A parallel, comparative study of intravenous iron versus intravenous ascorbic acid for erythropoietin-hyporesponse anaemia in haemodialysis patients with iron overload

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Abstract

Background. Functional iron deficiency may develop and cause erythropoietin resistance in haemodialysis patients with iron overload. Controversy remains as to whether intravenous iron medication can improve this hyporesponse due to decreased iron availability, or whether iron therapy will aggravate haemosiderosis. Intravenous administration of ascorbic acid has been shown to effectively circumvent resistant anaemia associated with iron overload in a small preliminary study. To elucidate further the possible mechanisms of this resistance, a parallel, comparative study was conducted to compare the effects of intravenous iron and ascorbate therapies in iron-overloaded haemodialysis patients.

Methods. Fifty haemodialysis patients with serum ferritin of >500 μg/l were randomly divided into two protocols. They were further stratified into controls (Control I, n = 11) and intravenous iron group (IVFE, n = 15) in protocol I; and into controls (Control II, n = 12) and intravenous ascorbic acid group (IVA, n = 12) in protocol II. Controls had a haematocrit of >30% and did not receive any adjuvant therapy. IVFE and IVA patients were hyporesponse to erythropoietin and functionally iron deficient. Ferric saccharate (100 mg dose) was administered intravenously post-dialysis on five consecutive dialysis sessions in the first 2 weeks; and ascorbic acid (300 mg dose) thrice a week for 8 weeks. Red cell and iron metabolism indices were examined before and following therapy.

Results. Mean values of haematocrit and transferrin saturation were significantly lower, and erythropoietin dose was higher in IVFE and IVA patients compared to controls. Intravenous iron therapy neither improved erythropoiesis nor reduced erythropoietin dose during 12 weeks. Iron metabolism indices significantly increased at 2 and 6 weeks, but decreased at 12 weeks returning to the baselines. In contrast, mean haematocrit significantly increased from 25.8 ± 0.5 to 30.6 ± 0.6% with a concomitant reduction of 20% in erythropoietin dose after 8 weeks of ascorbate therapy. Serum ferritin modestly fell but with no statistical significance. The enhanced erythropoiesis paralleled a rise in transferrin saturation from 27 ± 3 to 48 ± 6% and serum iron from 70 ± 11 to 107 ± 19 μg/dl (P < 0.05).

Conclusions. Short term intravenous iron therapy cannot resolve the issue of functional iron deficiency in haemodialysis patients with iron overload. Intravenous administration of ascorbic acid not only facilitates iron release from storage sites, but also increases iron utilization in the erythron. Our study draws attention to a potential adjuvant therapy, intravenous ascorbic acid, to treat erythropoietin-hyporesponse anaemia in iron-overloaded patients.

Key words: erythropoietin hyporesponsiveness; functional iron deficiency; haemodialysis; intravenous ascorbic acid; iron overload

Introduction

To ensure optimal response to recombinant human erythropoietin (rHuEpo), patients must have sufficient available iron to satisfy the requirements for erythropoiesis. Thus inadequate iron storage/availability is the most common cause of sub-optimal response to rHuEpo. Guidelines for management of hyporesponsiveness to rHuEpo [1–3] indicate that a serum ferritin level of 100 μg/l is the diagnostic threshold for iron deficiency in haemodialysis (HD) patients. Fishbane et al. [4] and our previous study [5] found that most HD patients have inadequate available iron for prompt erythropoiesis when current guidelines for serum ferritin and transferrin saturation (TS) are used, and that continuous use of i.v. iron can reduce rHuEpo requirements [4,6–8]. Cumulative data suggest that it is essential to maintain serum ferritin over 300 μg/l for
optimal response to rHuEpo since there is great need for iron in Epo-stimulated erythroid progenitor cells [5,9–12]. Nevertheless, some HD patients with increased iron stores exhibit rHuEpo hyporesponsiveness due to impaired iron availability. Functional iron deficiency is present when rHuEpo accelerates erythropoiesis to an extent that the body’s iron demand exceeds the capacity of tissue iron released to transferrin [5,8,11,12]. Theoretically intensive iron supplementation, almost always in the form of i.v. iron, is a treatment option in such patients. However, it is still a matter of debate whether functional iron deficiency in iron-overloaded patients can be overcome by i.v. iron therapy [6,13,14], or whether the potential hazard of haemosiderosis will be worsened following iron supplementation. An adjuvant therapy without hazardous side effect is needed to improve the response to rHuEpo. Intravenous administration of ascorbic acid has been shown to circumvent rHuEpo resistance in four iron-overloaded HD patients in a preliminary study [15]. These preliminary results prompted us to conduct a parallel, comparative study to further assess the effects of i.v. iron supplementation and ascorbic acid for the treatment of hyporesponsiveness to rHuEpo in HD patients with iron overload. In addition, the possible mechanism of action of vitamin C is investigated.

Subjects and methods

Patients

Iron overload was defined as a serum ferritin level >500 μg/l [16] and functional iron deficiency as TS <30% [11,12]. Inclusion criteria were: HD therapy for ≥6 months, duration of rHuEpo treatment ≥6 months, stable haematocrit (Hct) values for four consecutive weeks, and a state of iron overload not caused by i.v. iron therapy before study entry for ≥3 months. Three months prior to enrollment and during the study, patients were excluded if any following events known to affect response of rHuEpo treatment had occurred: blood loss, blood transfusion, intercurrent infection, chronic inflammatory disease, sickle cell disease, hemoglobinopathy, and treatment with ACE inhibitors. Finally, 50 patients on chronic HD participated in the study. Dialysis was performed for 4–4.5 h thrice a week using cellulose acetate hollow-fibre (FB 130T; Nipro®, Nissho, Japan), blood flow of 250–300 ml/min, and dialysate flow of 500 ml/min. The study was approved by the local ethics committee, and informed consent was obtained from all patients.

Study design

The study included two protocols with study and control groups for each. We randomly divided 26 patients (13 men and 13 women, mean age of 57 years) into protocol I and 24 patients (15 men and 9 women, mean age of 55 years) into protocol II. Patients in the study groups were hyporesponsive to rHuEpo (Hct value <30% despite rHuEpo doses >400 U/kg/month) and functionally iron deficient. The control groups just required monthly doses <400 U/kg to maintain Hct value ≥30%.

In protocol I, 11 patients belonged to the control group (Control I) and 15 to the study group (IVFE). IVFE patients received i.v. infusion of 100 mg ferric saccharate (Ferrum Hausmann®, Switzerland), diluted in 50 ml of 0.9% sodium chloride, postdialysis on five consecutive dialysis sessions during the first 2 weeks of the study. The follow-up period was 12 weeks. In protocol II, 12 patients belonged to the control group (Control II) and 12 to the study group (IVA). Ascorbic acid, each 300 mg, was administered intravenously postdialysis thrice a week for 8 weeks to IVAA patients. Controls in both protocols did not receive any adjuvant therapy. The rHuEpo dose ranged from 4000 to 8000 U/week given subcutaneously in 2–3 doses. In order to minimize the number of dosage adjustments to allow direct comparison of the Hct response, rHuEpo dose was maintained during the initial 4 weeks. After one month, the dose was decreased by 2000 U/week if Hct increased to greater than 31%. The dose was adjusted biweekly to maintain a Hct value of 30–31%.

Laboratory measurements

Blood was drawn before HD. Haemoglobin (Hb) and Hct were determined every 2 weeks. Iron metabolism indices including serum iron, total iron binding capacity (TIBC) and serum ferritin were measured before, 2, 6, and 12 weeks after the start of the study in protocol I, and before, 2, 4, and 8 weeks after the start of the study in protocol II. Hb and Hct were determined with a computerized Coulter counter. Serum iron was measured by a colorimetric method (Hitachi 736-60 autoanalyzer, Japan), TIBC by TIBC Microtest (Daichi, Japan), and ferritin by a radioimmunoassay (Incstar, MN, USA). TS was calculated by dividing serum iron by TIBC × 100. Plasma oxalate was measured with soluble oxalate oxidase, as described by Petraru et al. [17], in IVAA patients. Briefly, tenfold diluted plasma was deproteinized by sulfoalicylic acid solution, then the supernatant was mixed with buffered charcoal suspension. The charcoal-treated sample combined with 3-dimethylaminobenzoic acid/3-methyl-2-benzothiazolione hydrazone hydrochloride reagent and peroxidase was incubated at room temperature for 30 min. The mixture then added with oxalate oxidase was incubated at room temperature for 60 min and the absorbance was measured at 590 nm with a spectrophotometer. Blanks were prepared similarly but substituting water for the oxalate oxidase.

To test for adjuvant effects, blood samples from all four groups were also evaluated for serum aluminium, intact PTH, inflammatory indices: C-reactive protein (CRP) and fibrinogen, and haemolytic indices: haptoglobin and reticulocyte index. Serum aluminium concentrations were determined in duplicate by graphite furnace atomic absorption spectrophotometry (GBC-906 spectrophotometer, Australia). The detection limit was 0.5 μg/l and the coefficient of variation was lower than 4%. Serum intact PTH levels were measured by a radioimmunoassay (Incstar, MN, USA), CRP by rate nephelometry (Beckman, Ireland), fibrinogen by the clotting method (Fibri-Prest Automatic, France), and haptoglobin by radial immunodiffusion (Nordmarken, Germany). Reticulocytes were measured by an automated flow cytometer and reticulocyte index was calculated using the formula: reticulocyte count (%) = × (patient’s Hct/normal Hct).

Statistics

Data were expressed as mean values ± SEM. The statistical analysis was conducted by analysis of variance for
within-group comparison among posttreatment values and baselines. We used the Mann-Whitney ranked sum test for the differences of between-group comparison and paired Student’s t test for the differences of within-group comparison. Frequency distribution was analyzed through χ² statistics. A P value < 0.05 was considered statistically significant.

**Results**

The four groups were similar with regard to age, gender, duration of HD and rHuEpo treatment, and distribution of renal failure causes (Table 1). No parameters causing poor responses to rHuEpo other than decreased iron availability existed in the IVFE and IVAA patients (Table 1). The four groups were equivalent with regard to the values of serum aluminium, intact PTH, CRP, fibrinogen, haptoglobin, and reticulocyte index.

<table>
<thead>
<tr>
<th>Causes of end-stage renal disease</th>
<th>IVFE</th>
<th>Control I</th>
<th>IVAA</th>
<th>Control II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic glomerulonephritis</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Chronic interstitial nephritis</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Hypertensive nephrosclerosis</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Unknown aetiology</td>
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<tr>
<th>Parameters</th>
<th>Protocol I</th>
<th>Protocol II</th>
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<tr>
<td>IVFE</td>
<td>Control I</td>
<td>IVAA</td>
</tr>
<tr>
<td>Serum aluminium, mg/l</td>
<td>28.5 ± 1.3</td>
<td>35.0 ± 3.2</td>
</tr>
<tr>
<td>Ferritin, mg/l</td>
<td>578 ± 38</td>
<td>773 ± 132</td>
</tr>
<tr>
<td>Serum iron, mg/dl</td>
<td>42.5 ± 4.7</td>
<td>50.2 ± 6.9</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>19 ± 5</td>
<td>20 ± 6</td>
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IVFE, patients receiving i.v. iron supplementation; IVAA, patients receiving i.v. ascorbic acid therapy; rHuEpo, recombinant human erythropoietin.

**Table 1. Characteristics of haemodialysis patients and baseline values of parameters pertaining to responsiveness to rHuEpo treatment**

**Effect of i.v. iron supplementation**

Basal serum ferritin was not significantly different between the IVFE group and Control I (Table 2). The
mean rHuEpo dose was significantly higher ($P<0.05$), and the lower Hct, Hb, and TS values were markedly lower ($P<0.01$) in the IVFE group compared to Control I. Intravenous iron supplementation neither improved erythropoiesis nor reduced the rHuEpo dose during 12 weeks of study. Hct values in the IVFE patients did not significantly increase (Figure 1a). The respective values of the iron metabolism indices at 2, 6, and 12 weeks (Figure 2) for serum ferritin were $701\pm 46$, $622\pm 39$ and $554\pm 34 \mu g/l$; serum iron $89\pm 11$, $69\pm 9$ and $50\pm 5 \mu g/dl$; and TS $39\pm 3$, $31\pm 4$ and $20\pm 2\%$. Iron metabolism indices significantly increased at 2 and 6 weeks, but decreased at 12 weeks returning to the basal values. In Control I (Table 2), the monthly rHuEpo dose was not significantly changed during the 12 weeks. The mean Hct, Hb, serum ferritin, serum iron, and TS values also maintained stationary throughout the study.

**Effect of i.v. ascorbate therapy**

In the IVAA group, basal serum ferritin did not differ from Control II (Table 2). However, basal Hct, Hb, and TS values were markedly lower ($P<0.01$), and the mean rHuEpo dose was significantly higher ($P<0.05$) than those in Control II. Hct values significantly increased at 2 weeks and all subsequent weeks in the IVAA group (Figure 1b). Mean Hct reached the value of $30.6\pm 0.6\%$ at study completion with a significant reduction of $20\%$ in rHuEpo dose (Table 2). During i.v. ascorbate therapy, iron parameter values at 2, 4, and 8 weeks were serum ferritin of $558\pm 103$, $536\pm 102$ and $472\pm 91 \mu g/l$; serum iron of $97\pm 14$, $102\pm 8$ and $107\pm 19 \mu g/dl$; and TS of $38\pm 5$, $41\pm 6$ and $48\pm 6\%$, respectively (Figure 3). Serum ferritin modestly decreased at study completion but without statistical significance compared to baseline ($P<0.05$). TS markedly increased at 2 weeks and all subsequent weeks. This was due to an increase in serum iron. In Control II (Table 2), rHuEpo dose remained unchanged for 8 weeks. There were also no significant changes in the Hct, Hb, serum ferritin, serum iron, and TS values.

**Plasma oxalate levels and follow-up after stopping ascorbate therapy**

In 10 IVAA patients, there was no significant change in plasma oxalate concentrations during 8 weeks of treatment. A modest increase was found at 8 weeks but it did not reach statistical significance compared to baseline (Table 3). Mean Hct and Hb declined significantly and rHuEpo dose increased 2 weeks after i.v. ascorbic acid treatment was stopped.

**Discussion**

Iron overload in dialysis patients may be represented by a serum ferritin level of $>500 \mu g/l$ proposed by Anastasiades et al. [16], $>800 \mu g/l$ by Macdougall et al. [13], or $>1000 \mu g/l$ by Eschbach et al. [18]. Iron overload not only increases cardiovascular and infectious
Ascorbate and iron for erythropoietin-hyporesponsive anaemia in iron overload

Ascorbate and iron for erythropoietin-hyporesponsive anaemia in iron overload

Fig. 2. Values of serum ferritin, serum iron, and transferrin saturation at baseline and following intravenous iron supplementation (100 mg of ferric saccharate on five consecutive dialysis sessions) in IVAA group. Analysis of variance was used for within-group comparison among post-treatment values and baselines. *P<0.05 vs baselines.

Fig. 3. Values of serum ferritin, serum iron, and transferrin saturation at baseline and following intravenous ascorbic acid (300 mg, thrice weekly for 8 weeks) in IVAA group. Analysis of variance was used for within-group comparison among post-treatment values and baselines. *P<0.05 vs baselines.

Table 3. Concentrations of plasma oxalate in 10 patients receiving 8 weeks of intravenous ascorbic acid. Haematocrit values, haemoglobin concentrations and rHuEpo dose 4 weeks after stopping treatment.

<table>
<thead>
<tr>
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<th>During treatment</th>
<th>After treatment</th>
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<tbody>
<tr>
<td></td>
<td>0 week</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Serum oxalate, µmol/l</td>
<td>35.8±5.9</td>
<td>45.6±6.7</td>
</tr>
<tr>
<td>Haematocrit, %</td>
<td>30.6±0.6</td>
<td>27.8±0.6*</td>
</tr>
<tr>
<td>Haemoglobin, g/dl</td>
<td>330±54</td>
<td>376±40*</td>
</tr>
</tbody>
</table>

morbidity, but sometimes may cause relative resistance to rHuEpo in HD patients. El-Reshaid et al. [19] showed that the rHuEpo dose required to maintain the target Hb level is nearly twice as large in patients with severe iron overload (ferritin >1100 µg/l) as in controls (ferritin 100–600 µg/l). The mechanism of this rHuEpo hyporesponsiveness remains obscure, but inadequate iron mobilization and defective iron utilization are the possible reasons. Ali et al. [20] found that marrow iron stores were depleted in 10 HD patients with a mean ferritin of 1336 µg/l. El-Reshaid et al. [19] also observed that TS decreased to a level of <30% in 6 of 11 iron-overloaded patients.

Several studies reported that patients with ferritin of >500 µg/l may have functional iron deficiency and respond to i.v. iron therapy [6,13,21]. However, the beneficial effect of iron therapy is not observed in our study or by Rosenlöf et al. [14] and Goch et al. [22]. After long-term i.v. administration of iron dextran in 36 patients on HD, Ali et al. [20] found a paradox of hepatosplenic iron overload and marrow iron depletion. They proposed that the bulk of i.v. iron dextran is taken up by the liver and spleen, and that the hepatosplenic stores fail to be mobilized to the bone marrow. Therefore, iron supplementation is not war-
Gastaldello et al. [15] reported that resistant anaemia due to functional iron deficiency was corrected by i.v. ascorbate therapy (500 mg, 1–3 times/wk) in four HD patients with iron overload (ferritin > 500 μg/l). Our data partially corroborated their findings. During the 8 weeks of i.v. ascorbate treatment, the mean Hct markedly increased with a significant reduction in rHuEpo dose at study completion. The latter was not found by Gastaldello et al. TS is a marker for functional iron deficiency, indicating balance between supply and demand of plasma iron [5]. TS was lower initially in the IVAA patients and significantly increased 2 weeks after ascorbate therapy. The data are consistent with the proposal that i.v. ascorbate acts as a mediator facilitating iron release from storage sites and its delivery to haematopoietic tissues. It can increase iron utilization in erythroid progenitor cells. The dual effects improve the erythroid response to rHuEpo and result in a haematocrit rise.

Vitamin C is essential for HD patients, however, the use of excessive amounts of this vitamin should be avoided. Vitamin C overdose may cause secondary oxalosis [23], leading to plasma levels of oxalate at which deposition of calcium oxalate in tissue can occur. The available data suggest that a daily dose of 200 mg of vitamin C should be sufficient to prevent ascorbate deficiency in dialysis patients [24]. Moreover, Costello et al. [25] declared that a daily dose of ascorbate not exceeding 150 mg is considered safe in uraemics. The available data suggest that a daily dose of 300 mg vitamin C should be sufficient to prevent ascorbate deficiency in dialysis patients [24]. Our human erythropoietin doses by the use of chronic intravenous iron supplementation in patients treated with erythropoietin.

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