mm Hg) and arterial oxygen saturation (96±0% versus 97±0%) were comparable in HAPE-prone and HAPE-resistant subjects, whereas endothelin-1 plasma levels were slightly higher in HAPE-prone subjects (14.5±1.1 versus 11.8±0.7 pg/mL, \( P=0.04 \)).

**Discussion**

We found that at high altitude, subjects prone to HAPE have higher endothelin-1 plasma levels than mountaineers resistant to this condition and that a direct relationship exists between both the altitude-induced increase in endothelin-1 plasma concentration and systemic pulmonary artery pressure, as well as the endothelin-1 plasma level and the pulmonary artery pressure measured at high altitude. These findings suggest that at high altitude, the exaggerated release of the potent pulmonary vasoconstrictor peptide endothelin-1 may represent one of the mechanisms underlying the exaggerated pulmonary vasoconstriction universally observed in HAPE-prone subjects.\(^{10}\)

Endothelin-1 could play a part in this exaggerated pulmonary vasoconstrictor response in several ways. First, the pulmonary vascular bed is very sensitive to the vasoconstrictor effects of endothelin.\(^{33}\) Second, endothelin-1 infusion, which increases its circulating plasma concentration to levels comparable to those observed in the present study, augments vascular resistance in dogs.\(^{24}\) Third, endothelin-1 potentiates the vasoconstrictor effects of sympathetic activation,\(^{25-27}\) and its own vasoconstrictor effects are potentiated by a defect in nitric oxide synthesis,\(^{28,29}\) 2 conditions associated with HAPE susceptibility.\(^{5,30}\) Taken together with the present finding of a direct relationship between endothelin-1 plasma concentration and pulmonary artery pressure, these observations are consistent with the hypothesis that in HAPE-prone subjects, endothelin-1 plays a part in the exaggerated pulmonary vasoconstrictor response to high-altitude exposure.

Hypoxia is a potent stimulus of endothelin synthesis both in vitro and in vivo. Short-term hypoxia stimulates endothelin-1 in rat lung preparations\(^{31,32}\) and endothelin-1 gene transcription and synthesis in cultured human vascular endothelial cells.\(^{33}\) Prolonged hypoxic exposure increases endothelin-1 plasma levels in rats,\(^{34}\) humans exposed to high-altitude,\(^{34}\) and patients with primary pulmonary hypertension.\(^{33}\) The present findings are consistent with the concept that hypoxia stimulates endothelin-1 synthesis in the human vasculature in vivo, because at high altitude there was an inverse relationship between endothelin-1 plasma concentration and arterial oxygen saturation. The observation that endothelin-1 levels were similar in HAPE-prone subjects who did and who did not develop pulmonary edema suggests that the exaggerated stimulation of endothelin-1 release in these subjects is a primary phenomenon and does not occur secondary to edema. HAPE susceptibility may be associated with a defect in endothelium-dependent nitric oxide synthesis,\(^{5,35}\) and endothelin release can be inhibited by nitric oxide.\(^{35,36}\) An impairment of this nitric oxide–induced inhibition could be another mechanism contributing to the augmented endothelin-1 plasma levels in HAPE-prone subjects.

Even though the exact underlying mechanisms of high-altitude pulmonary edema are incompletely understood, endothelin-1 could contribute to HAPE susceptibility by augmenting capillary hydrostatic pressure\(^{37}\) and/or by increasing microvascular permeability.\(^{38,39}\) The lack of a correlation between venous endothelin-1 plasma levels and systemic arterial pressure argues against an important contribution of this peptide to the systemic vascular tone at high altitude. This observation is in accordance with findings in rats, indicating that hypoxia increases endothelin-1 mRNA in the right atrium and the lungs but not in the systemic vascular bed.\(^{35}\)

Finally, at low altitude, endothelin-1 plasma concentration in HAPE-prone subjects was also slightly but significantly higher than in HAPE-resistant subjects, whereas systolic pulmonary artery pressure was comparable in both groups. It is possible that under normoxic conditions, this slight elevation of endothelin-1 may not be sufficient to induce detectable pulmonary vasoconstriction. Alternatively, echocardiography may not be sensitive enough to detect small differences in pulmonary artery pressure that have been found between HAPE-prone and HAPE-resistant subjects with invasive measurements of pulmonary artery pressure.\(^{40}\)

In conclusion, we have shown that subjects susceptible to HAPE have higher venous endothelin-1 plasma levels than mountaineers resistant to this condition. The exact role played by this peptide in the pathogenesis of HAPE is not yet clear and awaits further investigation. The availability of orally active endothelin receptor antagonists should allow researchers to directly test its role in the near future.

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