High blood soluble receptor p80 for tumour necrosis factor-α is associated with erythropoietin resistance in haemodialysis patients

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Abstract

Background. Inflammation is one of the major causes of resistance to erythropoietin (rHuEpo) treatment. Tumour necrosis factor-α (TNF-α), one of the most potent proinflammatory cytokines, is known to inhibit human erythropoiesis directly in vitro. Although blood levels of soluble receptors for TNF-α (sTNFRs) are elevated in haemodialysis (HD) patients, the role of sTNFR for rHuEpo responsiveness in HD patients remains to be clarified.

Methods. We measured serum sTNFR (p55 and p80) levels in 83 stable outpatients undergoing regular HD (age 62 ± 1, HD duration 15 ± 1 years). After dividing the patients into three groups according to rHuEpo dose: low (L) < 60, n = 31; moderate (M) ≥ 60 to < 120, n = 31; high (H) ≥ 120 U/kg/week rHuEpo, n = 21), we examined the relationship between serum sTNFR levels and the degree of renal anaemia and rHuEpo dosage.

Results. Haemoglobin was significantly higher in patients receiving low rHuEpo dosage (L, 10.5 ± 0.2; M, 9.7 ± 0.1; H, 9.5 ± 0.2 g/dl, P < 0.01 vs M and H groups). There were no differences in blood TNF-α, sTNFR p55, C-reactive protein, albumin, ferritin, or intact parathyroid hormone levels among the three groups. Body mass index and creatinine generation rate, a marker of whole-body muscle volume, were significantly reduced in group H (P < 0.01). Serum sTNFR p80 levels were significantly higher in group H (4.88 ± 0.45 ng/ml) than in L (3.73 ± 0.14 ng/ml) and M (3.67 ± 0.21 ng/ml) groups (P < 0.05). The blood interleukin (IL)-6 level was also increased in patients requiring high rHuEpo doses (L, 5.5 ± 0.5; M, 6.4 ± 0.5; H, 10.2 ± 2.0 pg/ml, P < 0.05 vs L and H groups). A stepwise regression analysis revealed that gender and sTNFR p80 were significant predictors of rHuEpo dosage. A significant direct relationship was found between rHuEpo dose and sTNFR p80 (r = 0.499) and IL-6 (r = 0.439) values in women (P < 0.01) but not in men.

Conclusions. These findings suggest that high blood sTNFR p80 may contribute to the development of rHuEpo resistance in female patients undergoing long-term HD.

Keywords: erythropoietin; female; interleukin-6; resistance; soluble TNF receptor

Introduction

Recombinant human erythropoietin (rHuEpo) has been increasingly used for the treatment of renal anaemia in the last decade. However, about 10% of haemodialysis (HD) patients are resistant to conventional rHuEpo therapy. In these patients, high doses of rHuEpo are required to maintain a satisfactory haemoglobin (Hb) level. Several factors including gender, iron deficiency, vitamin deficiency, severe secondary hyperparathyroidism, aluminium toxicity, and inflammation have been shown to be associated with the rHuEpo resistance. However, the factors most likely to contribute to the development of rHuEpo resistance in dialysis patients remain to be clarified.

Recently, inflammation has been shown to play an important role in the development of dialysis-related long-term complication and mortality. Beguin et al. [1] first determined that the serum fibrinogen concentration, an acute-phase reactant, provided a good predictor for initial responses to rHuEpo therapy in HD patients. In addition, Bergström and colleagues [2] reported that the mean doses of weekly rHuEpo in patients with serum C-reactive protein (CRP) > 2.0 mg/dl were 80% higher than those with serum CRP < 2.0 mg/dl. Gunnell et al. [3] also demonstrated...
a significant positive relationship between blood CRP levels and the rHuEpo/haematocrit ratio. These findings suggested that the acute-phase inflammatory response seems to be involved in the rHuEpo sensitivity of HD patients.

Inflammatory stimuli elicit the release of a variety of cytokines from circulating monocytes and macrophages that cause systemic changes. Tumour necrosis factor-α (TNF-α) is one of the most potent proinflammatory cytokines. The endogenous formation of TNF-α leads to the expression of TNF receptors (p55 and p80) on the cell surface, which then shed into circulating blood through proteolytic cleavage and circulate as soluble molecules. Recently, these circulating soluble receptors for TNF-α (sTNFRs) have been shown to be correlated with disease activity, malnutrition, and mortality in many diseases. Excretion of sTNFRs is mainly through the kidney, and blood levels progressively increase along with the degree of renal dysfunction [4]. In HD patients, the production of TNF-α leads to sTNFR levels that are approximately 10-fold higher than in those of normal subjects [5]. However, it is unclear whether increased blood sTNFR contributes to rHuEpo resistance.

The aim of the present study was to investigate the role of proinflammatory cytokines in the development of renal anaemia and rHuEpo resistance. We measured serum TNF-α, sTNFRs, and interleukin-6 (IL-6) levels, and compared these to the severity of anaemia and to rHuEpo dose. We found positive correlations between rHuEpo dose and blood sTNFR p80 and IL-6 values in our patients.

Subjects and methods

Patients

We selected 83 of 331 patients receiving regular HD in one dialysis centre (Maruyama Hospital, Hamamatsu, Japan). We excluded patients with the complications of hospitalization, acute infections, malignancy, collagen disease, or liver cirrhosis, because these conditions could secondarily increase proinflammatory cytokines. As a result, 267 patients who had been undergoing regular HD for more than 3 years fulfilled the criteria, and 87 of these consented to join the study. There were no patients with serum aluminium levels >5 μg/l. No patient had occult blood stools. The study group comprised 47 men and 36 women, with a mean age of 62 ± 1 years (range 39–79 years). Nine patients had renal disease due to systemic disease, including five with diabetes mellitus, and four with autosomal dominant polycystic kidney disease. The remainder had primary renal disease. The rHuEpo dosage was adjusted to maintain Hb values above 10.0 g/dl. No patients had received blood transfusion within 1 year. All patients appropriately received i.v. iron supplement to maintain 100 mg/ml of blood ferritin levels. The patients received i.v. rHuEpo at the end of each HD session. The mean doses were calculated and expressed as U/kg/week. We divided all patients into three subgroups according to required rHuEpo doses: (low L), <60; moderate (M), 60 to <120; high (H), ≥120 U/kg/week.

All patients were in steady-state conditions with no complaints at the time of the study.

HD-related factors

All patients had been subjected to regular HD for 4–5 h three times per week at a blood flow rate of 170–200 ml/min. HD duration was 3–31 years, with a mean of 15 years. All patients used bicarbonate dialysate (30 mEq/l, Kindaly AF-2P, Fuso, Osaka, Japan) at a dialysis flow rate of 500 ml/min. All HD treatments were performed using one of the following membranes: low-flux ultrafiltration rate (UFR < 20 ml/min/h) modified regenerated cellulose hollow-fibre (MRC, Terumo, Tokyo, Japan, n = 22; Asahi Medical, Tokyo, Japan, n = 24); medium-flux (UFR 20–40 ml/min/h) cellulose triacetate hollow-fibre (CTA, Nipro Medical, Osaka, Japan, n = 26; Teijin-Gambro, Tokyo, Japan, n = 2) in 28 patients; and high-flux (UFR > 40 ml/min/h) polysulphone synthetic hollow-fibre (PS, Toray Medical, Tokyo, Japan, n = 27; Asahi Medical, Tokyo, Japan, n = 4) in 31 patients. All patients had been using the same type of dialysate membrane for a minimum of 1 year. No re-used dialysate membranes were employed. Neither bacteria nor pyrogen was detected in the dialysate prepared from water obtained by reverse osmosis. We used endotoxin removal filters to obtain concentrations in the dialysate below 20 EU/l by routine analysis with Limulus amoebocyte lysate assay (Wako Junyaku endotoxin measurement kit, Tokyo, Japan). No patient showed any sign of fever or infection. Blood samples were drawn from the arterial side of the arteriovenous fistula at the start and at the end of the dialysis session in the morning after the 2-day interval. Serum was transferred into plastic tubes and stored at −82°C until measurement.

Analytical procedures

Because laboratory data were stable during the 3 months of the study, we conducted analytical determinants using a single sample. Serum urea nitrogen, creatinine, total protein, albumin, total cholesterol, Hb, and bicarbonate were measured by standard laboratory techniques using an automatic analyser. The efficiency of dialysis was assessed from urea reduction rate (URR), calculated from the monthly blood tests and based on the formula (1–post-BUN/pre-BUN) × 100, and from the delivered dose of dialysis (Kt/Vareal) using a single-pool urea kinetic model. Normalized protein catabolic rate (PCRn), an indirect indicator of protein intake, was calculated from dialysis urea removal and serum urea levels. CRP was measured by laser nephelometer. The creatinine generation rate was calculated from changes in body weight and blood creatinine levels during a single HD session [6]. Serum ferritin and β2-microglobulin were determined by the latex agglutination method. Transferrin was measured by Nitroso-PSAP and the thrombin time method using an autoanalyser. Serum TNF-α, sTNFR (p55 and p80), and IL-6 levels were measured using predialysis samples by enzyme-linked immunosorbent assay (ELISA) (QuantiKine HS Human TNF-α Immunoassay, R&D Systems, Minneapolis, MN, USA; sTNFR (55 kDa) ELISA and sTNFR (80 kDa) ELISA, Bender MedSystems, Vienna, Austria; Human IL-6 Ultrasensitive ELISA, Biosource International, Camarillo, CA, USA). Body mass index (BMI) was calculated from the estimated dry weight (kg) and height (m).
Statistical analysis

Values were expressed as means ± standard error. Differences between groups were analysed by a one-way analysis of variance (ANOVA) followed by the Bonferroni-Dunn test. A multiple stepwise regression analysis was applied to examine the relationship between rHuEpo dose and 11 factors, including gender, time on HD, ferritin, PS membrane, TNF-α, sTNFR p55, sTNFR p80, IL-6, CRP, albumin, and PCRn. P values less than 0.05 were considered statistically significant. Statistical calculations were performed with GB-Stat software (Dynamic Microsystems, Silver Spring, MD, USA).

Results

Patient profiles

The mean dose of rHuEpo was significantly different among the three groups (P < 0.001, Table 1). Although there was no difference in patient age between group L (59 ± 2 years, n = 31) and group H (64 ± 2 years, n = 21), group M (65 ± 2 years, n = 31) had a slightly but significantly higher age than group L (P < 0.02). The prevalence of females was significantly higher in group H (P < 0.01). HD duration was identical in groups L (15 ± 1 years) and H (18 ± 2 years), but was shorter in group M (13 ± 1 years, P < 0.03 vs group H). There was no difference in the prevalence of diabetes between groups.

Clinical parameters (Table 1)

HB values were significantly lower in group H compared with groups M and L (P = 0.002, Table 1). In contrast, there were no differences in serum iron, transferrin saturation, or ferritin among the three groups. No differences were found in reticulocyte counts.

Estimated dry weight was significantly lower in group H compared with groups L and M (P = 0.001). Total cholesterol and BMI values in group H were significantly lower than those in groups L and M, but PCRn levels were identical between the three groups (Table 1). Blood albumin and transferrin levels were not significantly different but tended to be lower in group H compared with group L. Serum creatinine levels were significantly decreased in group H compared with groups L (P = 0.001) and M (P < 0.05). Creatinine generation rate, a marker of whole-body muscle volume, was also significantly reduced in group H compared with groups L and M (P < 0.0001). A significant negative relationship was found between creatinine generation rate and rHuEpo dose (r = −0.443, P < 0.0001).

There was no difference in pre-dialysis urea nitrogen, Kt/V_urea, or URR among the three groups. The prevalence of high-flux PS membrane was also identical between the groups.

Blood cytokine levels (Table 2)

Mean blood CRP levels were within normal ranges (<0.5 mg/dl) in all groups. Blood TNF-α levels in HD patients (11.41 ± 0.45 pg/ml, n = 83) were significantly increased compared with those in normal volunteers (2.50 ± 0.43 pg/ml, n = 8, P < 0.001). Similarly, HD patients had significantly higher levels of sTNFR p55 (3.91 ± 0.10 vs 0.21 ± 0.03 ng/ml) and of p80 (4.02 ± 0.16 vs 0.43 ± 0.04 ng/ml) than in normal subjects. Serum IL-6 values were below the detectable range.

Table 1. Clinical parameters in HD patients receiving different doses of rHuEpo

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>31</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>Male/female</td>
<td>22.9</td>
<td>21.11</td>
<td>5.16*</td>
</tr>
<tr>
<td>rHuEpo-related factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rHuEpo (U/kg/week)</td>
<td>17±4</td>
<td>89±3*</td>
<td>170±9**</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.5±0.2</td>
<td>9.7±0.1*</td>
<td>9.5±0.2*</td>
</tr>
<tr>
<td>Reticulocyte (%)</td>
<td>15±3</td>
<td>12±2</td>
<td>18±2</td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>76±37</td>
<td>129±62</td>
<td>65±17</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>95±5</td>
<td>90±4</td>
<td>84±5</td>
</tr>
<tr>
<td>TIBC (µg/dl)</td>
<td>304±10</td>
<td>269±9</td>
<td>287±13</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>32.4±2.2</td>
<td>34.6±1.7</td>
<td>30.9±2.2</td>
</tr>
<tr>
<td>Nutrition-related factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.8±0.05</td>
<td>3.8±0.05</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>Transferrin (µg/dl)</td>
<td>201±8</td>
<td>172±7*</td>
<td>186±10</td>
</tr>
</tbody>
</table>
| BMI (kg/m²)                     | 20.2±0.6 | 19.4±0.3 | 17.7±0.5*#
| Total cholesterol (mg/dl)       | 154±6 | 151±5    | 136±5*|
| Crt generation rate             | 19.4±0.7 | 18.2±0.5 | 15.2±0.7*#|
| PCRN (g/kg/day)                 | 0.97±0.03 | 1.02±0.03 | 0.96±0.05|
| Dialysis-related factors        |      |          |      |
| BUN (mg/dl)                     | 70.1±2.8 | 72.2±2.1 | 67.9±3.2|
| Crt (mg/dl)                     | 11.7±0.4 | 10.7±0.3* | 9.2±0.5*#
| Kt/V_urea                       | 1.28±0.06 | 1.30±0.04 | 1.38±0.09|
| URR (%)                         | 71.0±1.4 | 72.2±1.1 | 72.6±3.1|
| β2-MG (µg/l)                    | 31.7±1.7 | 32.1±1.4 | 28.9±1.9|
| Intact PTH (pg/ml)              | 261±59 | 191±34   | 222±52|
| PS membrane (%)                 | 38.7 | 38.7     | 33.3 |

*P < 0.01 compared with Low group; **P < 0.05 compared with Moderate group; Data are means ± SEM.

Table 2. Blood cytokine levels in patients receiving different doses of rHuEpo

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>31</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.12±0.04</td>
<td>0.34±0.11</td>
<td>0.39±0.17</td>
</tr>
</tbody>
</table>
| TNF-α (pg/ml) | 11.3±0.6 | 10.4±0.6 | 13.1±1.3#
| sTNFR p55 (ng/ml) | 3.86±0.19 | 3.90±0.16 | 3.91±0.19#
| sTNFR p80 (ng/ml) | 3.73±0.14 | 3.67±0.21 | 4.88±0.45**#
| IL-6 (pg/ml) | 5.5±0.5 | 6.4±0.5 | 10.2±2.0**#

CRP, C-reactive protein; TNF-α, tumour necrosis factor-alpha; sTNFR, soluble receptor for tumour necrosis factor; IL-6, interleukin-6. *P < 0.01 compared with Low group; **P < 0.05 compared with Moderate group. Data are means ± SEM.
TNF-α in rHuEpo-resistant dialysis patients

(<0.1 pg/ml) in normal subjects (n = 8), contrasting with elevated levels in HD patients (7.98 ± 0.59 pg/ml). The cytokine values did not correlate with the patient age. In contrast, the time on HD was positively correlated with serum sTNFR p55 (r = 0.285, P = 0.008), p80 (r = 0.432, P < 0.0001), and IL-6 (r = 0.285, P = 0.008), but not with TNF-α levels.

Although serum TNF-α levels were slightly higher in group H, there were no significant differences among the three groups. Blood sTNFR p55 was also identical among patients receiving different rHuEpo dosages. In contrast, serum sTNFR p80 was significantly increased in group H compared with groups L (P = 0.002) and M (P < 0.02). Serum IL-6 values were also significantly higher in group H than in groups L (P < 0.01) and M (P < 0.04). rHuEpo was correlated with blood sTNFR p80 levels (r = 0.363, P < 0.001) and with IL-6 (r = 0.344, P = 0.001) respectively (Figures 1 and 2). The rHuEpo/Hb ratio was also significantly correlated with circulating sTNFR p80 (r = 0.388, P < 0.001) and IL-6 (r = 0.351, P = 0.001).

Similar observations were made in HD patients having serum ferritin values > 100 ng/ml (mean Hb, 9.8 g/dl, ferritin 157 ng/ml, n = 44). In these patients, rHuEpo dose was also positively correlated with serum TNF-α (r = 0.298, P < 0.05), sTNFR p80 (r = 0.441, P = 0.003) and IL-6 (r = 0.452, P = 0.002), but not with sTNFR p55.

Serum albumin levels were negatively correlated with serum sTNFR p80 (r = −0.398, P = 0.0001) and with IL-6 (r = −0.386, P = 0.002). A significant correlation was also found between PCRn and sTNFR p80 (r = −0.221, P < 0.05) but not IL-6 (r = −0.111, P = NS). Serum sTNFR p80 levels were significantly and inversely related to total cholesterol (r = −0.321, P < 0.003), serum creatinine (r = −0.301, P = 0.005) and creatinine generation rate (r = −0.373, P = 0.004).

Similarly, circulating IL-6 was significantly associated with total cholesterol (r = −0.243, P < 0.03), creatinine (r = −0.301, P = 0.005) and creatinine generation rate (r = −0.373, P = 0.0004).

A stepwise multiple regression analysis revealed that gender (female) and sTNFR p80 were independently associated with rHuEpo dose, whereas time on HD, PS membrane, ferritin, TNF-α, sTNFR p55, IL-6, CRP, serum albumin, and PCRn were not related to rHuEpo dose (Table 3).

**Effect of gender (Table 4)**

Since female gender was an independent factor determining rHuEpo dosage in the stepwise multiple regression analysis, we compared circulating cytokine levels between males (n = 47) and females (n = 36). The rHuEpo dosage was higher in females than in males (P < 0.01). Similarly the rHuEpo to Hb ratio was also increased in women compared with men (P < 0.01). There was no difference in serum sTNFR p55 and sTNFR p80 (Fig. 1).

![Fig. 1. A positive association between rHuEpo dose and sTNFR p80 but not with sTNFR p55. There was a positive relationship between blood sTNFR p80 and rHuEpo dose (n = 83, r = 0.363, P = 0.001).](image)

![Fig. 2. A positive relationship between serum IL-6 and rHuEpo dose. There was a linear relationship between serum IL-6 values and rHuEpo dosage in dialysis patients (n = 83, r = 0.344, P = 0.001).](image)

rHuEpo, recombinant human erythropoietin; IL-6, interleukin-6.
Table 3. Stepwise regression analysis of various laboratory variables on rHuEpo dose

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female = 0, male = 1)</td>
<td>-0.292</td>
<td>13.814</td>
<td>7.836</td>
</tr>
<tr>
<td>sTNFR p80</td>
<td>0.294</td>
<td>4.784</td>
<td>7.914</td>
</tr>
</tbody>
</table>

R = 0.475, F-to-remove = 4.697. rHuEpo, recombinant human erythropoietin; sTNFR, soluble receptor for tumour necrosis (TNF). In contrast to these parameters, time of haemodilution, polysulphone membrane, ferritin, TNF-α, sTNFR p55, interleukin-6, C-reactive protein, serum albumin, and normalized protein catabolic rate failed to be independent factors for rHuEpo dosage.

Table 4. Effect of gender on rHuEpo treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>Female</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>47</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>10.1 ± 0.2</td>
<td>9.7 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>rHuEpo (U/kg/week)</td>
<td>62 ± 7</td>
<td>111 ± 12</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>rHuEpo/haemoglobin</td>
<td>6.5 ± 0.9</td>
<td>11.7 ± 1.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>10.2 ± 0.4</td>
<td>13.0 ± 0.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>sTNFR p55 (ng/ml)</td>
<td>3.85 ± 0.15</td>
<td>3.99 ± 0.15</td>
<td>NS</td>
</tr>
<tr>
<td>sTNFR p80 (ng/ml)</td>
<td>3.65 ± 0.14</td>
<td>4.51 ± 0.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>7.4 ± 0.9</td>
<td>8.8 ± 0.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

rHuEpo, recombinant human erythropoietin; TNF-α, tumour necrosis factor-α; sTNFR, soluble receptor for TNF; IL-6, interleukin-6. Data are means ± SEM.

IL-6 values between male and female. In contrast, serum TNF-α and sTNFR p80 levels were significantly higher in women than men (P < 0.01).

A significant positive relationship was found between rHuEpo dose and serum sTNFR p80 (r = 0.499, P = 0.002) and IL-6 values (r = 0.439, P = 0.007) in women. The rHuEpo/Hb ratio was also significantly correlated with sTNFR p80 (r = 0.524, P = 0.0008) and IL-6 (r = 0.941, P = 0.002). Serum sTNFR p80 was strongly and negatively correlated with albumin (r = -0.552, P = 0.0004) and PCRn (r = -0.479, P = 0.003) in females. Serum IL-6 was also associated with serum albumin (r = -0.469, P = 0.004) but not with PCRn in female patients.

In contrast there was no association between rHuEpo dosage and serum sTNFR p80 and IL-6 levels in men. PCRn was significantly but weakly correlated with sTNFR p55 (r = 0.359, P < 0.05), but was not correlated with sTNFR p80 or IL-6. Serum albumin was negatively correlated with sTNFR p80 (r = -0.295, P < 0.05) but not with sTNFR p80 in male patients.

Discussion

There is abundant evidence that TNF-α may modulate haematopoietic processes in bone marrow. Nude mice overexpressing the human TNF gene promptly developed severe anaemia with reticulocytopenia [7]. In addition, mice bearing TNF-producing tumours were resistant to rHuEpo treatment [8]. TNF-α directly inhibits early haematopoiesis through TNF receptors in vitro [9]. Specifically, the TNF receptor p80 largely mediates antiproliferative effects against exogenous rHuEpo in cultured primitive haematopoietic progenitor cells [10]. In HIV-infected patients, increases in blood sTNFRs but not TNF-α levels were associated with an increased dose of rHuEpo [11]. TNF-α blockade with a chimeric monoclonal antibody was reported to increase Hb levels dose-dependently in patients with active rheumatoid arthritis [12]. However, the relationship between soluble TNF receptors and rHuEpo dose remains to be clarified in dialysis patients.

In this study we selected stable outpatients with no complicating diseases that could affect circulating cytokine values. We found that HD patients requiring >120 U/kg/week rHuEpo had higher circulating sTNFR p80 than patients taking <60 U/kg/week. In contrast, serum TNF-α and sTNFR p55 levels did not differ among the three groups. Multiple regression analysis showed that female gender and sTNFR p80 were significant predictors of rHuEpo dose. Serum sTNFR p80 levels were significantly higher in females than in males. A significant direct relationship was found between blood sTNFR p80 levels and rHuEpo dose in women but not in men. These findings suggest that high sTNFR p80 may be associated with the rHuEpo resistance often seen in female patients [13].

Macdougall et al. [14] observed that the elevation of blood IL-6 level was also associated with the degree of renal anaemia, and that reductions in IL-6 corrected the anaemia in patients with end-stage chronic renal failure. In addition, Goichoechea et al. [15] noted a significant correlation between IL-6 production from peripheral blood mononuclear cells and rHuEpo dose in HD patients. Although they found a significant relationship between serum IL-6 values and rHuEpo dose, a multiple regression analysis revealed that IL-6 was not an independent factor for rHuEpo dosage. In agreement with this finding, exogenous IL-6 induced anaemia mainly by intestinal blood loss rather than by suppression of erythrocytosis in bone marrow in rats [16].

In two recent reports, circulating CRP levels were demonstrated to predict rHuEpo dose in dialysis patients [2,3]. In the present study, however, serum CRP values did not correlate with rHuEpo dosage. The reasons for this discrepancy remain unknown, but may be partly due to differences in the patient profile. We selected only stable and afebrile HD patients with mean CRP values within the normal range (mean 0.27 ± 0.06; range 0.00–2.10 mg/dl), whereas patients with higher CRP concentrations were examined in the previous studies. Barany et al. [2] found that HD patients with CRP levels of 2.0 mg/dl or greater required higher weekly rHuEpo dosages. Hyporesponsiveness to rHuEpo due to an inflammatory process was also associated with an increase in blood fibrinogen greater than 400 mg/dl [17]. Thus, further studies...
are needed to determine the role of sTNFR p80 in rHuEpo resistance using HD patients with evident inflammatory status and malignancies.

As previously shown in a large population of HD patients [13], we found that rHuEpo dosage was higher in female than in male HD patients. In addition we found that serum sTNFR p80 levels were significantly higher in women than in men. An association between sTNFR p80 and rHuEpo dose was obvious in our female patients. Together these findings suggest that higher sTNFR p80 levels may be involved in the more marked rHuEpo resistance found in female HD patients. It is well known that females have a larger volume of fat mass. Since soluble TNF receptors can be produced by adipose tissue in humans [18], adipocyte-derived sTNFR p80 may, in part, increase circulating values in women. Additional studies with many subjects will be needed to determine the effect of gender on the TNF-α system in dialysis patients.

It is unclear why serum sTNFR p80 levels were elevated in our population of HD patients. Dialysate quality has been shown to affect circulating cytokine levels and rHuEpo dosage [19]. In the current study, however, we used endotoxin removal filters and reverse osmosis in all patients, and dialysate endotoxin levels were always <20 EU1. It is therefore unlikely that dialysate endotoxin contamination contributed to the increase in circulating TNF receptors in our patients. In addition, there was no difference in age or adequacy of HD, measured by URR and Kt/V urea, between L and H groups. However, the higher concentrations of sTNFRs in patients with a longer HD duration suggest the possibility that uraemia-induced oxidant or carbonyl stress may stimulate the secretion of these receptors from T cells or macrophages.

We found that higher serum sTNFR p80 levels were associated with lower serum albumin, total cholesterol and PCRn values, indicating that elevations of blood sTNFR p80 might be associated with anorexia and malnutrition in HD patients, as was described previously [20]. In addition, sTNFR p80 levels were negatively correlated with serum creatinine and creatinine generation rate, which are markers of whole-body muscle volume. These findings suggest that high circulating sTNFR p80 concentrations may indicate poor nutritional status. Owen and Lowrie [21] found a direct association between CRP and rHuEpo dosage and its inverse association with albumin in dialysis patients. A similar relationship between serum sTNFR p80, albumin and Hb was found in children with protein–energy malnutrition [22]. Since chronic experimental administration of TNF causes anorexia and weight loss [23], these data suggest that TNF-dependent deterioration of nutritional status might further worsen erythropoiesis in our HD patients.

Chronic inflammation is known to affect iron availability. The consequences of inflammation are usually high ferritin concentrations, low iron levels, low to normal transferrin saturation, and functional iron deficiency in the presence of adequate to high iron stores. In this study, transferrin saturation was above 20%. In addition, a significant association between serum sTNFR p80 and rHuEpo dosage was present even in patients with serum ferritin > 100 ng/mL. It is unlikely, therefore, that iron deficiency could affect the association in this study.

In summary, we found that blood sTNFR p80 and IL-6 levels were directly associated with rHuEpo dosage in HD patients. A stepwise regression analysis revealed an independent association of sTNFR p80 and female gender with rHuEpo dose. It follows from these findings that the TNF-α-induced augmentation of the inflammatory response may, in part, contribute to the development of rHuEpo resistance, especially in female HD patients.

Acknowledgement The present work was supported by the Japanese Congress of Renal Anemia in 2000 (Tokyo, Japan).

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*Received for publication: 4.8.00
Accepted in revised form: 27.2.01*