Platelet count and function at high altitude and in high-altitude pulmonary edema

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HIGH-ALTITUDE PULMONARY EDEMA (HAPE) is an enigmatic disease occurring in susceptible individuals at altitudes >2,500 m above sea level (5, 8). It is a noncardiogenic, hydrostatic edema caused by an excessive rise in pulmonary artery pressure at normal left atrial pressure (20, 22) that leads to an increased capillary pressure (20). HAPE has been characterized as a noninflammatory high-permeability-type leak caused by stress failure of pulmonary capillaries (28, 31).

In an isolated perfused rabbit lung, a rapid rise of transmural pressures to 50 Torr induced ruptures of the epithelial and endothelial barrier (29). Electronmicrographs of such rabbit lungs show activated platelets adhering to exposed basement membranes. Thus activation of blood coagulation and in particular markers of platelet activation should be detectable in early stages of HAPE. Previous investigations had, however, shown that activation of platelets assessed by circulating levels of β-thromboglobulin and platelet factor 4 and plasminatic coagulation assessed by markers of thrombin and fibrin formation are only detectable in advanced cases of HAPE and appear therefore to be a consequence rather than a cause of HAPE (2–4, 6, 7).

Functional assays of platelet adhesion and aggregation and measurements of soluble P (sP)-selectin, another marker of platelet activation, have not yet been prospectively investigated in HAPE-susceptible individuals during exposure to high altitude. To further investigate the possibility of platelet activation in beginning HAPE, we performed these analyses in a placebo-controlled double-blind study that was designed to evaluate the prevention of HAPE in susceptible individuals by tadalafil, a phosphodiesterase 5 (PDE5) inhibitor, and dexamethasone after rapid ascent to an altitude of 4,559 m. Platelets also express PDE5 (27), and PDE5 inhibitors influence platelet aggregation by increasing intracellular cGMP and activate protein kinase G (13, 30), which made our study with tadalafil especially interesting. In addition, the same examinations were performed in a control group of nonsusceptible individuals with identical exposure to high altitude. We hypothesized that subjects developing HAPE would have increased platelet activation and higher plasma levels of sP-selectin, with the highest values shown in those with the greatest increases in pulmonary artery pressure. Such findings would support the concept of structural damage accounting for the leak in HAPE.

METHODS

Subjects. A total of 40 nonacclimatized volunteers (4 women, 36 men, age 40 ± 9 yr) were included in the study. Thirty of them were HAPE susceptible; i.e., they had suffered at least one episode of HAPE before this study. They were recruited directly by the investigators and by advertisements in alpine club journals. Ten volunteers, who never had suffered HAPE before, served as controls. All volunteers had to be in healthy condition and were not allowed to take any medication, especially no aspirin, clopidogrel, or antihypertensive drugs, throughout the study period. They gave their written, informed consent to the study, which had been approved by the ethical committee of the University of Zurich.

Study design. Baseline measurements at low altitude were done at the University Hospital of Zurich in Switzerland (490 m, average barometric pressure 710 Torr). In the evening between 5:00 and 9:00 PM and the next morning between 7:00 and 9:00 AM, the following investigations were performed: clinical examination, chest x-ray, lung function test, noninvasive hemodynamic examinations with echocardiography, measurement of the dynamic cerebral blood flow autoreg-
Blood sampling.

After the baseline investigations in Zurich, HAPE-susceptible subjects were assigned in a random and double-blind fashion to either 10 mg two times a day tadalafil, 8 mg two times a day dexamethasone, or placebo, starting on the day before ascent to high altitude. Within 4 wk after the initial examination at low altitude, the subjects ascended in <1 day to 4,559 m (Capanna Regina Margherita, average barometric pressure of 440 Torr). They were taken by cable car from Alagna, Italy (1,200 m), to an altitude of 3,200 m (Punta Indren), from where they climbed within 1.5 h to an altitude of 3,650 m (Capanna Gnifetti), where they stayed overnight. The next morning, they climbed under professional guidance within 4 h to the Capanna Regina Margherita, where all research equipment used at low altitude had been installed. In the evening (5:00 to 8:00 PM) of the arrival day at high altitude, i.e., after a rest of at least 5 h, and the next morning (7:00 to 10:00 AM), the volunteers were clinically examined, and all of the above-mentioned investigations performed at low altitude (490 m) were repeated at this high altitude of 4,559 m.

Blood sampling. Blood was drawn by clean venipuncture from an antecubital vein (S-Monovette, Sarstedt, Nümbrecht, Germany). First, 4.5 ml of blood were drawn into 0.5 ml of CTAD-PPACK anticoagulant [stock solution containing 25 ml of citrate-theophylline-adenosine-dipyridamole (Becton Dickinson, Rutherford, NJ) plus 5 mg of phenyl-prolyl-argininechloromethylketone (Calbiochem, La Jolla, CA), giving a PPACK concentration of 382 µM]. Another 4.5-ml tube containing 0.129 M buffered sodium citrate (pH 5.5) was used for platelet function analyzer (PFA-100) analysis, and a K3-EDTA blood sample was used for blood cell count. CTAD-PPACK samples were immediately centrifuged at 2,000 g for 30 min at 4°C, and the plasma was aspirated, frozen in liquid nitrogen, and stored below −60°C until measurement of prothrombin fragment 1 + 2 (Enzygnost-F1 + 2, Dade Behring, Marburg, Germany) and thrombin-antithrombin III complexes (Enzygnost-TAT, Dade Behring). Blood cell count, including platelet count, was performed with a Cobas Micros 60 S/N (Axon Lab, Dättwil, Switzerland). Blood samples assigned to PFA-100 analyses were stored at room temperature for 30–60 min before measurements.

**Platelet aggregatory function.** PFA-100 (Dade Behring) was used to test platelet adhesion and aggregation under high shear stress conditions in vitro (18). The PFA-100 instrument is composed of a microprocessor-controlled device and single-use cartridges. The test cartridges consist of a sample reservoir, a capillary, and a membrane coated with 2 mg of type I collagen and either 10 mg of epinephrine bitartrate (EPI) or 50 mg of ADP. Blood is pipetted into the reservoir and aspirated through a capillary with a diameter of 200 µm with constant negative pressure, resulting in high shear forces (5,000–6,000 s⁻¹). The capillary ends in a membrane aperture with a diameter of 150 µm. Platelets adhere to the collagen and become activated by either EPI or ADP and aggregate, which ultimately leads to a complete stop of blood flow by an occluding platelet plug. The time from the beginning of the test until formation of the occluding platelet plug was measured in seconds as closure time. Measurements were performed in duplicate, and mean values were calculated. The instrument was tested in a decompression chamber (ETH, Zurich, Switzerland) at the simulated altitude of 4,600 m before flying it to the high-altitude research laboratory, and it was found to give reliable, reproducible results under both conditions.

**Measurement of sP-selectin.** sP-selectin, a marker of in vivo platelet activation, was measured in EDTA plasma by a commercially available ELISA kit (R&D Systems, Abingdon, UK).

**Statistical analysis.** Values are presented as means ± SD. Statistical analyses were performed using Wilcoxon’s signed rank test. Unless otherwise indicated, a P value of <0.05 was considered statistically significant.

**RESULTS**

Platelet counts at low and high altitude for all subjects are shown in Fig. 1. They were slightly higher in the evening compared with in the morning. At high altitude, the platelet count decreased by >20% compared with at low altitude.

Platelet aggregation as measured with PFA-100 closure times using epinephrine as an activating agent is shown in Fig. 2. At low altitude, closure times were shorter in the morning, indicating increased platelet aggregability. At high altitude,
Closure times were decreased by ~27% in the evening and 18% in the morning compared with low-altitude values. With ADP as an activating agent (ADP cartridge), PFA-100 closure times were in general shorter than with EPI cartridges, which is shown in Fig. 3. Similar to EPI, ADP closure times were shorter in the morning at low altitude and were much shorter at high altitude (~26% in the evening and 14% in the morning) compared with at low altitude.

Eight HAPE-susceptible subjects effectively developed HAPE during their stay at high altitude; seven of these had been administered placebo, and one had been administered tadalafil. Platelets were not involved in HAPE development, which is shown in Table 1. Platelet count and closure times with EPI or ADP were not different in the control group and the group developing HAPE, and the different treatments did not affect any parameter.

Plasma levels of sP-selectin were measured in the morning. The results are shown in Fig. 4. A 250% increase of this marker of platelet activation was observed at high altitude compared with at low altitude. sP-selectin values were similar in controls and subjects developing HAPE, and medication in HAPE-susceptible individuals had no influence on these values, as shown in Table 2.

Whereas platelets were activated by high altitude, plasma coagulation was not, which is summarized in Table 3.

### Table 1. Platelet count and aggregatory function (EPI and ADP closure times) in HAPE-susceptible subjects treated with either tadalafil, dexamethasone, or placebo and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>450 m Evening</th>
<th>450 m Morning</th>
<th>4559 m Evening</th>
<th>4559 m Morning</th>
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</thead>
<tbody>
<tr>
<td><strong>Platelet count, ×10^3 μl</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HAPE-susceptible subjects</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With tadalafil</td>
<td>7</td>
<td>259±39</td>
<td>246±44</td>
<td>196±33†</td>
<td>172±30†</td>
</tr>
<tr>
<td>With dexamethasone</td>
<td>9</td>
<td>237±44</td>
<td>241±27</td>
<td>204±45*</td>
<td>199±42*</td>
</tr>
<tr>
<td>With placebo</td>
<td>8</td>
<td>259±68</td>
<td>253±68</td>
<td>206±56*</td>
<td>189±53*</td>
</tr>
<tr>
<td>Those who developed HAPE</td>
<td>8</td>
<td>272±70</td>
<td>249±73</td>
<td>206±52†</td>
<td>186±51*</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>222±31</td>
<td>210±35</td>
<td>170±23‡</td>
<td>166±23†</td>
</tr>
<tr>
<td><strong>EPI closure time, s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>HAPE-susceptible subjects</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With tadalafil</td>
<td>7</td>
<td>135±41</td>
<td>129±32</td>
<td>99±19</td>
<td>97±24*</td>
</tr>
<tr>
<td>With dexamethasone</td>
<td>9</td>
<td>148±23</td>
<td>127±22</td>
<td>98±12†</td>
<td>99±7†</td>
</tr>
<tr>
<td>With placebo</td>
<td>8</td>
<td>137±19</td>
<td>125±35</td>
<td>117±24</td>
<td>103±19</td>
</tr>
<tr>
<td>Those who developed HAPE</td>
<td>8</td>
<td>133±18</td>
<td>131±37</td>
<td>116±23</td>
<td>110±26</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>137±15</td>
<td>127±15</td>
<td>94±27‡</td>
<td>110±25</td>
</tr>
<tr>
<td><strong>ADP closure time, s</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAPE-susceptible subjects</td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With tadalafil</td>
<td>7</td>
<td>98±19</td>
<td>94±19</td>
<td>66±8‡</td>
<td>70±10†</td>
</tr>
<tr>
<td>With dexamethasone</td>
<td>9</td>
<td>97±15</td>
<td>89±12</td>
<td>72±7‡</td>
<td>70±8†</td>
</tr>
<tr>
<td>With placebo</td>
<td>8</td>
<td>96±10</td>
<td>89±12</td>
<td>81±5‡</td>
<td>78±13</td>
</tr>
<tr>
<td>Those who developed HAPE</td>
<td>8</td>
<td>96±12</td>
<td>89±13</td>
<td>79±7*</td>
<td>75±9*</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>96±23</td>
<td>88±10</td>
<td>68±6‡</td>
<td>75±12*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of subjects. HAPE, high-altitude pulmonary edema; EPI, epinephrine. *P < 0.05, †P ≤ 0.01, ‡P ≤ 0.001 compared with corresponding values at 450 m (Wilcoxon’s test).

Fig. 3. PFA-100 closure times with ADP as an activating agent (ADP cartridge) in all subjects at low (450 m) and high (4,559 m) altitude in the evening and the next morning.

Fig. 4. Soluble P (sP)-selectin in controls and high-altitude pulmonary edema-susceptible individuals in the morning at low (450 m) and high (4,559 m) altitude, respectively (n = 39).
monitoring of thrombin formation by the measurement of prothrombin fragment 1+2 as well as the formation of thrombin-antithrombin III complexes was not significantly affected by high-altitude exposure, neither in controls nor in HAPE-susceptible individuals treated with tadalafil, dexamethasone, or placebo.

DISCUSSION

We could not confirm our hypothesis because we found no association of in vivo and in vitro platelet activation with HAPE. All subjects showed, independent of clinical findings and medication, a consistent fall in platelet count, an increase in sP-selectin, a marker of in vivo platelet activation, and a decreased platelet count suggest platelet consumption or damage due to high capillary pressures could lead to exposure of basement membrane and activation of platelets as shown in animal models (24, 32). In recent years, it has become evident that PFA-100 is also capable of detecting activated platelets (24, 32). One mechanism might be the increased shear stress imposed on platelets by the accelerated blood flow, since the PFA-100 method has initially been used instead of the cumbersome classical bleeding time to detect platelet hypofunction and von Willebrand disease (24, 32). In recent years, it has become evident that PFA-100 is also capable of detecting activated platelets (11, 25). Our results confirm PFA-100 measurements on animals exposed to hypobaric hypoxia, which also showed an increased platelet aggregation (23). This indicates an increased adhesion of platelets to collagen and increased platelet aggregation, leading to a more rapid closure of the pore by a platelet plug, despite lower platelet counts as described above. So far, this is only a phenomenological description; the pathophysiological mechanisms and signaling pathways behind it remain to be elucidated.

Where have all the platelets gone? The acute onset on the first day at high altitude of an increased platelet aggregation and a decreased platelet count suggest platelet consumption or sequestration somewhere in the circulation. The marked increase of sP-selectin at high altitude in this study supports such a concept of platelet consumption. There is a growing body of evidence that P-selectin, an adhesion molecule of the integrin family, is a component of the α-granule membrane, which appears on the platelet surface on activation, where it is cleaved off to produce the soluble form (sP-selectin). It is a reliable marker of in vivo platelet activation and is not affected by ex vivo activation (9, 16). The marked increase of P-selectin at high altitude contrasts with the results of previous studies (2), in which plasma levels of β-thromboglobulin did not increase in comparable settings. This discrepancy raises the question of whether sP-selectin is a more sensitive marker of platelet activation, whether in addition it reflects endothelial cell activation at high altitude with an increased release from endothelial Weibel-Palade bodies (10), or whether its clearance is reduced in hypoxia.

The platelet count was substantially lower on the day of arrival at high altitude and the following day. This may be an acute effect in the first 24 h, since a stay of more than 2 days had been found to increase platelet counts (14, 15, 26). It is, however, a controversial issue because others have shown that the platelet count decreases after exposure to high altitude (12), and it has also been found that decompression from depth has the same effect (21). Other studies, including one from our group under the same conditions (2, 27), found no consistent change in platelet count at high altitude. Our measurements were done after several hours of rest on each occasion, which makes an exercise-induced change (1) rather unlikely. The circadian variation with lower platelet counts in the morning vs. in the evening corroborates our earlier observation at low altitude (25).

The major findings of this study are the shorter closure times with both ADP and EPI at high altitude. The PFA-100 method has initially been used instead of the cumbersome classical bleeding time to detect platelet hypofunction and von Willebrand disease (24, 32). In recent years, it has become evident that PFA-100 is also capable of detecting activated platelets (11, 25). Our results confirm PFA-100 measurements on animals exposed to hypobaric hypoxia, which also showed an increased platelet aggregation (23). This indicates an increased adhesion of platelets to collagen and increased platelet aggregation, leading to a more rapid closure of the pore by a platelet plug, despite lower platelet counts as described above. So far, this is only a phenomenological description; the pathophysiological mechanisms and signaling pathways behind it remain to be elucidated.

Table 2. Plasma soluble P-selectin values taken in the morning at low (450 m) and high (4,559 m) altitude in HAPE-susceptibles subjects and controls

<table>
<thead>
<tr>
<th>n</th>
<th>At 450 m</th>
<th>At 4,559 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAPE-susceptible subjects</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>With tadalafil</td>
<td>7</td>
<td>61 ± 24</td>
</tr>
<tr>
<td>With dexamethasone</td>
<td>9</td>
<td>54 ± 19</td>
</tr>
<tr>
<td>With placebo</td>
<td>8</td>
<td>47 ± 15</td>
</tr>
<tr>
<td>Those who developed HAPE</td>
<td>8</td>
<td>51 ± 17</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>45 ± 14</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of subjects. *P < 0.01, †P < 0.001, compared with low altitude.

Table 3. PTF 1+2 and TAT complexes (TAT) at low and high altitude

<table>
<thead>
<tr>
<th>n</th>
<th>Morning</th>
<th>Evening</th>
<th>Morning</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTF 1+2, mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAPE-susceptible subjects</td>
<td>24</td>
<td>0.84 ± 0.19</td>
<td>0.78 ± 0.19</td>
</tr>
<tr>
<td>With tadalafil</td>
<td>7</td>
<td>0.91 ± 0.21</td>
<td>0.79 ± 0.19</td>
</tr>
<tr>
<td>With dexamethasone</td>
<td>9</td>
<td>0.83 ± 0.17</td>
<td>0.70 ± 0.11</td>
</tr>
<tr>
<td>With placebo</td>
<td>8</td>
<td>0.78 ± 0.20</td>
<td>0.85 ± 0.25</td>
</tr>
<tr>
<td>Those who developed HAPE</td>
<td>8</td>
<td>0.85 ± 0.22</td>
<td>0.85 ± 0.25</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>0.74 ± 0.12</td>
<td>0.67 ± 0.16</td>
</tr>
<tr>
<td>TAT, μg/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HAPE-susceptible subjects</td>
<td>24</td>
<td>0.89 ± 0.51</td>
<td>0.85 ± 0.47</td>
</tr>
<tr>
<td>With tadalafil</td>
<td>7</td>
<td>1.01 ± 0.69</td>
<td>0.80 ± 0.33</td>
</tr>
<tr>
<td>With dexamethasone</td>
<td>9</td>
<td>0.96 ± 0.56</td>
<td>1.02 ± 0.62</td>
</tr>
<tr>
<td>With placebo</td>
<td>8</td>
<td>0.72 ± 0.23</td>
<td>0.72 ± 0.39</td>
</tr>
<tr>
<td>Those who developed HAPE</td>
<td>8</td>
<td>0.92 ± 0.67</td>
<td>0.70 ± 0.38</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>1.19 ± 0.92</td>
<td>0.54 ± 0.39</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = no. of subjects. PTF 1+2, prothrombin fragment 1+2; TAT, thrombin-antithrombin III.
evidence that the pulmonary capillaries are the deposition and sequestration sites of platelets activated by various stimuli such as hypoxia (12, 23) but also endotoxinemia (17). One may speculate that such (pre)capillary platelet deposits could contribute to the increased pulmonary artery pressure and the uneven lung perfusion with overperfusion of unaffected vascular beds.

The two investigated drugs, dexamethasone and taladafil, had no influence on platelet count or function. Although this was expected for dexamethasone, it is somewhat surprising that taladafil had no effect because platelets express PDE5, the specific ligand of taladafil, on their surface. Because the mode of action of PDE5 inhibitors seems to be complex and probably biphastic (19), we may have missed the time points where an effect could have been observed.

In conclusion, we found that hypobaric hypoxia induced by an ascent to 4,559 m results in platelet activation and sequestration with a decrease in platelet count. This phenomenon is an ascent to 4,559 m results in platelet activation and sequestration (19), we may have missed the time points where an action could have been observed.

Platelet activation is, therefore, not a pathophysiological mechanism with a decrease in platelet count. This phenomenon is an ascent to 4,559 m results in platelet activation and sequestration sites of platelets activated by various stimuli such as hypoxia (12, 23) but also endotoxinemia (17). One may speculate that such (pre)capillary platelet deposits could contribute to the increased pulmonary artery pressure and the uneven lung perfusion with overperfusion of unaffected vascular beds.

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