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Chapter 4

Plasma Thrombopoietin Levels in Patients with Chronic Kidney Failure

Submitted for publication
Plasma Thrombopoietin Levels in Patients with Chronic Kidney Failure

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ABSTRACT
Thrombopoietin (Tpo) is the most important regulator of thrombopoiesis. It is produced mainly by the liver and the kidney. To investigate the influence of kidney failure on overall Tpo production, we measured Tpo levels in 23 patients on haemodialysis (HD) and 16 patients on chronic peritoneal dialysis (CAPD). Plasma glycoplasticin levels and platelet counts were measured as parameters of platelet mass and/or platelet turnover.
Platelet counts were significantly lower in the HD group, both before, 207±98 x10⁹/L (p<0.001) and after haemodialysis 202±102 x10⁹/L (p<0.001) when compared to healthy controls, 293±79 x10⁹/L. No significant difference was found between platelet count in patients on CAPD and healthy donors. Mean plasma Tpo levels of HD patients were higher both before 23±18 A.U./ml (p<0.0001) and after dialysis 25±26 A.U./ml (p<0.0001), as compared to Tpo levels in healthy controls (11±8 A.U./ml). Patients on CAPD had significantly higher Tpo concentrations, 29±25 A.U./ml than healthy controls (p<0.0001). There was no difference in Tpo level between the HD and CAPD group. No correlation was found between Tpo concentration and platelet count, haematocrit, creatinin or uraemia levels. Glycoplasticin was significantly higher in HD patients and CAPD patients when compared to healthy controls. There was no correlation between glycoplasticin and Tpo level or platelet count. These results confirm the increased platelet turnover in patients with chronic kidney failure. Moreover this study shows that the kidney does not seem to play a major role in the overall Tpo production in the body.
INTRODUCTION
Thrombopoietin (Tpo) is the most important regulator of thrombocytopoiesis [1-6]. Tpo is produced constitutively in the liver and to a lesser extent in the kidney. In the kidney, Tpo mRNA is confined to the proximal tubulus cells [7]. The influence of kidney failure on circulating Tpo concentrations is unknown. In contrast, the effect of kidney failure on platelet homeostasis and platelet function has been studied extensively. Circulating toxic substances (e.g. uraemia) in patients with kidney failure negatively affect platelet function [8]. Platelet counts, although within the normal range, are significantly lower in patients on haemodialysis as compared to healthy controls [8]. In addition, an increased platelet turnover has been reported [9-11]. A decreased number of circulating platelets may be caused by decreased circulating Tpo levels as a result of a diminished Tpo production. It is known that in kidney failure the production of Epo is impaired, which can lead to a decreased production of red cells resulting in anemia. Epo is produced by cells of the distal tubuli in the kidney [9,12].
To investigate whether an impaired kidney function affects Tpo production, two patient groups were analysed, patients on chronic intermittent haemodialysis (HD) and patients on chronic ambulatory peritoneal dialysis (CAPD). Tpo levels were measured both before and after haemodialysis. In addition, plasma glycocalcin concentrations were measured as an indicator of platelet turnover [13].

MATERIALS AND METHODS

Patients and blood collection:
A group of 23 patients on chronic haemodialysis participated in the study. Their mean age was 56 years (range 27-79), with 13 males and 10 females. The CAPD-group consisted of 16 patients with a mean age of 53 years (range 28-70), with 13 being male. All patients were in a stable condition and normally hydrated on clinical grounds. Of these patients, 22 required rHuEPO for correction of their anemia (15 HD patients and 7 CAPD patients). None of the patients suffered from polycystic kidney disease. All patients gave informed consent. Samples were collected in EDTA-containing tubes and were processed 2-6 hours after collection. In the HD group, samples were taken both before and after dialysis. The samples were centrifuged and plasma was frozen at -20°C until use. Platelet count, haemoglobin, haematocrit, creatinin and urea levels of the patients were routinely assessed. Haemodialysate was obtained from five patients. The dialysate was concentrated with a Centricon-3 (Amicon, USA) concentrator, according to the manufacturers’ recommendations. The concentrated dialysate was stored at -20°C until before use. This procedure was also applied to saline spiked with known concentrations of Tpo, which served as controls.

Enzyme-linked immunosorbent assay for Tpo
A Tpo assay that has been described in detail elsewhere was used for determination of the Tpo levels in all blood samples [14]. The intra- and inter-assay variance of this test is 5-7%
Tpo and chronic kidney failure

Figure 1: Platelet counts and plasma Tpo levels in patients with renal failure. Box plots are shown that represent the platelet counts (A) and the plasma Tpo level (B) in controls and the different subgroups of patients with renal failure. Boxes represent the interquartile range containing 50% of all values. The whiskers extend to the highest and lowest value and the line across the box indicates the median. Outliers and extremes are not shown. *: significantly different from control values (p<0.001)

and 7-13%, respectively. Blood samples of one patient were tested in the same plate. A pool of plasma was used as standard, with the first standard dilution arbitrarily set at 100 AU.

Normal values as determined in a group of 193 healthy individuals (mean age 39±11, range 17-69), ranged from 4-32 AU/ml (2.5<sup>th</sup> - 97.5<sup>th</sup> percentile).

Glycocalicin assay
Glycocalicin (GC) concentrations were measured with a sandwich ELISA as previously described [15]. The assay is based on two non cross-reactive MoAbs, one of which is used to capture glycocalicin. A biotinylated one is used for detection. The supernatant of a platelet concentrate was used as a standard and arbitrarily set at 100 AU.

Normal GC plasma levels as determined in 95 healthy individuals were between 144 and 444 AU/ml [15]. To determine whether the time span between blood collection and plasma separation influenced GC levels, EDTA-anticoagulated blood of four different donors was processed at different time points.

Data Analysis
Results were statistically analysed with the software application SPSS for Windows version 8.0 (SPSS INC.). The Mann-Whitney U test was used to show differences between groups. Wilcoxon test was used to compare data before and after dialysis. Spearman’s test was applied to show possible correlations. All levels are expressed as mean ± SD.

RESULTS

Tpo data
Figure 1 depicts the platelet counts (1A) and the plasma Tpo levels (1B) in patients with renal failure and control donors. Platelet counts in the HD group were significantly lower both be-
fore, 207±98 x10^9/L (range 73-403) and after haemodialysis 202±102 x10^9/L (range 73-111) when compared to 270±62 x10^9/L (range 146-510) of normal controls (p<0.001). No statistical difference was found between platelet counts of CAPD patients 293±79 x10^9/L (range 139-452) and controls.

The Tpo concentration before haemodialysis was 23±18 AU/ml (range 8-78) and rose after haemodialysis to 25±26 A.U./ml (range 10-105), a small but significant difference (p<0.05). These levels were significantly higher when compared to Tpo levels in healthy donors, 11±8 A.U./ml (range 3-60; p<0.0001). Patients on CAPD also had significantly higher Tpo levels 29±25 A.U./ml (range 14-148) than healthy donors. There was no significant difference between plasma Tpo levels in the CAPD group and the haemodialysis patients.

No correlation was present between plasma Tpo concentration and platelet count (figure 2), creatinin or ureum level in any group. The change in Tpo level after haemodialysis did not correlate with the change in haematocrit. The change in platelet count as a result of haemodialysis did correlate (p<0.01, r_s=0.75) with the rise in haematocrit in the HD group, as did the increment in haemoglobin level (p<0.001, r_s=0.95), which served as control (data not shown). Patients on rHuEpo treatment had no significant difference in platelet count or Tpo level when compared to patients without rHuEpo treatment.

**Glycocalcin data**

Results are shown in figure 3. GC levels were 1088±670 A.U./ml before and 1020±543 A.U./ml after haemodialysis, and 972±197 A.U./ml in the CAPD group, which is significantly higher in all cases compared to healthy controls (293±75 A.U./ml, p<0.001).

There was no correlation between Tpo and GC level. GC and platelet count showed a significant correlation in the HD group after dialysis (p<0.001, r_s=0.79) but in none of the other groups. Plasma GC concentrations did not correlate with creatinin or ureum level in the dialysis groups, nor was there a correlation between the change in glycocalcin level and haematocrit due to the haemodialysis.
Figure 3: Plasma GC levels in patients with renal failure. Box plots representing plasma GC levels in controls and in the different subgroups are shown. With regard to the box plots, the same legend as in Fig. 1 applies.

To exclude that the increment in plasma GC concentration in the patients was due to delayed processing of samples, control samples were processed at different time points. As shown in figure 4, no significant differences were present up to 7 hours of storage at room temperature.

**Dialysate data**

No Tpo was detected in non-concentrated or concentrated dialysate samples. Tpo added to these samples as a control was recovered completely (data not shown).

**DISCUSSION**

Thrombopoietin is constitutively produced by the liver and the kidney. Circulating platelets and bone-marrow megakaryocytes are responsible for removal of Tpo from the circulation, thus regulating the Tpo concentration in blood [16].

Although the kidney is one of the Tpo-producing organs, the current study shows that loss of kidney function did not result in a decreased circulating concentration of Tpo. On the contrary, in patients on HD and CAPD, with normal platelet counts, a small but significant increase in plasma Tpo concentration was found. A recent study by Stockelberg et al. [17] also showed no decreased Tpo-levels in patients on HD for end-stage kidney failure.

In our study most patients (22/39) required rHuEpo to maintain normal red cell levels. Thus, loss of parenchyma was to such an extent that sufficient amounts of Epo could not be produced by the kidney, and probably no sufficient Tpo either.

An indication for the role of the kidney in Tpo production was recently obtained from a study in which wild-type mice were transplanted with a liver from Tpo knockout mice. These animals showed a 60% decrease in circulating platelets [18]. Measured with a less sensitive assay, Tpo concentrations in these mice were below the detection limit of the assay. This indicates that the liver is responsible for most of the Tpo production.

Apparently, the loss of Tpo production by the liver cannot be overcome by the other Tpo-producing organs. The normal or increased plasma Tpo levels in our study confirms the small contribution of the kidneys towards the total Tpo production.
Platelet turnover seems increased in the studied patients, since plasma GC concentrations were found to be increased. GC has previously been shown to be a marker for platelet turnover [13]. An artificial increment of GC concentration due to delayed processing of samples as reported earlier [13] is unlikely because control samples were stable for up to 24 hours. The finding of elevated GC levels as a reflection of platelet turnover is in accordance with a study from Himmelfarb et al. [19], who showed that the percentage of reticulated platelets is increased in dialysis patients. Reticulated platelets are young platelets, and an increment in reticulated platelets reflects enhanced thrombopoiesis. In contrast to the study mentioned above, in the current study no difference in GC and Tpo level in patients on HD as compared to patients on CAPD was observed. The increased Tpo concentration might be responsible for the enhanced platelet production.

The patients with kidney failure studied in this report had normal platelet counts, albeit significantly lower than in healthy controls. Since no correlation exists between platelet counts in the normal range and Tpo level [14], the small increment in Tpo level is not likely to be caused by diminished uptake by platelets.

Since the kidney is not involved in the clearance of Tpo, which is supported by the finding that Tpo levels did not correlate with creatinin of ureum levels, the loss of kidney function is not responsible for the elevated Tpo levels. Platelet activation occurs in patients on haemodialysis [19]. We previously showed that platelet activation during coagulation results in Tpo release [14,20]. Indeed, Tpo levels were slightly but significantly increased after haemodialysis, indicating that platelet activation might occur, leading to Tpo release and a subsequent increase in circulating Tpo. However, we were unable to show a difference in GC level before and after dialysis. Also, Tpo and GC levels did not differ between HD and CAPD group. Apparently, GC levels reflect platelet turnover rather than platelet activation.

In conclusion, in contrast to the effect kidney failure has on the circulating amount of Epo, the Tpo concentration does not seem to be affected in a major way, i.e. there is no Tpo deficiency. Thus, compared to the liver, the kidney does not seem to play a major role in the constitutive production of Tpo.
REFERENCES


The data do not support the concept that Tpo levels are responsible for the observed Tpo levels.

We previously showed that platelet activation increases Tpo levels in circulating Tpo. However, we were unable to correlate Tpo levels with platelet activation. This is partly because Tpo levels reflect platelet turnover. Tpo production is not constant, and Tpo levels are influenced by a variety of factors. In conclusion, in contrast to the effect of liver injury on Tpo levels, the effect of apheresis on Tpo levels is not apparent. Thus, compared to the liver, the kidney does not appear to play a major role in the constitutive production of Tpo.