Pentoxifylline Improves Hemoglobin Levels in Patients with Erythropoietin-resistant Anemia in Renal Failure

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Abstract. It was hypothesized that pentoxifylline might improve the response to recombinant human erythropoietin (rh-Epo) in anemic renal failure patients. Sixteen patients with ESRD and rh-Epo–resistant anemia, defined by a hemoglobin of <10.7 g/dl for 6 mo before treatment and a rh-Epo dose of ≥12,000 IU/wk, were recruited. They were treated with oral pentoxifylline 400 mg o.d. for 4 mo. Ex vivo T cell generation of tumor necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ) from the patients was assessed before treatment and 6 to 8 wk after therapy. A total of 12 of 16 patients completed the study. Before therapy, the 12 patients’ mean hemoglobin concentration was 9.5 ± 0.9 g/dl. After 4 mo of pentoxifylline treatment, the mean hemoglobin concentration increased to 11.7 ± 1.0 g/dl (P = 0.0001). Baseline ex vivo T cell expression of TNF-α decreased from 58% ± 11% to 31% ± 23% (P = 0.0007) after therapy. Likewise, IFN-γ expression decreased from 31% ± 10% to 13% ± 10% (P = 0.0002). Pentoxifylline therapy may significantly improve the hemoglobin response in patients with previously rh-Epo–resistant anemia in renal failure. This may occur due to inhibition of proinflammatory cytokine production, which could interfere with the effectiveness of rh-Epo.

The widespread use of recombinant erythropoietin (rh-Epo) has transformed the management of anemia in ESRD. Hemoglobin concentration improves in 90% to 95% of patients treated. Nevertheless, there is a small but important minority of patients who show an inadequate response to rh-Epo, and in a subset of these, no obvious cause (such as iron deficiency) can be found (1). Failure to respond to rh-Epo may be due to enhanced immune activation, which is known to occur in renal failure patients (2,3). Some proinflammatory cytokines (IFN-γ, TNF-α, and IL-1) suppress erythropoiesis in vitro (4,5). We have recently shown that T cells from renal failure patients responding poorly to rh-Epo generate more IFN-γ and TNF-α compared with both good responders to rh-Epo and healthy controls (6).

Pentoxifylline has been used for more than 20 yr in the treatment of peripheral vascular disease because of its potent hemorrheological properties (7). Subsequently, pentoxifylline was found to have antiinflammatory properties, mediated via inhibition of phosphodiesterase (8). In vitro, pentoxifylline inhibits monocyte production of TNF-α (9) and T cell production of IFN-γ (10,11). TNF-α is thought to play a central role in the pathogenesis of many diseases, prompting the experimental use of pentoxifylline in a number of clinical trials. Beneficial effects have been reported in idiopathic dilated cardiomyopathy (12), childhood type 1 diabetes (13), and systemic vasculitis (14–16). Modest clinical effects have also been observed in rheumatoid arthritis (17). To date, however, this drug has not been tested in patients with rh-Epo–resistant anemia, and the aim of the study presented here was to test the hypothesis that pentoxifylline inhibits proinflammatory cytokine production in vivo, giving rise to enhanced erythropoiesis.

Patients and Methods

Study Patients
We recruited 16 patients with ESRD who exhibited a poor response to rh-Epo. These patients all had hemoglobin concentrations of ≤10.7 g/dl for 6 mo before treatment, despite receiving high doses of rh-Epo ≥12,000 IU/wk. The percentage of patients in our unit with hemoglobin levels of ≤10 g/dl and rh-Epo doses of ≥12,000 IU/wk is 10.4%. Known factors that might inhibit the patients’ response to rh-Epo had previously been excluded from our recruited subjects, including iron deficiency, underdialysis, hyperparathyroidism, and acute infection. One patient was receiving monthly blood transfusions. Eleven of 16 patients were undergoing hemodialysis, 4 of 16 patients were managed by peritoneal dialysis, and 1 patient had a failing renal transplant. The study was approved by the Research Ethics Committee of King’s College Hospital, and informed consent was obtained from all subjects.

Study Protocol
Patients satisfying the above inclusion criteria were prescribed 400 mg pentoxifylline once daily after baseline determination of hemoglobin and T cell cytokines. The patients were given oral pentoxifylline for 12 wk, and T cell cytokine generation was determined immediately before the first dose. Repeat measurement of the T cell
cytokines was performed after treatment with pentoxifylline for 6 to 8 wk. The dose of pentoxifylline selected for this study is slightly lower than that used in other clinical trials of this drug (400 mg three times a day) (12,18) and in clinical practice for peripheral vascular disease (400 mg two to three times a day). This is because the drug may accumulate in renal failure, and once-daily therapy may also improve compliance. The patients’ complete blood count is normally checked monthly as part of their standard clinical treatment. This allowed the retrospective assessment of hemoglobin over the previous 6 mo and the monthly monitoring of hemoglobin while the patients received pentoxifylline therapy.

**Cell Preparation and Culture Conditions**

Blood from peritoneal dialysis patients was collected in the morning and from hemodialysis patients immediately before a dialysis session. PBMC were isolated as described previously (6). PBMC were cultured in the presence of ionomycin (400 ng/ml; Sigma Chemical, Poole, Dorset, UK) plus PMA (10 ng/ml; Sigma). The protein transport inhibitor, brefeldin-A (5 μg/ml; Sigma) was included in all cultures.

**T Cell Cytokine Measurement by Flow Cytometry**

The method developed by Jung et al. (19) and Prussin and Metcalfe (20) was used for cytokine determination. After 18 h culture (37°C, 5% CO₂), the PBMC were washed twice with PBS (Life Technologies, Paisley, UK) containing 0.1% BSA (Sigma) (200 g, 5 min, 4°C). The cells were initially stained with mouse anti-human PerCP-conjugated CD3 (Becton Dickinson, Cowley, Oxford, UK). Control cell cultures.

**Statistical Analyses**

Statistical analyses were carried out by Prism version 3.0 statistical software (GraphPad Software, San Diego, CA; http://www.graphpad.com). Results are expressed as mean ± SD. Differences between the pre- and posttreatment variables were analyzed by Student’s paired t-test. Differences between demographic data of recruited patients and the renal unit patients were analyzed by the Mann-Whitney test. Correlation analysis was carried out by Spearman’s rank correlation. Results were considered significant at \( P < 0.05 \).

**Results**

**Effect of Pentoxifylline on Hemoglobin Concentration**

A total of 12 of 16 patients who started the study continued therapy for 4 mo. Two patients were noncompliant, one patient developed nausea, and one patient developed confusion unrelated to pentoxifylline therapy. Table 1 shows the demographic and laboratory data for the 12 patients who completed the study. The patients’ ferritin levels ranged from 184 to 1215 μg/L (median, 390 μg/L). It is unlikely that severe iron deficiency was present and responsible for the patients’ poor response to erythropoietin. The age of our patients was not dissimilar from the age of patients in our renal unit (54 ± 10 years).

**Table 1. Demographic and laboratory data of patients on pentoxifylline adjuvant therapy**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Gender</th>
<th>Cause of Renal Failure</th>
<th>HD or PD</th>
<th>Time on Dialysis (mo)</th>
<th>Time on rh-Epo (mo)</th>
<th>Rh-Epo Dose (IU/kg/wk)</th>
<th>ACE Inhibitor</th>
<th>Ferritin (μg/L)</th>
<th>PTH (pg/ml)</th>
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<tbody>
<tr>
<td>1</td>
<td>38</td>
<td>F</td>
<td>Polycystic kidneys</td>
<td>HD</td>
<td>121</td>
<td>45</td>
<td>171.0</td>
<td>No</td>
<td>548</td>
<td>69</td>
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<tr>
<td>2</td>
<td>58</td>
<td>M</td>
<td>Renal vascular disease</td>
<td>HD</td>
<td>29</td>
<td>29</td>
<td>365.0</td>
<td>Yes</td>
<td>189</td>
<td>1134</td>
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<tr>
<td>3</td>
<td>61</td>
<td>M</td>
<td>Unknown</td>
<td>HD</td>
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<td>72</td>
<td>428.0</td>
<td>Yes</td>
<td>1215</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>F</td>
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<td>HD</td>
<td>63</td>
<td>34</td>
<td>341.0</td>
<td>Yes</td>
<td>458</td>
<td>95</td>
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<td>34</td>
<td>32</td>
<td>118</td>
<td>Yes</td>
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<td>305</td>
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<tr>
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<td>M</td>
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<td>PD</td>
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<td>8</td>
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<td>No</td>
<td>273</td>
<td>132</td>
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<tr>
<td>7</td>
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<td>M</td>
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<td>PD</td>
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<td>15</td>
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<tr>
<td>8</td>
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<td>18</td>
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<td>1070</td>
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<tr>
<td>10</td>
<td>53</td>
<td>M</td>
<td>Diabetic nephropathy</td>
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<td>4</td>
<td>295.0</td>
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<td>317</td>
<td>ND</td>
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<tr>
<td>11</td>
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<td>Hypertension</td>
<td>HD</td>
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<td>12</td>
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<td>12</td>
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<td>F</td>
<td>Diabetic nephropathy</td>
<td>HD</td>
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<td>36</td>
<td>492.0</td>
<td>Yes</td>
<td>594</td>
<td>1143</td>
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</table>

\[\text{Mean ± SD 54 ± 10}\]
Weights 60 ± 16 yr, \( P = 0.1069 \), respectively), whereas the rh-Epo dose was significantly different (study patients: 294 ± 125 IU/kg/wk versus renal unit patients: 104 ± 91 IU/kg/wk, \( P < 0.0001 \)).

Hemoglobin levels increased significantly from 9.5 ± 0.9 g/dl at the start of treatment to 11.7 ± 1.0 g/dl (\( P = 0.0001 \)) after 4 mo of pentoxifylline therapy (Figure 1). These patients had refractory anemia for at least 6 mo before receiving pentoxifylline (Figure 1). One patient (patient 2, Table 1) was undergoing monthly blood transfusions before pentoxifylline therapy, but these became unnecessary when the patient’s hemoglobin increased to 11.7 g/dl.

All but one of the patients were maintained on the same dose of subcutaneous rh-Epo during the study period. A dose reduction of rh-Epo was required for one patient (patient 11, Table 2), from 18,000 IU to 12,000 IU weekly, when his hemoglobin increased from 9.1 g/dl to 12.6 g/dl at 2 mo. For the 4 mo before receiving pentoxifylline, his hemoglobin (checked monthly) ranged from 8.7 to 9.2 g/dl, and after pentoxifylline, his hemoglobin was 12.6 g/dl at 2 mo, 12.9 g/dl at 3 mo, and 12.8 g/dl at 4 mo, despite the reduction in his dose of rh-Epo.

### Effect of Pentoxifylline on Circulating White Blood Cell Numbers

Table 2 shows the white blood cell parameters of the patients before and 4 mo after treatment. Pentoxifylline therapy had no effect on the numbers of circulating lymphocytes, neutrophils, monocytes, eosinophils, or basophils.

### Effect of Pentoxifylline on Ex Vivo T Cell Cytokine Production

In the 12 patients studied, ex vivo T cell expression of TNF-\( \alpha \) decreased from 58% ± 11% to 31% ± 23% (\( P = 0.0007 \)) after 6 to 8 wk of pentoxifylline therapy. Figure 2 shows the individual pre- and postpentoxifylline TNF-\( \alpha \) values. Similarly, IFN-\( \gamma \) expression decreased from 31% ± 10% to 13% ± 10% (\( P = 0.0002 \)) (Figure 3). There was a significant correlation between change in hemoglobin (%) and TNF-\( \alpha \) generation (%), as illustrated in Figure 4 (\( r_s = 0.7145 \), \( P = 0.0118 \), \( n = 12 \)). Although there was a trend in the change in hemoglobin (%) and IFN-\( \gamma \) generation (%), this did not reach statistical significance (Figure 5, \( r_s = 0.4406 \), \( P = 0.1542 \), \( n = 12 \)).

### Discussion

This study describes a potential new use for an old drug. Pentoxifylline is licensed for the treatment of peripheral vascular disease, but its role as adjuvant therapy to rh-Epo has not previously been described. Nevertheless, like aspirin, it is a drug with ubiquitous properties, including anti–TNF-\( \alpha \) (9) and anti–IFN-\( \gamma \) (10) actions, as well as antioxidant (21) and antiapoptotic effects (22). We selected pentoxifylline for investigation in rh-Epo–resistant patients because these patients show enhanced T cell generation of TNF-\( \alpha \) and IFN-\( \gamma \) (6) compared with good responders to rh-Epo. Although this was conducted as an open-label study, the patients were carefully selected as those who had remained persistently anemic, with hemoglobin less than 10.7 g/dl despite receiving high doses of

**Table 2. White blood cell parameters of patients on pentoxifylline adjuvant therapy**

<table>
<thead>
<tr>
<th></th>
<th>Pre-Pentoxifylline Therapy</th>
<th>Post-Pentoxifylline Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>White cell count (( \times 10^9/L ))</td>
<td>6.52 ± 2.22</td>
<td>6.68 ± 2.56</td>
</tr>
<tr>
<td>Lymphocytes (( \times 10^9/L ))</td>
<td>1.03 ± 0.41</td>
<td>0.93 ± 0.34</td>
</tr>
<tr>
<td>Monocytes (( \times 10^9/L ))</td>
<td>0.57 ± 0.16</td>
<td>0.60 ± 0.29</td>
</tr>
<tr>
<td>Neutrophils (( \times 10^9/L ))</td>
<td>3.14 ± 2.58</td>
<td>3.38 ± 3.23</td>
</tr>
<tr>
<td>Eosinophils (( \times 10^9/L ))</td>
<td>0.45 ± 0.33</td>
<td>0.30 ± 0.21</td>
</tr>
<tr>
<td>Basophils (( \times 10^9/L ))</td>
<td>0.05 ± 0.05</td>
<td>0.05 ± 0.05</td>
</tr>
</tbody>
</table>

* Samples were taken for hematological analysis at the start of pentoxifylline therapy and 4 mo post-pentoxifylline therapy. Results are expressed as the mean ± SD.
rh-Epo. This clinical state had persisted for a minimum of 6 mo in all patients, and indeed, most of them had failed to achieve their target hemoglobin level for a year before pentoxifylline therapy was initiated. It is therefore unlikely that the rise in hemoglobin in this cohort is the result of regression to the mean. One patient (patient 2) was heavily transfusion dependent before commencing pentoxifylline therapy and became transfusion-independent after treatment with this drug. Within 1 to 2 mo of initiating pentoxifylline, this cohort of patients showed a significant increase in hemoglobin concentration. This study is to our knowledge the first demonstration that pentoxifylline therapy downregulates the ability of T cells to generate IFN-γ and TNF-α. Oral administration of pentoxifylline to healthy volunteers reduced TNF-α secretion from PBMC (18). In vitro studies on human whole blood have shown that pentoxifylline inhibits the production of IFN-γ.

Figure 2. Effect of pentoxifylline therapy on T cell generation of TNF-α in 12 patients with a poor response to erythropoietic therapy. PBMC were isolated from whole blood, then stimulated in culture for 18 h before cytokine determination by flow cytometry. Each circle represents the percentage of T cells expressing TNF-α for each patient. The horizontal bar represents the mean values before and after therapy.

Figure 3. Effect of pentoxifylline therapy on T cell generation of IFN-γ in 12 patients with a poor response to erythropoietic therapy. PBMC were isolated from whole blood, then stimulated in culture for 18 h before cytokine determination by flow cytometry. Each circle represents the percentage of T cells expressing IFN-γ for each patient. The horizontal bars represent the means before and after therapy.

Figure 4. Effect of pentoxifylline therapy on percentage decrease of T cell generation of TNF-α versus percentage increase in hemoglobin levels in 12 patients with a poor response to erythropoietic therapy. Each square represents one patient.

Figure 5. Effect of pentoxifylline therapy on percentage decrease of T cell generation of IFN-γ versus percentage increase in hemoglobin levels in 12 patients with a poor response to erythropoietic therapy. Each square represents one patient.
A study on purified human T cells reported that pentoxifylline reduced stimulated TNF-α, IL-5, and IL-10 production, but not IFN-γ secretion (23). In a murine model of allergic pulmonary inflammation, pentoxifylline treatment reduced IFN-γ levels in bronchial lavage and decreased expression of IFN-γ from stimulated spleen cells (24). This demonstrates that the drug can have an in vivo action on IFN-γ generation, which is consistent with our findings.

IFN-γ antagonizes the antiapoptotic effect of erythropoietin on erythroid colony-forming units (the developmental precursors of red blood cells in the bone marrow) (25). TNF-α inhibits erythropoiesis in vitro (5) via an indirect mechanism requiring IFN-β (26). Both of these proinflammatory cytokines have been implicated in the anemia of chronic disease, a complication of inflammatory conditions and malignancy (27). In rheumatoid arthritis patients with anemia of chronic disease, administration of an anti–TNF-α antibody caused a significant rise in hemoglobin levels (28). There was a decrease in the numbers of apoptotic erythroid cells isolated from the bone marrow. Before therapy, anti–TNF-α antibody decreased the formation of erythroid colonies in vitro, but there was no effect after therapy. These findings support the concept that antagonizing the action of TNF-α is successful in treating inflammatory anemia. Anti–TNF-α therapy requires parenteral administration and has been associated with adverse skin reactions (29), increased risk of tuberculosis (30), and lymphoma (30). Pentoxifylline represents a more practical alternative for treatment of renal anemia because it is administered orally and has a low incidence of side effects. Pentoxifylline has a milder effect than other immunosuppressive drugs (such as cyclophosphamide) because the drug had no effect on the numbers of circulating lymphocytes. It is possible that the increase in proinflammatory cytokine generation in our rh-Epo–resistant patients is having a positive biologic effect; hence, inhibiting their generation may result in an adverse clinical action. In the context of renal anemia, we believe these cytokines have a negative action as they antagonize the proliferation of red blood cells (25,26).

Although this is an uncontrolled study, it was conducted under rigorous scientific conditions, and the temporal association with the commencement of pentoxifylline therapy suggests that this is a real effect. The patients in this study had experienced erythropoietin-resistant anemia for a minimum of 6 mo before receiving pentoxifylline, and some of them had shown inadequate hemoglobin levels for up to 1 yr before pentoxifylline therapy. No other interventions or clinical events coincided with the improvement in anemia, and there is a biologic rationale for this effect. The reduction in TNF-α generation from T cells significantly correlated with the rise in hemoglobin in the rh-Epo–resistant patients.

Although the reduction in both TNF-α and IFN-γ levels was associated with a rise in hemoglobin concentration, this study did not show that they were causally related. A poor response to erythropoietin is associated with increased mortality (31), and hemoglobin levels are inversely associated with death and hospitalization levels in patients with renal failure (32). Anemia is also thought to contribute to the risk of congestive heart failure in these patients (33). Correction of the hemoglobin levels with pentoxifylline may therefore have benefits on patient outcome. Because this project was an open-label interventional study, its findings should be interpreted cautiously, although the preliminary results warrant further investigation in a controlled, randomized study.

References


25. Dai CH, Price JO, Brunner T, Krantz SB: Fas ligand is present in human erythroid colony-forming cells and interacts with Fas induced by interferon gamma to produce erythroid cell apoptosis. *Blood* 91:1235–1242, 1998


Access to UpToDate on-line is available for additional clinical information at http://www.jasn.org/