The role of iron in erythropoiesis in the absence and presence of erythropoietin therapy

Lawrence Tim Goodnough

Department of Medicine and Pathology and Immunology, Washington University School of Medicine, St Louis, Missouri, USA

Abstract

Preoperative autologous blood donation has served as a model for blood loss anaemia. Studies in these patients, along with clinical trials of i.v. iron and recombinant human erythropoietin (rHuEPO) therapy, have furthered our understanding of the relationship between erythropoietin, iron, and erythropoiesis. With supplemental oral iron, the endogenous erythropoietic response to routine autologous blood donation and to the anaemia of chronic illness has been shown to be modest, but predictable. In more aggressive donation and more severe anaemia, the endogenous erythropoietic response is more substantial, but still predictable. Studies in patients undergoing aggressive phlebotomy whilst receiving rHuEPO demonstrate a wide variation in response to rHuEPO dose. This variability is not related to age or gender and suggests factors such as iron-restricted erythropoiesis may be responsible. Supporting evidence arises from the superior erythropoietic response observed in patients with haemochromatosis. These patients maintain very high serum iron and transferrin saturation levels. In response to serial phlebotomy these patients can mount an endogenous erythropoietin response up to five-times greater than healthy individuals. When treated with rHuEPO, patients with haemochromatosis respond with much greater RBC expansion volumes than patients receiving rHuEPO and iron supplementation. Studies show no difference in the degree of endogenously stimulated erythropoiesis between patients with measurable iron stores and those without. However, when treated with rHuEPO, increased erythropoiesis has been observed in patients with measurable iron stores compared with those without. This suggests that, while oral iron supplementation may be sufficient to keep pace with endogenously stimulated erythropoiesis, it may not be adequate to prevent iron-restricted erythropoiesis during rHuEPO therapy. Some studies have suggested that i.v iron may prevent iron-restricted erythropoiesis during rHuEPO therapy although further research is needed. The availability of better tolerated i.v. iron preparations provides an ideal opportunity to study the value of iron therapy in patients with acute blood loss, particularly those undergoing rHuEPO therapy.

Keywords: anaemia; chronic kidney disease; erythropoietin; iron

Introduction

In a review nearly 20 years ago, the knowledge gained regarding the erythropoietic response to anaemia was summarized from studies of normal individuals and patients with haemochromatosis subjected to repeated phlebotomy [1]. Under conditions of basal erythropoiesis in normal subjects, plasma iron turnover (as an index of erythropoietic response) is little affected by the transferrin saturation level. Patients with haemochromatosis who underwent serial phlebotomy were observed to mount erythropoietic responses up to eight-times above basal rates; such high erythropoietic response rates were attributed to the maintenance of very high serum iron and transferrin saturation levels in these patients [2], whereas healthy individuals have been shown to have difficulty providing sufficient iron to support rates of erythropoiesis greater than three-times basal rates [3]. These observations led to the identification of ‘relative iron deficiency.’ This occurs when increased erythropoietin iron requirement exceeds the available supply of iron, even in the presence of storage iron [4]. The recent practice of multiple phlebotomies via autologous blood donation in patients scheduled for elective surgery, is also a model for blood-loss anaemia [5]. This review will summarize the relationship between erythropoietin, iron, and erythropoiesis in patients with blood-loss anaemia, with or
without recombinant human erythropoietin (rHuEPO) therapy [5].

**Erythropoiesis mediated by endogenous erythropoietin**

Under routine conditions, patients undergoing pre-operative autologous blood donation (PAD) usually donate once weekly [6]. Oral iron supplements are routinely prescribed. This iatrogenic blood loss is accompanied by a significant increase in endogenous erythropoietin compared with basal levels. However, these erythropoietin levels remain within the normal range (4–26 mU/ml) [7] and the erythropoietic response that occurs under these conditions is modest [6,8]. A summary of selected prospective, controlled trials of patients undergoing routine PAD is presented in Table 1 [9–14]. Calculated estimates [15] of red blood cell (RBC) volume expansion (erythropoiesis in excess of basal rates) were determined to be 220–351 ml (11–19% RBC expansion) [11,12] or the equivalent of 1–1.75 U of blood [16].

In patients subjected to more aggressive phlebotomy (up to 2 U weekly), the endogenous erythropoietin response is more substantial [11–14]. In one clinical trial [12], a linear-logarithmic relationship was demonstrated between change in haemoglobin (Hb) level and the endogenous erythropoietin response [17]. Erythropoietin-mediated erythropoiesis in this setting is 397–568 ml (19–25% RBC expansion), or the equivalent of 2–3 U of blood [16].

**rHuEPO-mediated erythropoiesis**

Clinical trials have demonstrated a dose–response relationship between rHuEPO dose and RBC expansion [14]. Table 2 details RBC volume expansion in 134 patients receiving rHuEPO therapy during aggressive phlebotomy [12–14,18,19], ranging from 358 to 764 ml (28–79% RBC expansion) over 25–35 days, or the equivalent of 2–9 U of blood [16]. The variability in response (erythropoiesis) to dose (rHuEPO) is not related to patient gender or age [20,21], suggesting that patient-specific factors such as iron-restricted erythropoiesis may account for the variability in response to rHuEPO therapy.

Studies in patients with anaemia of the chronic diseases of osteoarthritis [22–24] and rheumatoid arthritis [25,26] are summarized in Table 3. RBC volume expansion ranged from 157–353 ml (11–24%) for endogenous erythropoietin-mediated erythropoiesis and 268–673 ml (21–40%) with rHuEPO therapy. These erythropoietic responses are indistinguishable from those of patients with anaemia resulting from blood loss alone (Tables 1 and 2).

rHuEPO-enhanced erythropoiesis can become iron-restricted even in patients with measurable storage iron [28] (Figure 1). Indeed, serum ferritin and transferrin saturation levels have been demonstrated to decline by up to 50% during rHuEPO therapy [28]. The superior rHuEPO-stimulated erythropoietic response in patients with haemochromatosis further supports the occurrence of iron-restricted erythropoiesis in patients treated with rHuEPO [19] (Table 2). In patients undergoing aggressive phlebotomy [14], treatment with rHuEPO increased the marrow erythropoietic index from 2.3- (with endogenous erythropoietin stimulation) to 2.9- and 3.6-fold with increasing rHuEPO doses. However, despite a 400% increase in rHuEPO dose, this translated into an increase in erythropoiesis of only 25% (Figure 2).

Two studies have reported no differences in erythropoiesis stimulated by endogenous erythropoietin between patients with or without measurable storage iron. Mean RBC expansion was 20 and 22%, respectively, in the first study [27], and 23 and 24%, respectively, in the second [23]. When patients were

---

### Table 1. Endogenous erythropoietin-mediated erythropoiesis

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Blood removed (donated)</th>
<th>Baseline RBC (ml)</th>
<th>Blood produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Units requested</td>
<td>Units donated</td>
<td>RBC (ml)</td>
</tr>
<tr>
<td>Standard phlebotomy</td>
<td>108</td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>3</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>3</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>41</td>
<td>3</td>
<td>2.9</td>
</tr>
<tr>
<td>Aggressive phlebotomy</td>
<td>30</td>
<td>≥3</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>≥3</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>≥3</td>
<td>2.9</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>6</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>6</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Data expressed as means. p.o., oral; i.v., intravenous.
Table 2. Erythropoiesis during blood loss and rHuEPO therapy

<table>
<thead>
<tr>
<th>Patients (n/sex)</th>
<th>Total rHuEPO dose (IU/kg)</th>
<th>Blood removed</th>
<th>Baseline RBC (ml)</th>
<th>Blood produced</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Units</td>
<td>RBC (ml)</td>
<td>Baseline</td>
<td>RBC (ml)</td>
</tr>
<tr>
<td>(10F) 900 s.c.</td>
<td>3.4</td>
<td>435</td>
<td>1285</td>
<td>358</td>
</tr>
<tr>
<td>(24) 900 i.v.</td>
<td>5.2</td>
<td>864</td>
<td>1949</td>
<td>621</td>
</tr>
<tr>
<td>(10F) 1800 s.c.</td>
<td>4.3</td>
<td>526</td>
<td>1293</td>
<td>474</td>
</tr>
<tr>
<td>(26) 1800 i.v.</td>
<td>5.5</td>
<td>917</td>
<td>2032</td>
<td>644</td>
</tr>
<tr>
<td>(11F) 3600 i.v.</td>
<td>4.9</td>
<td>809</td>
<td>1796</td>
<td>701</td>
</tr>
<tr>
<td>(12F) 3600 i.v.</td>
<td>5.9</td>
<td>1097</td>
<td>2296</td>
<td>1102</td>
</tr>
<tr>
<td>(23) 3600 i.v.</td>
<td>5.4</td>
<td>970</td>
<td>2049</td>
<td>911</td>
</tr>
<tr>
<td>(18) 3600 i.v.</td>
<td>5.6</td>
<td>972</td>
<td>2019</td>
<td>856</td>
</tr>
<tr>
<td>(1M) 4200 s.c.</td>
<td>8</td>
<td>1600</td>
<td>2241</td>
<td>1764</td>
</tr>
</tbody>
</table>

Data expressed as means. p.o., oral; i.v., intravenous; s.c., subcutaneous.

Table 3. Erythropoietin and erythropoiesis in patients with anaemia* of chronic disease

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>RBC removed (U)</th>
<th>RBC produced (ml)</th>
<th>Expansion (%)</th>
<th>Iron therapy</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Osteoarthritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Placebo 6</td>
<td>2.6</td>
<td>157</td>
<td>11</td>
<td>p.o.</td>
<td>23</td>
</tr>
<tr>
<td>3 Placebo</td>
<td>3.3</td>
<td>220</td>
<td>18</td>
<td>p.o.</td>
<td></td>
</tr>
<tr>
<td>rHuEPO (1800 IU/kg i.v.)**</td>
<td>10</td>
<td>3.7</td>
<td>268</td>
<td>21</td>
<td>p.o.</td>
</tr>
<tr>
<td>rHuEPO (1800 IU/kg i.v.)</td>
<td>9</td>
<td>5.2</td>
<td>560</td>
<td>43</td>
<td>p.o. + i.v.</td>
</tr>
<tr>
<td>rHuEPO (3600 IU/kg i.v.)</td>
<td>8</td>
<td>4.0</td>
<td>289</td>
<td>22</td>
<td>p.o.</td>
</tr>
<tr>
<td>rHuEPO (3600 IU/kg i.v.)</td>
<td>12</td>
<td>5.0</td>
<td>515</td>
<td>40</td>
<td>p.o. + i.v.</td>
</tr>
<tr>
<td>2. Placebo 77</td>
<td>3.0</td>
<td>353</td>
<td>24</td>
<td>p.o.</td>
<td>24</td>
</tr>
<tr>
<td>rHuEPO (3600 IU/kg i.v.)</td>
<td>75</td>
<td>4.5</td>
<td>673</td>
<td>44</td>
<td>p.o.</td>
</tr>
<tr>
<td>3. Placebo 26</td>
<td>None</td>
<td>4</td>
<td>0.3</td>
<td>p.o.</td>
<td>25***</td>
</tr>
<tr>
<td>rHuEPO (1200 IU/kg s.c.)</td>
<td>26</td>
<td>None</td>
<td>18</td>
<td>1</td>
<td>i.v.</td>
</tr>
<tr>
<td>rHuEPO (1200 IU/kg s.c.)</td>
<td>26</td>
<td>None</td>
<td>219</td>
<td>14</td>
<td>p.o.</td>
</tr>
<tr>
<td>II Rheumatoid arthritis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo 6</td>
<td>2.3</td>
<td>271</td>
<td>25</td>
<td>p.o.</td>
<td>26</td>
</tr>
<tr>
<td>rHuEPO (3600 IU/kg i.v.)</td>
<td>4</td>
<td>4.8</td>
<td>624</td>
<td>37</td>
<td>p.o.</td>
</tr>
<tr>
<td>rHuEPO (1800 IU/kg i.v.)</td>
<td>11</td>
<td>2.6</td>
<td>291</td>
<td>27</td>
<td>i.v.</td>
</tr>
<tr>
<td>rHuEPO (800 IU/kg s.c.)</td>
<td>11</td>
<td>2.5</td>
<td>337</td>
<td>27</td>
<td>i.v.</td>
</tr>
</tbody>
</table>

Data expressed as means. *With measurable storage iron; **rHuEPO is total dosage of rHuEPO administered; ***perisurgical therapy without autologous phlebotomy. p.o., oral; i.v., intravenous; s.c., subcutaneous.

Fig. 1. The relationship between initial storage iron (mg) and RBC volume expansion (ml/kg) in patients who received rHuEPO therapy. Linear regression analysis demonstrated a significant correlation ($r = 0.6$, $P = 0.02$). Reproduced with kind permission [28].

Fig. 2. Bone marrow erythropoietic index in patients receiving rHuEPO and undergoing aggressive phlebotomy. Erythropoietic response (ml/kg/day) was estimated for each treatment group, according to the formula: bone marrow erythropoietic index = [$RBC$ expansion] + [baseline $RBC$ production] - [baseline $RBC$ production]. Reproduced with kind permission [28].
administered rHuEPO therapy, those without measurable storage iron experienced a modest reduction in erythropoiesis compared with those having measurable storage iron. This difference reached statistical significance in one study (P < 0.05) [24] but not the other (P = 0.07) [27]. These studies indicate that oral iron supplementation is sufficient to keep pace with endogenous erythropoietin-mediated RBC expansion, but may not be sufficient to prevent iron-restricted erythropoiesis during rHuEPO therapy.

Iron therapy

In healthy individuals, i.v. iron supplementation will allow up to a 5-fold increase in erythropoietic response to anaemia caused by significant blood loss [29]. A limiting factor to i.v. iron therapy in patients not undergoing rHuEPO therapy may be that much of the iron administered is transported into the reticuloendothelial system (RES) as storage iron, where it is less readily available for erythropoiesis [30]. In iron-deficient patients, 50% of i.v. iron is incorporated into Hb within 3–4 weeks [31], whereas in patients with anaemia of chronic disease or renal failure, i.v. iron is less rapidly mobilized from the RES [32].

The value of i.v. iron administration in patients receiving rHuEPO therapy outside the setting of renal dialysis has yet to be established. In a clinical trial in patients with osteoarthritis [22], significantly greater erythropoietic responses were seen with i.v. iron therapy compared with patients supplemented with oral iron only (Table 3). However, a subsequent study found no difference in RBC production between oral and i.v. iron therapy in patients before orthopaedic surgery [24]. Another study found that i.v. iron supplementation was not accompanied by a corresponding erythropoietic response to increasing doses of rHuEPO therapy; a 2-fold increase in rHuEPO dose was associated with only a 32% increase in RBC production [18], similar to the dose–response relationship utilizing oral iron supplementation [14]. I.v. iron administered to healthy subjects treated with rHuEPO prevented iron restriction and abolished the marked reduction in serum ferritin. In these individuals, rHuEPO therapy increased the reticulocyte Hb content (a measure in grams per litre of the Hb contained in all reticulocytes), although the total number of reticulocytes generated over 8 days after therapy was not affected [33]. Finally, perioperative exposure to allogeneic blood does not differ between autologous blood donors with measurable storage iron and those without, regardless of whether iron was administered orally [23,27] or i.v. [24].

Conclusion

PAD has served as a model of blood-loss anaemia. Studies in these patients, along with clinical trials of i.v. iron and rHuEPO therapy, have furthered our understanding of the relationship between erythropoietin, iron, and erythropoiesis. The availability of safer i.v. iron preparations allows an opportunity to study the value of iron therapy in patients with acute blood loss, particularly those undergoing rHuEPO therapy. Given the relatively rare but potentially severe side effects of i.v. iron, its use outside the settings of renal dialysis and in Jehovah’s Witness patients needs to be defined by controlled clinical trials.

References


