Inverse relationships between haemoglobin and ristocetin-induced platelet aggregation in haemodialysis patients under erythropoietin therapy

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Introduction

Prolongation of the skin bleeding time (BT) reflects defective formation of the primary haemostatic plug, and is a well-established estimate of uraemic bleeding tendency [1]. Decreased erythrocyte numbers result in less efficient radial transport of platelets towards the vessel wall, which is at least partially responsible for impaired platelet–vessel wall interactions. The crucial role of raising haematocrit in correcting bleeding diathesis in uraemia was evidenced by shortening of the BT that followed transfusion of packed red blood cells [2, 3], or amelioration of the anaemia by recombinant human erythropoietin (rHuEpo) without any evidence of activation of intravascular coagulation [4–7]. Another reliable measure of platelet–vessel wall interactions is their aggregation in response to ristocetin.

Herein we report an unexpected inverse correlation between Hb concentration and whole-blood ristocetin-induced platelet aggregation found in rHuEpo-treated haemodialysis (HD) patients. The study also describes changes in other haematological parameters or arterial blood pressure.

Conclusions. Considering the role of von Willebrand factor and fibrinogen in mediating ristocetin-induced platelet aggregation, and enhanced synthesis and/or release of these macromolecules in response to uraemia or inflammation, we suggest that exaggerated whole-blood platelet aggregability to ristocetin points to blunted erythropoiesis in HD patients on rHuEpo therapy.

Key words: erythropoietin; ketanserin; platelet aggregation; ristocetin; uraemia
some consequences of pharmacological inhibition of ristocetin-induced platelet aggregation by ketanserin, which is an antagonist of platelet and vascular smooth muscle serotonin (5-hydroxytryptamine, 5-HT) 5-HT2 receptors [14].

**Subjects and methods**

*Patients*

We studied 28 non-diabetic chronic HD patients. Dialysis dose was adjusted to Kt/V > 1.3. They all were treated with rHuEpo (Eprex®, Cilag AG International, Switzerland) injected subcutaneously. Relevant clinical data of these patients are outlined in Table 1. In sixteen of them, BP was compared with dihydralazine alone (n = 10) or in combination with metoprolol (n = 6) and by increased ultrafiltration during HD. The patients showed BT < 10 min, were clinically stable with normal iron stores, and had no evidence of functional iron deficiency. Although measurements of blood PTH were not available, there was no biochemical or clinical evidence of severe secondary hyperparathyroidism. No patient was receiving any medication known to affect haemostasis, except for unfractionated heparin during HD. Dialysis was always performed using the double-needle technique, with cuprophane capillary dialysers, and with bicarbonate as buffer in the dialysate. Vascular access was in all cases a Cimino-Brescia arteriovenous fistula.

Within this group of patients, there was a subgroup of 16 subjects (Table 1) to whom ketanserin (Sufrexal, Janssen Pharmaceutica, Belgium) at an oral dose of 10–20 mg twice daily was administered. The studies were repeated after a 2-week (n = 12) and a 4-week (n = 4) ketanserin treatment. The rationale of such a design and some haematopoietic and the subgroup of these subjects treated with ketanserin comparison with the patients studied.

*Methods*

Haematological parameters were determined by standard laboratory techniques. Bleeding time was measured using the method of Ivy modified by Mielke et al. [16]. Whole-blood platelet aggregation in response to ristocetin (0.6 g/l, Sigma, USA) was monitored by measuring electric impedance using Chronolog aggregometer (Chrono-Log Corp., Havertown, PA, USA) according to the method of Wilsoncroft et al. [17]. The extent of the aggregation was evaluated by measuring the maximal extension of the aggregation curve at 6 min after the addition of the agonist and expressed in ohms (Ω). Serum Epo concentration was determined using an enzyme-linked immunosorbent assay kit (EPO-ELISA, Boehringer Mannheim, Germany; normal range 0.4–9.0 U/l).

**Study design**

A complete blood count and whole-blood platelet aggregation in response to ristocetin were measured in fasting patients in the morning before HD session. Bleeding time was measured and blood was taken by free flow through a 19-G butterfly needle at the onset of HD, and anticoagulated with 3.8% sodium citrate for platelet function studies. In the ketanserin-treated patients the measurements were repeated after the drug was withdrawn. Serum for Epo determination was prepared conventionally and stored at —40°C until assayed.

For platelet aggregation tests where standard reference ranges were not available, studies were also performed in 21 age-matched non-uraemic healthy individuals (normals), and in 25 HD patients not treated with rHuEpo (uraemics), for comparison with the patients studied.

**Table 1. Clinical characteristics of the rHuEpo-treated HD patients and the subgroup of these subjects treated with ketanserin**

<table>
<thead>
<tr>
<th>Total</th>
<th>Ketanserin treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>28</td>
</tr>
<tr>
<td>Male vs. female</td>
<td>18/10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42.8 ± 15.3</td>
</tr>
<tr>
<td>CRF causes</td>
<td></td>
</tr>
<tr>
<td>primary glomerulonephritis (n)</td>
<td>18</td>
</tr>
<tr>
<td>interstitial nephritis (n)</td>
<td>6</td>
</tr>
<tr>
<td>ADPKD (n)</td>
<td>2</td>
</tr>
<tr>
<td>unknown (n)</td>
<td>2</td>
</tr>
<tr>
<td>HD duration (months)</td>
<td>20.7 ± 13.7</td>
</tr>
<tr>
<td>rHuEpo therapy duration (weeks)</td>
<td>26.2 ± 10.2</td>
</tr>
<tr>
<td>rHuEpo dose (IU week)</td>
<td>4714 ± 1462</td>
</tr>
</tbody>
</table>

Data are mean ± SD.

ADPKD, adult dominant poly cystic kidney disease.

The Hb concentration in the group of 28 patients was 10.1 ± 1.4 g/dl and BT was 6.1 ± 2.0 min. Ristocetin-induced platelet aggregation (15.2 ± 14.4 Ω) was not different from the value found either in the normals (11.6 ± 2.5 Ω) or the uraemics (11.4 ± 2.8 Ω). The regression line between ristocetin-induced platelet aggregation and Hb concentration showed a significant inverse logarithmic trend (r = —0.392, P < 0.05) (Figure 1).

Ketanserin administration produced a significant fall in serum immunoreactive Epo concentration (21.8 ± 10.0 U/l pre, and 13.5 ± 8.4 U/l post, P < 0.0002), a fall in the Hb level (10.2 ± 1.5 g/dl pre, and 9.4 ± 1.7 g/dl post, P < 0.001), and prolongation of the BT (5.8 ± 1.9 min pre, and 11.2 ± 8.0 min post, P < 0.02). Ristocetin-induced platelet aggregation value of 20.4 ± 17.1 Ω was significantly more intensive (P < 0.05) than in the normals and uraemics, and decreased to 10.4 ± 7.1 Ω (P < 0.02) following the ketanserin trial. Once more an inverse correlation between depressed ristocetin-induced platelet aggregation and lowered Hb concentration (r = —0.590, P < 0.02) was found (Figure 2).
Fig. 1. Log-log plot of Hb concentration and ristocetin-induced platelet aggregation (PA) in HD patients treated with rHuEpo.

As shown in Figure 3, a strong positive linear correlation between the extent of pre-ketanserin platelet aggregation and the decrease in the intensity of aggregation that followed ketanserin treatment was observed ($r = 0.919$, $P < 0.000005$).

The platelet count remained within normal limits in both groups of patients. Furthermore, neither the platelet count nor BP were affected by ketanserin (data not shown).

Discussion

The inverse correlations between the values of ristocetin-induced platelet aggregation and the concurrent Hb levels found in this study suggest that some of the factors involved in this complex aggregation process exert an inhibitory effect on erythropoiesis and therefore blunt the therapeutic response to rHuEpo.

Considering the elements involved in platelet aggregation in response to ristocetin, the level of GP Ib antigen in the platelets was found to be significantly reduced in uraemia [18]. Interestingly, an increase of expression of these molecules during rHuEpo therapy was reported [19]. If vWF antigen and ristocetin cofactor activity are considered, both elevated [12,19–21] or stable [11,22,23] values assessed in different stages of rHuEpo therapy were found. There is also a large body of clinical studies on changes of fibrinogen during rHuEpo therapy. Stable [5,11,23] as well as rising [24,25] plasma levels of these platelet-bridging molecules were found. Recently, it has been shown [26] that, in contrast to platelet aggregation in response to other agonists, ristocetin-induced clumping is dependent on an influx of Ca$^{2+}$ via GP IIb/IIIa (the integrin $\alpha_{IIb}\beta_{3}$) rather than on a discharge from intraplatelet Ca$^{2+}$ stores. Inability of this integrin that functions as a fibrinogen receptor to undergo a conformational change during platelet activation has been reported in uraemia [27].

High plasma fibrinogen and vWF levels are well-established markers of acute phase reactions. More specifically, elevated levels of endothelium-derived vWF reflect a state of chronic endothelial cell injury often accompanying uraemia [28,29], or their activation during rHuEpo treatment [21,30]. Considering the above-described mechanisms of ristocetin-induced platelet aggregation, augmentation of this process may also reflect the presence of inflammation. This, often subclinical and not easily diagnosed state is usually followed by development of anaemia due to increased generation of numerous inflammatory cytokines which may either suppress Epo synthesis or induce a resistance of erythroid progenitors to the hormone [31].

Normal BT values in the whole group and improved ristocetin-induced platelet clumping encountered pre-treatment in ketanserin-treated patients give evidence of effective platelet-vessel wall interactions under rHuEpo therapy. The fact of significantly more intensive platelet aggregability in this subgroup of patients in comparison to the whole group could have been due to its greater homogeneity in regard to rHuEpo therapy.
Haemoglobin level versus ristocetin-induced platelet aggregation
treatment duration. In this population, 13 of 17 patients included were treated for as long as 8 months, which further confirms our findings that ristocetin-induced platelet aggregation is especially exaggerated in long-term rHuEpo patients (unpublished data). The BT was prolonged and ristocetin-induced platelet aggregation became depressed following ketanserin treatment. While ketanserin was shown to prolong BT by antagonising vascular α-adrenergic receptors or by preventing interaction between 5-HT and catecholamines in the vascular bed [32], the precise mechanism by which this agent reduces platelet aggregation remains speculative. In view of the stable platelet count, a 25% fall in fibrinogen concentration that followed ketanserin treatment in 15 HD patients treated with rHuEpo for 8 months (our unpublished data) may be causative, and of possible antithrombotic benefit. However, we have recently shown that ketanserin has also a unique property of decreasing serum Epo levels and inhibiting erythropoiesis in both HD patients treated with rHuEpo[15], and in some HD patients with polycystic kidneys and intrinsically high haemacrits [33] as well as in kidney recipients with post-transplant erythrocytosis [34]. This effect is most probably due to diminished endogenous Epo synthesis. Finally, as a result of these dual actions of ketanserin, the inverse correlation between the values of Hb and platelet aggregation in response to ristocetin has become stronger (Figure 2). It is of note that ketanserin is more effective in decreasing platelet clumping in HD patients showing the most exaggerated sensitivity to ristocetin (Figure 3). Unfortunately, the undesirable effect of lowering blood Epo concentration limits its use as an antiplatelet agent in these already anaemic patients.

Although no relationships between rHuEpo dose and Hb levels, or between the former and platelet aggregability to ristocetin were found, based on this report, further studies should be undertaken to determine if enhanced ristocetin-induced platelet aggregation is an indicator of a state of hyporesponsiveness to rHuEpo in uraemic patients. It would also be of value to determine if this parameter can serve as a predictor of erythropoietic response in patients in whom the hormone is started. This would allow avoidance of dose escalation and would improve the cost-effectiveness of rHuEpo therapy.

Acknowledgements. This work was supported by a grant no. 54 3 798 provided by the Bialystok Medical School. We thank Dr Christian Hörig, Janssen Pharmaceutica Belgium, a division of Johnson & Johnson, for the donation of ketanserin. Miss W. Truszwicz RN and other nurses of the Dialysis Unit of our Department for excellent patient care, and Mrs I. Klagisz and E. Jamiołkowska for expert technical support are also gratefully acknowledged.

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Received for publication: 8.4.96
Accepted in revised form: 22.7.96