Synergistic effect of desferrioxamine and recombinant erythropoietin on erythroid precursor proliferation in chronic renal failure

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Abstract

**Background.** Desferrioxamine (DFO) has been suggested to improve erythropoiesis in end-stage renal failure independently of its aluminium (Al)-chelating effect. A possible synergistic effect of DFO and recombinant human erythropoietin (r-HuEpo) could be very useful in treating anaemia of chronic renal failure.

**Methods.** In order to verify whether a synergistic action of DFO and r-HuEpo exists, we enrolled 11 patients undergoing chronic haemodialysis and r-HuEpo treatment. All had a negative DFO test, very low serum Al levels (<20 μg/l), ferritin >100 ng/ml and iPTH <200 pg/ml. Samples were drawn for a basal erythroid precursor (burst-forming unit-Erythroid, BFU-E) evaluation. After isolation by Ficoll–Hypaque, a 14 day incubation was carried out with: (i) r-HuEpo 3 U/ml; (ii) r-HuEpo 30 U/ml; and (iii) r-HuEpo 30 U/ml + DFO 167 μg/ml. Patients then received 5 mg/kg DFO infused during the last hour of each dialysis session for 12 weeks. New BFU-E evaluations were performed after 2, 6 and 12 weeks of treatment. BFU-E colonies were counted in duplicate with an inverted microscope after 14 days.

**Results.** High dose r-HuEpo achieved greater proliferation than low dose r-HuEpo cultures during all phases of the study. At baseline, r-HuEpo and DFO culture had a greater number of colony units than high dose r-HuEpo culture (103.7 ± 50.2 vs 95.1 ± 50.5, NS). This increase became significant after 2 weeks (145 ± 59.3 vs 122.9 ± 59.6, P < 0.02), and remained so at 6 (167.4 ± 60.3 vs 149 ± 55.6, P < 0.01) and 12 weeks (191 ± 64.5 vs 155.1 ± 56.3, P < 0.01). An increased proliferation was observed after DFO therapy in all culture studies: low dose r-HuEpo culture increased from 69.4 ± 38.2 to 86.6 ± 48.5, 115 ± 39 and 123 ± 46; high dose r-HuEpo culture increased from 95.1 ± 50.5 to 122.9 ± 59, 149 ± 55.6 and 155.1 ± 56.3 and r-HuEpo plus DFO culture from 103.7 ± 50.2 to 145 ± 59.3, 167 ± 60.3 and 191 ± 64.5 at 2, 6 and 12 weeks, respectively (all P < 0.01 by ANOVA). Haemoglobin, reticulocytes and soluble transferrin receptor were slightly increased, while ferritin decreased. Hypochromic erythrocytes were variable.

**Conclusions.** DFO increases erythroid precursor proliferation and has a synergistic in vivo effect with r-HuEpo in patients with chronic renal failure. Further investigations are needed to evaluate whether such an effect may have clinical application.

Key words: chronic uraemia; desferrioxamine; erythroid precursors; erythropoiesis

Introduction

The anaemia of chronic renal failure (CRF) patients is due mainly to inadequate erythropoietic hormone levels. In addition, anaemia may be worsened by aluminium overload resulting from the dialysate or use of aluminium-containing phosphate binders. The use of desferrioxamine (DFO) in the treatment of aluminium (Al) overload is a well defined strategy; it results in improvement of anaemia and a reduced requirement for recombinant human erythropoietin (r-HuEpo) [1].

However, DFO may improve the anaemia in CRF patients without Al intoxication or in dialysis populations with only moderate serum Al levels [2]. DFO has proved to be very effective in patients without Al overload [3,4], including heterogeneous clinic conditions such as rheumatoid arthritis [5], favism [6] or myelodysplastic syndromes [7]. Most of these pathological conditions share a chronic inflammatory state.

A direct effect of DFO in improving anaemia, unrelated to Al chelation [3,4,8] has been discussed controversially [9,10]. New papers strengthen the argument for a direct effect. After the first noteworthy work of
Giordano and colleagues [5], DFO was confirmed to improve anaemia in rheumatoid arthritis, probably by enhancing the action of endogenous erythropoietic hormone [11].

Iron chelators, e.g. defipirone, may improve erythropoiesis in patients affected by myelofibrosis or post-transfusional haemosiderosis [12]. Patients on chronic dialysis, also affected by iron overload, may show a relative resistance to r-HuEpo therapy [13]. This resistance could be overcome by using an iron chelator, improving the bioavailability of iron. In fact, iron chelators may improve erythropoiesis by increasing the delivery of the element from the storage pool to the haematopoietic tissue, enhancing the response to r-HuEpo in iron overload conditions [14].

Moreover, both r-HuEpo and DFO may increase the transferrin receptor expression on the cell surface and transferrin cellular uptake [15]. The two drugs show similar effects on iron metabolism, and a synergistic action may be hypothesized [16].

In a previous study, we showed that DFO increased burst-forming unit-erythroid (BFU-E) proliferation in uraemic patients [17]. The present study was carried out, in vitro and in vivo, to verify whether a synergistic action of DFO and r-HuEpo on BFU-E proliferation exists. Preliminary data seemed to support such a synergy [18].

Materials and methods

Patients

We enrolled 11 patients, four males and seven females, on chronic haemodialysis, with a mean age of 52 ± 11 months.

They were affected by chronic pyelonephritis (n = 5), autosomal polycystic kidney disease (APKD) (n = 2), membranous glomerulonephritis (n = 2), diabetes type 1 (n = 1) and renal vasculitis (n = 1).

None of the patients suffered from other chronic inflammatory, haematological or neoplastic diseases. All patients were treated with a constant weekly dose of r-HuEpo during the previous 4 months. All had serum ferritin > 100 ng/ml and intact parathyroid hormone (iPTH) levels < 200 pg/ml. Moreover, although serum AI levels were in the 'normal' range for uraemic patients (between 12 and 47 mg/l), and intact parathyroid hormone (iPTH) levels < 200 pg/ml. Moreover, although serum AI levels were in the 'normal' range for uraemic patients (between 12 and 47 mg/l), and intact parathyroid hormone (iPTH) levels < 200 pg/ml. Moreover, although serum AI levels were in the 'normal' range for uraemic patients (between 12 and 47 mg/l), and intact parathyroid hormone (iPTH) levels < 200 pg/ml.

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115 ± 39 and 123 ± 46; high dose r-HuEpo culture increased from 95.1 ± 50.5 to 122.9 ± 59, 149 ± 55.6 and 155.1 ± 56.3; and high dose r-HuEpo + DFO culture increased from 103.7 ± 50.2 to 145 ± 59.3, 167 ± 60.3 and 191 ± 64.5, respectively (all \( P < 0.01 \) by ANOVA).

Moreover, in each evaluation, the combined culture always showed the greatest increase, with significant values compared with high dose r-HuEpo culture after 2 (145 ± 59.3 \( \text{vs} \) 122.9 ± 59.6, \( P < 0.02 \)), 6 (167.4 ± 60.3 \( \text{vs} \) 149 ± 55.6, \( P < 0.01 \)) and 12 (191 ± 64.5 \( \text{vs} \) 155.1 ± 56.3, \( P < 0.01 \)) weeks. After 12 weeks, no evidence of a plateau of proliferation was noted.

Culture studies performed at baseline and after 2 and 6 weeks of placebo treatment in the five patients did not show any significant variation in erythroid precursor proliferation. BFU-E colonies in the placebo group were 65 ± 14, 74 ± 22 and 69 ± 41 at baseline and after 2 and 6 weeks of NaCl 0.9% therapy, respectively.

Biochemical parameters

Serum Al was in the normal range for uraemic subjects at baseline as well as after the DFO test (4–36 and 51–63 mg/l, respectively). Haematological parameters are shown in Table 1.

Haematocrit and haemoglobin slightly increased and were significant at week 6 (\( P < 0.05 \)). The increase in reticulocytes was significant after 12 weeks (1.4 \( \text{vs} \) 2.3, \( P < 0.01 \)). Hypochromic erythrocytes decreased slightly, as did ferritin (379 ± 147 \( \text{vs} \) 305 ± 99 \( \text{vs} \) 317 ± 118 \( \text{vs} \) 291 ± 85, \( P < 0.02 \)). Transferrin and TIBC remained unchanged. On the other hand, sTR increased significantly at week 6 and 12 and sEpo at week 6 (see Table 1). Moreover, in each evaluation, the combined culture always showed the greatest increase, with significant values compared with high dose r-HuEpo culture after 2 (145 ± 59.3 \( \text{vs} \) 122.9 ± 59.6, \( P < 0.02 \)), 6 (167.4 ± 60.3 \( \text{vs} \) 149 ± 55.6, \( P < 0.01 \)) and 12 (191 ± 64.5 \( \text{vs} \) 155.1 ± 56.3, \( P < 0.01 \)) weeks. After 12 weeks, no evidence of a plateau of proliferation was noted.

Table 1. Haematological parameters

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>2nd week</th>
<th>6th week</th>
<th>12th week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct (%)</td>
<td>29.7 ± 5.5</td>
<td>29.9 ± 4.7</td>
<td>30.5 ± 7.3**</td>
<td>29.9 ± 5.2</td>
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<tr>
<td>Hb (g/dl)</td>
<td>9.38 ± 1.2</td>
<td>9.27 ± 1.1</td>
<td>9.7 ± 1.5**</td>
<td>9.8 ± 2.1**</td>
</tr>
<tr>
<td>HE (%)</td>
<td>9.9 ± 4</td>
<td>8.9 ± 8.2</td>
<td>5.7 ± 7.7**</td>
<td>6.7 ± 6.9</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>1.41 ± 0.4</td>
<td>1.72 ± 0.2</td>
<td>2.01 ± 1.2</td>
<td>2.3 ± 1.8*</td>
</tr>
<tr>
<td>Transferrin (mg%)</td>
<td>162 ± 40</td>
<td>189 ± 60</td>
<td>194 ± 85</td>
<td>141 ± 76</td>
</tr>
<tr>
<td>Ferritin (mg%)</td>
<td>379 ± 147</td>
<td>305 ± 99*</td>
<td>317 ± 118*</td>
<td>291 ± 85*</td>
</tr>
<tr>
<td>TIBC (µg/dl)</td>
<td>243 ± 42</td>
<td>258 ± 72</td>
<td>273 ± 113</td>
<td>265 ± 55</td>
</tr>
<tr>
<td>sTR (ng/ml)</td>
<td>1.6 ± 0.11</td>
<td>1.53 ± 0.43</td>
<td>1.79 ± 0.5**</td>
<td>1.9 ± 1.4**</td>
</tr>
<tr>
<td>sEpo (ml U/ml)</td>
<td>4.7 ± 2.2</td>
<td>4.7 ± 4.1</td>
<td>5.4 ± 3.0**</td>
<td>5.3 ± 2.5</td>
</tr>
</tbody>
</table>

Data are mean ± SD; *\( P < 0.01 \) vs basal; **\( P < 0.05 \) vs basal.

HE, hypochromic erythrocytes; sTR, soluble transferrin receptor; sEpo, serum erythropoietin.

Discussion

The present study confirms that DFO increases erythroid precursor proliferation in CRF, as reported by us [17]. It also shows a synergistic action between DFO and r-HuEpo. It needs to be stressed that at all time points of the study, the combination culture of r-HuEpo and DFO always showed the highest BFU-E proliferation (Figure 1). After 12 weeks of treatment with low dose (5 mg/kg) DFO three times a week, a plateau of the effect was still not reached. Therefore, better results may be achieved by prolonging the treatment.

Recently, DFO has been shown to induce erythropo-
The efficiency of iron delivery from the storage pool is greatly reduced in patients with anaemia of chronic disease, the effectiveness of iron overload patients. In fact, in the uraemic failure subject. Iron chelators could facilitate the release of iron from the storage pool, making it available to the bone marrow. This property is also relevant acute phase. A tremendous increase in iron demand may occur when r-HuEpo therapy is administered to a renal failure subject. Iron chelators could facilitate the release of iron from the storage pool, making it available to the bone marrow. This property is also relevant acute phase. Iron chelators could facilitate the release of iron from the storage pool, making it available to the bone marrow. This property is also relevant acute phase. Iron chelators could facilitate the release of iron from the storage pool, making it available to the bone marrow. This property is also relevant acute phase. Iron chelators could facilitate the release of iron from the storage pool, making it available to the bone marrow. This property is also relevant acute phase. Iron chelators could facilitate the release of iron from the storage pool, making it available to the bone marrow. This property is also relevant acute phase.
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