Thrombopotein: its ups and downs
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Chapter 7

The Role of Thrombopoietin in Disorders with Thrombocytosis; Polycythaemia Vera, Essential Thrombocythaemia and Reactive Thrombocytosis
The Role of Thrombopoietin (Tpo) in Disorders with Thrombocytosis: Polycythaemia Vera, Essential Thrombocythaemia and Reactive Thrombocytosis

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\textbf{ABSTRACT}

The exact role of thrombopoietin (Tpo), the main regulator of thrombocytopoiesis, and its receptor, Mpl, in disorders with thrombocytosis is unknown. The current study shows that plasma Tpo levels were within the normal range or slightly elevated in patients with essential thrombocythaemia (ET), polycythaemia vera (PV) and reactive thrombocytosis (RT). In view of the highly elevated platelet counts in these patients, Tpo levels are relatively high. Analysis of the platelet Tpo content showed that this parameter was decreased in PV and ET compared with RT. Moreover, platelet counts and plasma Tpo levels were inversely correlated in these myeloproliferative disorders but were positively correlated in RT. In line with published observations on diminished Tpo-receptor expression on platelets from patients with PV or ET, we postulate that in these disorders Tpo clearance seems to be decreased due to a diminished Tpo uptake. In contrast, in RT, Tpo production might be increased under the influence of inflammatory cytokines.
INTRODUCTION

Thrombopoietin (Tpo) is the main regulator of thrombocytopoiesis and is therefore, together with its receptor, a likely candidate to be involved in the aberrant overproduction of platelets. Under normal conditions, Tpo is produced at a constant rate. According to the model that was initially proposed by Kuter and Rosenberg [1], the amount of circulating Tpo is mainly dependent on the total mass of megakaryocytes and platelets. These cells carry the Tpo-receptor, Mpl, on their surface and bind and internalise Tpo [2-6]. Several factors can influence this balance. Mutations in the gene encoding Tpo (in upstream AUG codons in the 5'UTR of the Tpo mRNA that normally function as translational repressors) have been found to underlie Tpo and subsequent platelet overproduction in four different families in which hereditary thrombocythaemia occurred [7-10].

So far, mutations leading to Tpo overproduction have not been found in other myeloproliferative syndromes with thrombocytosis. In polycythaemia vera (PV) and essential thrombocythaemia (ET), normal but also slightly elevated plasma Tpo levels have been reported. Similarly, normal or increased Tpo levels have been reported in reactive thrombocytosis (RT) [11]. In view of the high number of platelets in these patients, Tpo levels are inappropriately high. Therefore, in these cases, either Tpo production is elevated, or Tpo clearance is impaired, as was previously postulated for PV by Moliterno et al. [12,13] and for ET by Horikawa et al. [14].

In the current study, plasma Tpo levels and the Tpo content of platelets, as a measure of platelet Tpo uptake and clearance, was determined in PV, ET and RT patients and compared with control values.

MATERIALS AND METHODS

Patients

Upon informed consent, 13 patients with polycythaemia vera (PV), 12 patients with essential thrombocythaemia (ET), 13 patients with reactive thrombosis (RT) and 14 healthy controls were enrolled in the study. The diagnoses PV and ET were established according to the Rotterdam Criteria of the thrombocythaemia vera study group [15]. In the RT group, thrombocytopoiesis occurred secondary to surgery (n=5), inflammation (n=6), lymphoma (n=1) or iron deficiency (n=1).

At the time of blood sampling, most of the PV and ET patients were undergoing therapy: phlebotomy in 10 of 13 PV patients, hydroxyurea in 1 of 13 PV and 5 of 12 ET patients, interferon in 1 ET patient. Mean age and m/f ratio for the different groups were: PV, 61±16 and 9/4; ET, 60±13 and 5/7; RT, 58±15 and 5/8; Control, 54±5 and 6/8.

Blood processing

Plasma and platelets were isolated from EDTA-anticoagulated blood. Plasma was stored at -20°C until use. Platelets were washed once with phosphate-buffered saline (PBS) containing 2% (w/v) BSA and 5 mM EDTA. Platelets were counted (Technicon H3 RTX™ System;
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Figure 1: Box plots representing plasma Tpo concentration and platelet Tpo content in the different patient groups. Circles represent extremes (cases with values higher that 3 box lengths). Boxes represent the interquartile range containing 50% of all values. The line across the box indicates the median, whereas the whiskers extend to the highest and lowest value. Panel A: plasma Tpo concentration in patients with polycythaemia vera (PV), essential thrombocythaemia (ET), reactive thrombocytosis (RT) and controls. Asterisks represent a significant difference compared to controls (* p<0.05; ** p<0.005). Panel B: platelet (plt) Tpo-content. Not shown in this figure is the platelet Tpo content of one ET patient with a value of 266 AU / 1000 plt. Triangles represent a significant difference compared to the RT group (△ p<0.01; △△ p<0.005).

Bayer, Tarrytown, NY, USA) and suspended in 250 μl of PBS with 20% High Performance ELISA-buffer (CLB, Amsterdam, the Netherlands) in a concentration not exceeding 500 x 10^6 platelets / ml. Subsequently, the platelets were disrupted by freezing at -20°C.

Tpo ELISA
The Tpo concentration in both plasma and the supernatant of the disrupted platelets was determined with a previously described sandwich ELISA [16]. To establish the platelet Tpo content, the Tpo concentration in the supernatant of the disrupted platelets was divided by the platelet count in the sample.

Statistical analysis
The software package SPSS for windows, release 7.5 (SPSS Inc.) was used for statistical analysis. Differences between PV, ET and RT patients were assessed with the one-way ANOVA. The Tukey analysis was used for pairwise comparisons between these groups. To assess whether platelet counts and plasma Tpo levels were correlated, Pearson's correlation coefficient (r_p) was calculated. A p-value <0.05 was considered significant.

RESULTS
Platelet counts in the PV, ET and RT group ranged from 469-1500, 414-1729 and 596-1200 x 10^9 / Litre, respectively, and were not significantly different between the three groups. In figure 1, the plasma Tpo concentration and the platelet-associated Tpo concentration in the different groups are shown. Plasma Tpo levels were significantly elevated compared to the controls in the ET (p<0.05) and the RT group (p<0.001), but not in the PV group (p=0.09). The
Figure 2: Platelet counts versus plasma Tpo levels. Platelet counts versus plasma Tpo levels are shown for patients with PV (●) and patients with ET (○) (panel A). The dotted line represents the linear regression line for the ET group ($r_p = -0.7$, $p<0.05$), the solid line represents the linear regression line for the combined group of patients with PV and ET ($r_p = -0.4$, $p<0.05$). Panel B shows the results for patients with RT. The line represents the linear regression line ($r_p = 0.7$, $p<0.05$).

amount of Tpo in platelets from the RT patients was significantly higher than that of the PV ($p<0.01$) and the ET patients ($p<0.005$). Platelet counts, plasma Tpo level and platelet Tpo content were not different between patients treated with either hydroxyurea or phlebotomy.

Figure 2 depicts the relation between platelet counts and plasma Tpo levels. Platelet counts were inversely correlated to plasma Tpo levels in the ET group ($r_p = -0.7$, $p<0.05$) and in the combined PV and ET ($r_p = -0.4$, $p<0.05$) (Fig 2a). This was not significant for the isolated PV group alone. In the RT group, a positive correlation was present between platelet counts and plasma Tpo levels ($r_p = 0.7$, $p<0.05$) (Fig 2b). No correlation between these two parameters was present in the control group.

DISCUSSION

In accordance with previous reports, plasma Tpo levels in PV, ET and RT patients were within the normal range or slightly increased compared to controls [11]. Theoretically, and according to the model proposed by Kuter and Rosenberg [1], in which platelet and megakaryocyte mass regulate Tpo levels, the concentration of circulating Tpo should be inversely related to the total platelet and megakaryocyte mass. The presence of relatively normal or even elevated plasma Tpo levels in combination with elevated platelet counts indicates that the plasma Tpo concentration is not properly down regulated. Tpo clearance from the circulation might be decreased, Tpo production might be increased, or both mechanisms might apply.

In the current study, a significantly lower platelet Tpo content in PV and ET patients was seen compared to that in RT patients, without a significant difference in platelet counts. In addition, platelet counts in PV and ET patients (notably in ET, not significant in PV), were negatively correlated with plasma Tpo levels whereas these parameters were positively correlated
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in the RT group. This suggests that the mechanism behind controlling plasma Tpo levels is different in the myeloproliferative disorders versus reactive thrombocytosis. Our findings of the decreased Tpo content in platelets from patients with PV and ET, are in line with the postulated diminished Tpo uptake due to a decreased expression of the Tpo receptor. A decreased Tpo-receptor expression has been reported for platelets of both PV [12,13,17] and ET patients [14,18] although Moliterno et al. [12] reported that expression of the Tpo receptor was not decreased in ET. Apart from a decreased expression of the Tpo receptor, the glycosylation of the Tpo-receptor in PV was also reported to be defective[13].

In RT, we postulate that another mechanism is involved in the rise in plasma Tpo concentration. The positive correlation between platelet count and plasma Tpo concentration, combined with the normal platelet Tpo content, is consistent with the hypothesis that Tpo production is increased. Previously, it has been reported that the concentration of various cytokines, such as interleukin-6 (IL-6), IL-1, tumor necrosis factor (TNF), and acute-phase reactants are increased in inflammatory conditions [19-21]. In case of IL-6, it has been shown that this cytokine can increase the Tpo production of liver-cell lines in vitro [22].

In summary, the mechanism behind the relatively increased plasma Tpo levels in MPS and RT seems to be different. In PV and ET, a decreased platelet Tpo content suggests a decreased Tpo uptake, whereas in RT, the positive correlation between platelet count and plasma Tpo level combined with a normal platelet Tpo content is in concordance with Tpo overproduction.

REFERENCES


