

Platelets and Blood Cells

Effect of altitude on thrombopoietin and the platelet count in healthy volunteers

Sabine Hartmann, Alexander Krafft, Renate Huch, Christian Breyermann

Perinatal Research Unit, Department of Obstetrics, Zurich University Hospital, Switzerland

Summary

Although there is evidence that altitude increases the platelet count, its effect on the platelet precursor stimulating factor, thrombopoietin (TPO), is unclear. Unlike erythropoietin, TPO appears largely unresponsive to exogenous signals. In a study in 16 healthy volunteers, we report the effects of altitude exposure at between 1000 and 1822 m for 1 or 2 weeks on TPO, the platelet count (+ indices), erythropoietin, hemoglobin, hematocrit and erythrocytes (+ indices). There were significant post-expo-

sure increases in TPO (57.9 vs 37.1 U/l; $P = 0.0006$), platelet count (219.1 vs 208.0 $\times 10^3$ /ml; $P = 0.031$) and erythropoietin (16.1 vs 9.9 U/l; $P = 0.0032$). There was a positive correlation between the increases in TPO and platelet count ($r = 0.52$, $P = 0.043$). Hemoglobin and hematocrit remained unchanged. Our results provide clear evidence for a relationship, presumably driven by hypoxia, between altitude exposure, TPO production and the platelet count.

Keywords

Thrombopoietin, erythropoietin, hypoxia, altitude

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Introduction

Thrombopoietin (TPO) is the most potent stimulant of megakaryopoiesis and thrombopoiesis. Like its red cell homologue, erythropoietin (EPO), TPO prevents apoptosis of its target cells. Its primary production site is the liver; subsidiary sites include the kidneys, spleen, bone marrow and brain (1). Levels are regulated by the megakaryocyte mass; they appear less responsive to exogenous signals than EPO (2). TPO is cleared from the circulation by the TPO receptor found on platelets, hematopoietic stem cells, megakaryocyte precursors and megakaryocytes, creating an inverse correlation between TPO levels and the platelet count (3).

In humans, the sole relevant study to date found no evidence that TPO mediates the increased platelet count observed in response to altitude (4). However, we recently observed an increased TPO level combined with an increased platelet count following altitude exposure in a patient with pregnancy-induced immune-mediated thrombocytopenia (ITP) (5). We wished to test this anecdotal observation in a study in healthy volunteers.

Materials and methods

Following approval by the cantonal ethics committee, blood samples were taken in 16 consenting healthy and sportive subjects (F: $n = 9$; M: $n = 7$) a few hours before and after altitude exposure (1000–1822 m) for 1 week ($n = 9$) or 2 weeks ($n = 7$). During these stays physical activity consisted mainly of recreational skiing. Baseline values were measured in Zurich (411 m). Post exposure blood samples were taken at baseline altitude immediately after returning from altitude, kept at 4°C and processed within one hour. Samples for full blood counts were taken in ethylenediaminetetraacetic acid treated tubes, samples for platelet studies were taken in sodium citrate (0.129 M; 3.8%) tubes (both BD Vacutainer® Systems, BD, Franklin Lakes NJ). Sodium citrate glass tubes are used to minimize artificial platelet activation. The following parameters were determined using a flow cytometric hematology analyser (Advia® 120, Bayer Diagnostics Europe Limited, Dublin): hemoglobin, hematocrit, erythrocytes + indices (mean corpuscular volume [MCV], red cell distribution width [RDW], hypochromic erythrocytes (%), hy-

Correspondence to:
Dr. Alexander Krafft
Perinatal Physiology Research Unit
Department of Obstetrics and Gynaecology
University Hospital Zurich
Frauenklinikstr. 10
8091 Zurich
Switzerland
Tel.: +41 12555148, Fax: +41 12554430
E-mail: alexander.krafft@usz.ch

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perchromic erythrocytes [%], microcytic erythrocytes [%], macrocytic erythrocytes [%]) and platelet count + indices (mean platelet component [MPC], platelet component distribution width [PCDW], mean platelet dry mass [MPM], and platelet dry mass distribution width [PMDW]). The platelet density method used by the flow cytometric hematology analyser is described elsewhere (6). EPO (serum) and TPO (plasma) were determined by enzyme-linked immunosorbent assay (R&D Systems Europe, Abingdon, UK). The plasma for TPO analysis was collected in lithium heparin treated glass tubes (BD Vacutainer® Systems, BD, Franklin Lakes NJ) and centrifugated for 15 minutes at 1000 g within 30 minutes of collection. To warrant minimal platelet contamination, an additional centrifugation step was done at 10000 g for 10 minutes at 4°C according to the TPO assay manufacturers guidelines. The aliquoted samples were stored at -80°C and measured when sampling from all volunteers was completed.

The statistical analysis (StatView, version 5.0.1, SAS Institute Inc., Cary NC) used the arithmetic mean, standard deviation, the Wilcoxon and Mann-Whitney tests for non-parametric multiple comparisons of the mean, and Spearman's rank correlation coefficient.

Results

There were significant increases in TPO ($P = 0.0006$), platelets ($P = 0.031$) and EPO ($P = 0.003$) (Table 1). Hemoglobin, hematocrit and platelet indices remained unchanged. There was significant correlation between the increases in TPO and platelets ($r = 0.52$, $P = 0.043$), but not between the increases in EPO and platelets ($r = 0.26$, $P = 0.32$). The increases in microcytic erythrocytes ($P = 0.01$) and the erythrocyte distribution curve ($P = 0.008$) remained within the normal range. The other erythrocyte indices did not alter significantly.

One patient showed an exceptionally high increase in serum EPO values (EPO baseline 7 U/l, post altitude exposure EPO 53 U/l). If one excludes this subject from analysis, the difference between baseline and post exposure EPO Levels remains statistically significant (10.1 ± 7.8 U/l vs. 13.6 ± 8.0 U/l; $P = 0.005$).

For detection of potential platelet activation, mean platelet component (MPC) and mean platelet dry mass (MPM) were measured before and after altitude exposure. The difference between pre and post exposure values was not statistically significant different for either MPC (25.85 ± 2.65 g/dl vs. 26.17 ± 1.84

g/dl; $P = 0.66$) or MPM respectively (1.90 ± 0.14 pg vs. 1.90 ± 0.14 pg; $P = 0.83$).

Discussion

Our results confirm our observations in a patient with ITP who showed a combined increase in TPO and platelets following altitude exposure (5). TPO, platelets and EPO all increased in 15 of the 16 healthy volunteers, despite ascent to a relatively low altitude (from 400 m to 1000–1822 m), compared to the study of Hudson et al (ascent from 600 m to 3600 m) (4). But our results seem to contradict the results of Hudson et al, which did not support a role of TPO as a mediator of increased platelet count in altitude (4). Admittedly our results confirm the reports of altitude-induced thrombocytosis (7).

Although hypoxia is a well-established determinant of EPO, previous evidence suggests that external signals have little effect on the synthesis of TPO: thus in human hepatoma cultures, hepatocytes express the TPO gene in a constitutive manner, irrespective of the platelet count (8).

Our study indicates an effect of altitude exposure on TPO presumably driven by hypoxia, since it shows a parallel increase of the two cytokines, TPO and EPO. Both share similarities not only in molecular structure but also in their sites of production. The liver is the primary site of EPO synthesis in the fetus, while the epithelial cells of the renal proximal tubule, where EPO is produced in the adult, also express TPO mRNA (8).

Our findings are in accordance with the results of Hudson et al who also reported an increase in platelets in 26 normal subjects 48 h and one week after ascending from 600 to 3600 m (from 251 to 367 ± 103 /ml to 398 ± 103 /ml). Besides hemoconcentration, which is a physiological step of acclimatization to altitude (9), the increase in platelet count could be explained by an increase in production or a decrease in elimination of TPO. Likewise Hudson et al showed a slight increase of TPO in the examined population (70.6 ± 12.7 vs. 92 ± 12 pg/ml) (4). Nevertheless, with the recent findings in mind, they drew a theoretically challengeable conclusion, i.e. TPO is not a mediator for increased platelet count in altitude.

Other possible explanations for a rise in platelet count, which have potentially to be taken into account, are an increased physical exercise during the stay at altitude, medication intake or a change of diet, which can interfere with platelet production and platelet function. None of the volunteers reported the use of medication whatsoever. Since the sport activity consisted mainly of recreational downhill skiing and all volunteers were average trained and not professional athletes, a substantial increase in physical exercise can be excluded as well as a pivotal change in their everyday diet.

Accidental platelet activation and consecutive release of TPO from c-mpl receptors was excluded by measuring mean MPC and MPM in the blood samples taken before and after altitude exposure. Especially MPC, which decreases after platelet activation is regarded as a useful parameter for assessing activated platelets (6).

Our study may have only limited clinical implications since the platelet count remains within the normal range after exposure to altitude. But bearing in mind the increase of platelet count in

Table 1: Haematology values [mean \pm standard deviation (range)] pre and post altitude exposure in 16 subjects. (* = $p < 0.05$; ** = 0.01 ; n.s. = statistically not significant).

| | Normal range | Baseline | Post altitude exposure | P |
|----------------------------------|--------------|-------------------------------|-------------------------------------|--------|
| Platelets ($10^3/\mu\text{l}$) | 130 – 400 | 208 ± 55.9 (112–310) | $219 \pm 55.4^*$ (127–362) | 0.031 |
| TPO (U/l) | 1 – 60 | 37.1 ± 23.6 (1–102) | $57.9 \pm 26.3^{**}$ (14–104) | 0.0006 |
| EPO (U/l) | 8 – 22 | 9.9 ± 7.6 (3–34) | $16.1 \pm 12.5^{**}$ (4–53) | 0.003 |
| Hb (g/dl) | 12.0 – 18.0 | 12.8 ± 1.4 (10.6–15.7) | 13.2 ± 1.5 n.s. (10.8–16.5) | 0.46 |
| Hct (%) | 37 – 52 | 37.6 ± 3.4 (32.4–44.3) | 38.1 ± 4.03 n.s. (31.8–48.1) | 0.98 |

the case of a patient with pregnancy-induced immune-mediated thrombocytopenia (ITP) (5) one could at least speculate about a stay at altitude as a therapeutic measure. A main drawback of the presented study might be the minor altitude the volunteers were

exposed to. But it confirms our earlier observations in a patient with ITP who was exposed to the same altitude. The study shows a relationship between the increases in TPO and platelet count in response to altitude exposure.

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