The required dose of erythropoietin during renal anaemia treatment is related to the degree of impairment in erythrocyte deformability

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Abstract

Introduction

Decreased red cell survival has been documented in patients with chronic renal failure [1–4]. Normal subjects should, however, easily compensate for the mild degree of haemolysis by an increase in erythropoiesis. The usual response to anaemia in man is an increase in erythropoietin production, leading to an increase in erythropoiesis. Renal disease results in a submaximal Epo response and consequently a hypoproliferative anaemia [5]. Normal red blood cells are highly deformable and easily traverse nutritive capillaries whose diameters are smaller than that of the cells. The ability of the red blood cell to undergo deformation is termed deformability [6]. Erythrocyte deformability has been found to be impaired in uraemia [7–9] and to be related to the degree of renal failure [7–10]. The erythrocyte survival time has been shown to correlate with the deformability in haemodialysis patients [11], and the degree of renal anaemia has also been found to be related to erythrocyte deformability in groups of patients with chronic renal failure [10], haemodialysis patients [12], and patients undergoing CAPD [13]. Thus, impaired erythrocyte deformability may give rise to shortened red cell survival time and may therefore be one factor contributing to the development of renal anaemia.

In the majority of haemodialysis patients, anaemia is rapidly corrected or avoided by recombinant human erythropoietin (rHuEpo) therapy, but the dose required varies greatly. The aim of the present study was to identify factors with an impact on the required rHuEpo doses in haemodialysis patients with special reference to erythrocyte deformability.

Subjects and methods

Study population

In all patients on chronic haemodialysis at the haemodialysis centre of the University Hospital of Uppsala, Sweden, patients fulfilling the following criteria were identified:

1. Age exceeding 18 years.
2. Haemodialysis treatment and subcutaneous rHuEpo therapy for at least 6 months.

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3. Unaltered dosage of rHuEpo during the last 2 months.
4. Stable Hb during 2 months, meaning that no value of Hb deviated from the last Hb value by more than 5%.
5. Serum concentration of ferritin exceeding 50 µg/l and concentrations of vitamin B12 and folic acid within normal ranges.
6. Serum calcium and alkaline phosphatase within the normal ranges.
7. No blood transfusions received during the last 6 months. Between a total of 77 haemodialysis patients, 25 subjects (17 men and 8 women) fulfilled the criteria. After explanation of the purpose of the study, all these patients gave their consent to participate in the study. Their mean age was 66 years (range 31–88 years). The primary renal diseases were nephrosclerosis in nine patients, diabetic nephropathy in five, chronic glomerulonephritis in three, congenital renal hypoplasia in two, renal artery stenosis in one, polycystic kidney disease in one, nephrectomia due to carcinoma in two, and unknown in two patients. The biochemical characteristics are given in Table 1. Nine of the patients were treated by haemodiafiltration and the remaining 16 by conventional bicarbonate haemodialysis using modified cellulose membranes in 15 cases and a synthetic membrane in one case. Dialysis treatments were given twice weekly in nine patients and thrice weekly in 16 patients. No patients were on aluminium-containing phosphate binders. Iron supplementation with iron sucrose was administered intravenously in connection with dialysis to eight of the patients. rHuEpo was administered subcutaneously at the end of the dialysis session by a member of the staff. Epoetin alpha was used in all cases. Blood samples were taken immediately before the start of a mid-week dialysis procedure.

Biochemical variables

The biochemical variables were analysed using standard laboratory techniques for hospital use. The reticulocytes were counted under a microscope after supravital staining and expressed as a percentage of the red blood cells, where at least 1000 cells had been counted. aluminium in serum was analysed by flameless atomic absorption spectrophotometry using a graphite furnace. Serum parathyroid hormone (PTH) was measured by an immunological technique that is claimed to detect exclusively intact PTH (Allegro Intact PTH immunoassay, Nichols Institute, San Juan Capistrano, CA, USA).

Table 1. Mean values of the variables measured and the R and P values from a simple regression analysis between the variable and the required weekly dose of rHuEpo

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood haemoglobin (g/dl)</td>
<td>12.0 ± 1.5</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1.7 ± 0.7</td>
<td>0.69</td>
<td>0.0001</td>
</tr>
<tr>
<td>S albumin (g/l)</td>
<td>37 ± 5</td>
<td>0.14</td>
<td>NS</td>
</tr>
<tr>
<td>S ferritin (µg/l)</td>
<td>412 ± 427</td>
<td>0.27</td>
<td>NS</td>
</tr>
<tr>
<td>S aluminium (µg/l)</td>
<td>9.7 ± 12</td>
<td>0.19</td>
<td>NS</td>
</tr>
<tr>
<td>S parathryoid hormone (ng/l)</td>
<td>198 ± 297</td>
<td>0.29</td>
<td>NS</td>
</tr>
<tr>
<td>Dialysate time (h/week)</td>
<td>9.0 ± 2.2</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>S urea (mmol/l)</td>
<td>27 ± 5</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Kt/V</td>
<td>3.3 ± 1.1</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma viscosity (mPa·s)</td>
<td>1.36 ± 0.09</td>
<td>0.42</td>
<td>0.035</td>
</tr>
<tr>
<td>Erythrocyte fluidity (1/Pa·s)</td>
<td>85 ± 11</td>
<td>0.48</td>
<td>0.014</td>
</tr>
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Fig. 1. The reticulocyte fraction as a function of the required weekly doses of rHuEpo in 25 haemodialysis patients (R = 0.69, P = 0.0001).

Results

The required dose of rHuEpo varied within the range 56–370 U/kg with a mean value of 186 ± 93 U/kg bodyweight and week. The mean values of the variables measured are given in Table 1. Simple regression analyses were performed between the required doses of rHuEpo and the variables measured. The R and P values are given in Table 1. The required dose of rHuEpo was significantly positively correlated to the reticulocyte fraction (R = 0.69, P = 0.0001, Figure 1) and to the plasma viscosity (R = 0.42, P = 0.035, Figure 2) and negatively to erythrocyte fluidity (R = 0.48, P = 0.014, Figure 3). When the rHuEpo dose was

Haemorheological variables

Inverse viscosity is termed fluidity. Erythrocyte fluidity has been shown to reflect erythrocyte deformability [15]. In order to assess erythrocyte fluidity, the erythrocytes were separated from plasma by centrifugation and re-suspended to a haematocrit of 55% in isotonic phosphate-buffered saline at pH 7.4. Erythrocyte fluidity was calculated as the inverse viscosity of the suspension, at a shear rate of 1 s⁻¹ at 37°C using a Low Shear 30 rotational viscometer (Contraves AG, Zürich, Switzerland). The plasma viscosity was determined at a shear rate of 38 s⁻¹ at the same temperature using the same instrument. The analyses were performed within 20 min after blood collection. In healthy subjects an erythrocyte fluidity of 101 ± 6 Pa⁻¹ s⁻¹ and plasma viscosity of 1.24 ± 0.04 mPa s have been found [16].

Statistical evaluation

Values are given as means ± SD. Unpaired two tailed t tests and simple and multiple stepwise regression analyses were used. The level of statistical significance was set at P < 0.05.
Erythrocyte deformability and rHuEpo requirement

normally distributed and consequently not suitable for regression analysis. Therefore the patients were divided into two groups with a CRP value lower or higher than 20 mg/l respectively. The Hb and serum albumin concentration were decreased and the reticulocyte fraction increased in the patients with a CRP concentration exceeding 20 mg/l (Table 2).

Discussion

The patients selected to participate in the present investigation had unaltered rHuEpo dose and stable Hb for at least 2 months prior to the investigation. They experienced no laboratory signs of known causes of rHuEpo hyporesponsiveness like iron deficiency, osteitis fibrosa, vitamin deficiency, or aluminium overload [17]. The weekly dose of rHuEpo needed to keep the Hb at a constant level did, however, vary greatly (56–370 U/kg/week).

The doses were very closely related to the reticulocyte fraction. The reticulocyte fraction can be taken as an estimate of erythropoietic activity [18]. The finding of a close correlation between these two variables consequently indicates that the bone marrow response to rHuEpo did not vary much between different patients. Thus, the differences in required doses of rHuEpo cannot be explained by different degree of bone marrow inhibition in these patients.

Since our reticulocyte count findings indicate that the red blood cell formation rate was principally dependent on the doses of rHuEpo given, differences in red blood cell disappearance rate seem to be the major factor explaining the differences in rHuEpo requirements. Blood loss, due to platelet dysfunction, haemodialysis, and periodic laboratory tests, is present in haemodialysis patients and may be an important variable as regards the red blood cell disappearance rate [5]. Haemolysis may be another reason. In the present investigation erythrocyte deformability, measured as erythrocyte fluidity, was the only variable significantly correlated with the required dose of rHuEpo in the stepwise multiple analysis of regression. It is possible that impaired erythrocyte deformability might give rise to haemolysis and thereby increased requirement of rHuEpo.

However, the possibility of an opposite causative

<table>
<thead>
<tr>
<th>CRP &lt; 20 mg/l</th>
<th>CRP &gt; 20 mg/l</th>
<th>P</th>
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<tbody>
<tr>
<td>S CRP (mg/l)</td>
<td>11 ± 1</td>
<td>38 ± 17</td>
</tr>
<tr>
<td>Required rHuEpo dose (U/kg/week)</td>
<td>166 ± 73</td>
<td>221 ± 118</td>
</tr>
<tr>
<td>B Hb (g/dl)</td>
<td>12.5 ± 1.3</td>
<td>11.1 ± 1.3</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1.5 ± 0.6</td>
<td>2.0 ± 0.6</td>
</tr>
<tr>
<td>S albumin (g/l)</td>
<td>39 ± 3</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>S ferritin (µg/l)</td>
<td>310 ± 278</td>
<td>595 ± 586</td>
</tr>
<tr>
<td>Plasma viscosity (mPa·s)</td>
<td>1.33 ± 0.08</td>
<td>1.40 ± 0.08</td>
</tr>
<tr>
<td>Erythrocyte fluidity (1/Pa·s)</td>
<td>88 ± 11</td>
<td>81 ± 11</td>
</tr>
</tbody>
</table>
relationship between the required rHuEpo doses and erythrocyte deformability must be borne in mind. Calcium ions are known to decrease membrane deformability [19] and changes in red blood cell calcium homeostasis have been described during renal anemia treatment with rHuEpo [20]. In a previous study [21] we demonstrated a decreased erythrocyte deformability during the time period when the Hb value was increasing in rHuEpo-treated haemodialysis patients. When target Hb was reached, and the doses of rHuEpo were reduced, erythrocyte deformability, however, improved and reached values equal to those seen before treatment [21]. In the present investigation the patients had been treated with rHuEpo for at least 6 months and had had stable Hb during the last 2. Thus, it seems unlikely that rHuEpo per se would cause decreased erythrocyte deformability in the present investigation. Such a proposition is supported by findings from other studies where unchanged [4,22] or improved [23,24] mechanical properties of red blood cells during renal anaemia treatment with rHuEpo have been demonstrated.

In a recent study increasing intensity of dialysis was found to result in a significant increase in the haematocrit [25]. A high intensity of haemodialysis might result in an improved erythrocyte deformability and thereby a low requirement of rHuEpo. Any significant correlation between Kt/V and erythrocyte fluidity or required rHuEpo doses was, however, not found in the present investigation. The probable reason for that is the remaining renal function varied between patients.

Improvement in anaemia has also been reported after parathyroidectomy [26]. However, in the present study no correlation was found between serum concentrations of PTH and rHuEpo requirements. Neither did Rao and collaborators [27] find any such correlation, but demonstrated that the rHuEpo requirement was dependent on the extent of bone marrow fibrosis. In the present study no patients with elevated alkaline phosphatase or serum calcium were included. The number of patients with bone marrow fibrosis due to secondary hyperparathyroidism can therefore be assumed to be low, which explains that no interrelationship between serum PTH and rHuEpo requirement was found. PTH has also been shown to cause an increase in the osmotic fragility of red blood cells, probably due to enhanced calcium entry into the cells [28]. No relationship between PTH and erythrocyte fluidity was, however, found in the present study.

Our findings support previous observations that inflammation is a factor associated with rHuEpo hyporesponsiveness [17]. In the present investigation the Hb value was lower in the group of patients with a CRP exceeding 20 mg/l despite a tendency towards higher weekly doses of rHuEpo. Acute and chronic infections cause reactive increases in fibrinogen and serum globulins which increase plasma viscosity [6]. Inflammation may therefore be the mechanism underlying the positive relationship between the plasma viscosity and the required rHuEpo doses. Cytokines associated with inflammation have been shown to inhibit erythropoiesis in vivo and in vitro. [29]. Our observation that the reticulocyte count was increased in the patients with a CRP exceeding 20 mg/l does, however, suggest an appropriate bone marrow response to rHuEpo in these patients. Again, blood loss or shortened red blood cell life span seem to be important factors for the increased requirement of rHuEpo in these patients. However, the finding must be interpreted with caution since the difference in reticulocyte count between the two groups was slight and a suboptimal method for the estimation of the variable was used.

In the present investigation, comprising selected haemodialysis patients without iron deficiency, aluminium intoxication, or any obvious signs suggestive for bone marrow fibrosis, the required doses of rHuEpo were closely related to the reticulocyte fraction. We therefore conclude that the interindividual differences in bone marrow response to rHuEpo were small in these patients. Our finding of a relationship between erythrocyte fluidity and the required doses of rHuEpo may indicate that different degrees of alterations in the mechanical properties of the red blood cells play a key role as regards the rHuEpo requirement. Based on the assumption that an increased CRP and plasma viscosity are indicative for inflammation, we also conclude that inflammation may be an additional reason for increased rHuEpo requirement.

Acknowledgements. This work was supported by grants from the Swedish Society of Medicine and E, K and G Selanders foundation.

References

Received for publication: 18.2.97
Accepted in revised form: 16.6.97