Evidence for Amelioration of Endothelial Cell Dysfunction by Erythropoietin Therapy in Predialysis Patients

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Evidence for the involvement of endothelial cells in the pathogenesis of erythropoietin-induced hypertension, and for endothelial cell damage in patients with chronic renal failure, has emerged and appears to be of major concern. We, therefore, investigated the effect of recombinant human erythropoietin (rHuEPO) therapy on endothelium-derived hormones in predialysis patients with progressive renal anemia.

At the entry to the trial, the serum thrombomodulin concentration (Tm) and plasma endothelin-1 concentration (ET-1) in the predialysis patients were significantly higher than those in age- and sex-matched normal subjects. Following a 16 week period of treatment with rHuEPO given intravenously once a week, patients' hematocrit increased from 27.1 ± 2.6% to 34.6 ± 3.2% (n = 16, P < .001). A positive correlation was found between Tm and serum creatinine concentration (Cr) (r = 0.61, P < .05 (n = 16)), but no correlation was found between ET-1 and Cr. Tm and Tm/Cr significantly decreased from 7.9 ± 2.8 ng/mL to 6.6 ± 2.4 ng/mL (P < .01, n = 16), and from 2.1 ± 0.7 (×10^-10) to 1.6 ± 0.7 (×10^-10), P < .01, n = 16), respectively. However, there was no change in ET-1 as a result of the rHuEPO therapy. Creatinine clearance (CCr), Cr, total amount of daily Tm excretion, Tm clearance/CCr, daily urinary protein and albumin excretion, and blood pressure also remained unchanged throughout the trial.

The present study indicates that correcting anemia by rHuEPO therapy reduces an abnormally elevated Tm in predialysis patients while blood pressure and renal function remain unchanged, suggesting that rHuEPO has a beneficial effect on endothelial cell dysfunction in chronic renal failure patients. This effect may be mediated via an improved oxygen supply to the endothelial cells due to the amelioration of anemia by rHuEPO. Am J Hypertens 1996;9:426-431

KEY WORDS: Erythropoietin, endothelial cells, thrombomodulin, endothelin, renal anemia, chronic renal failure.
circulating soluble Tm has been found in patients with chronic renal failure.1-3 Tm levels are positively correlated with the severity of the impaired renal function.4-6 The former plays a pivotal role in the anticoagulant pathway, while the latter is involved in the regulation of peripheral vascular resistance. An elevated circulating soluble Tm has been found in patients with impaired renal function.7; Tm levels are positively correlated with the severity of the impaired renal function. Abnormally elevated ET-1 levels have been found in patients with chronic renal failure.8,9 Since Tm and ET-1 are released from endothelial cells when they are damaged, elevated circulating Tm and ET-1 levels in renal diseases are considered to arise from damage to vascular endothelial cells rather than reduced urinary excretion capacity.10-12 In view of these observations, it seems reasonable that an elevation of circulating Tm and ET-1 reflects the severity of endothelial cell damage, and that it can be regarded as a useful indicator for monitoring vascular endothelial cell damage.

Chronic renal failure patients at the predialysis stage have progressive anemia and benefit from rHuEPO therapy.13-17 However, the correction of their anemia may have both beneficial or deleterious effects on renal function and blood pressure.18,19 In this regard, the issue of whether the correction of anemia by rHuEPO influences endothelial cell function is of major concern. Assuming that an elevation of circulating Tm and ET-1 could reflect endothelial cell damage and/or dysfunction, the present study investigated the effect of rHuEPO therapy on the endothelium-derived hormones, Tm and ET-1, in predialysis patients.

METHODS

Patients Enrolled in this study were 20 outpatients (10 women, 10 men, average age 57, range 32 to 70) with chronic renal failure at the pre-dialysis stage. The underlying causative renal diseases were chronic glomerulonephritis in eight, diabetic nephropathy in eight, nephrosclerosis in two, but were unknown in two. We used a plasma creatinine concentration of 2 to 5 mg/dL as an inclusion criterion. The mean Ccr at entry was 15 ± 4 mL/min/1.73 m², and the hematocrit less than 30%. The cause of anemia in all patients was secondary to chronic renal failure. Nine patients had hypertension, which was treated with Ca antagonists, β-blockers, α-blockers and angiotensin converting enzyme (ACE) inhibitors. All patients were placed on a strict diet for predialysis chronic renal failure that consists of total calorie intake of 32 cal/kg/day, protein of 0.6 g/kg/day, NaCl of 5 g/day and K of 1.0 g/day. The dietary and medication regimens were kept constant for at least 2 months prior to the study and attempts were made to keep them so throughout the study period. Four patients (two men and two women) dropped out of the study after it started, due to their inability to receive periodical rHuEPO injections. All patients gave informed consent before the study.

Methods Six thousand units of rHuEPO (β-erythropoietin, Chugai Pharmaceutical Co. Ltd., Japan) were injected into the predialysis patients intravenously once a week on an outpatient basis over 16 weeks. In order to maintain serum iron and ferritin concentrations at adequate level, iron was given intravenously as deemed appropriate, the target level for serum iron was set at 100 µg/dL and for ferritin at 100 ng/mL. Blood samples were taken regularly every 4 weeks to measure CBC, serum iron concentration, TIBC, UIBC, ferritin, blood urea nitrogen (BUN) concentration, and creatinine concentration.

Measurements of Blood Parameters Serum creatinine concentration (Cr) and blood urea nitrogen concentration (BUN) were measured using an autoanalyzer. Various blood hormone measurements, including plasma renin activity (PRA), α-human atrial natriuretic peptide (h-ANP), epinephrine (ADN), norepinephrine (NADN), dopamine concentration (DPN), serum thrombomodulin concentration (Tm) and plasma endothelin-1 concentration (ET-1) were performed at the beginning and end (16 weeks) of the treatment. To determine the normal range of Tm and ET-1, blood samples were taken from 28 age- and sex-matched subjects (average age 54, ranging from 35 to 68, 15 men and 13 females) with normal renal function. The Tm in the normal subjects was 3.5 ± 0.7 FU/mL and the ET-1 was 1.32 ± 0.4 pg/mL. Tm was measured by the one-step sandwich method of EIA. ET-1, PRA and h-ANP were measured by RIA. ADN, NADN and DPN were measured by HPLC.

Blood pressure and urinalysis Blood pressure was determined every week in the outpatient clinic with patients in the sitting position. The mean of three consecutive blood pressure measurements was calculated and used as the determined blood pressure value. Twenty-four-hour urine samples were collected by spontaneous micturition and used for the determination of Cr, Na and K concentrations, and Tm. Creatinine clearances (Ccrr) were calculated using the standard clearance formula C = UV/P (where C is the clearance, U is the urinary creatinine concentration, and V the daily urine volume), and expressed as milliliters per minute per 1.73 square meters of body surface.
I- HGURE 1. Serum thrombomodulin (Tm) and plasma endothelin (ET-1) concentration in predialysis patients and normal subjects. *P < .001 compared to the respective value of normal subjects.

**Statistical Analysis** All results are expressed as mean ± SD, unless otherwise indicated. Student t test procedures for paired samples or independent samples were used as appropriate for the statistical analysis. Differences were considered statistically significant at P less than .05.

**RESULTS**

Figure 1 shows the Tm and ET-1 levels of 16 predialysis patients and 28 normal subjects. The levels of Tm and ET-1 in the predialysis patients are substantially greater than the respective values of the normal subjects.

Figure 2 shows the relationship between the endothelium derived hormones, Tm and ET-1, and Cr at the entry to the study. A positive correlation was found between Tm and Cr. No correlation was found between ET-1 and Cr. In addition, no correlation was found between systolic blood pressure and ET-1, and between diastolic blood pressure and ET-1 (data not shown).

Figure 3 shows how Tm was affected by the rHuEPO therapy. At the entry to the trial, Tm was 7.9 ± 2.8 ng/mL (n = 16), which was significantly higher than that in age- and sex-matched normal healthy subjects (3.5 ± 0.7 ng/mL (n = 28), P < .001). After the treatment with rHuEPO, Tm was significantly decreased.

**DISCUSSION**

Tm is a glycoprotein located at the surface of endothelial cells. It is involved in anticoagulant pathways in which it intracellularly activates protein C. ET-1 is secreted from endothelial cells, and is a potent vasoconstrictor that constricts afferent and efferent arterioles in the kidney and lowers GFR and RBF. It acts on renal mesangial cells, reducing Kf and GFR. ET-1 also stimulates cell growth, presumably contributing to the pathogenesis of renal diseases including glomerulonephritis.

Using these two endothelium derived hormones as markers for endothelial cell function, the present study showed that Tm and ET-1 are substantially higher in predialysis patients than in healthy subjects.
and that correcting anemia by rHuEPO therapy lowered Tm in such patients, whereas renal function remained unchanged throughout the study period. One must be critical, however, of the assumption that these hormones are proper indicators of endothelial cell damage. An elevated circulating Tm has been found in a variety of renal diseases, including glomerulonephritis and lupus nephritis. In addition, circulating Tm is positively correlated with the severity of the impaired renal function. Elevated circulating ET-1 levels have also been found in patients with advanced renal failure and this is positively correlated with the severity of impaired renal function. Furthermore, Tm is present in the systemic circulation, and is increased by endothelial cell damage. These observations raise two possibilities: first, that there is underlying endothelial cell damage in some patients with renal diseases; and second, that Tm and ET-1 could be good indicators for endothelial cell damage. Also one must be aware of the fact that the changes in circulating Tm and ET-1 may not be due only to their release from endothelial cells, as renal function also affect the circulating levels of these hormones. Their levels could be determined on the basis that the lower the renal capacity became, the higher the Tm and ET-1 blood concentration would be. In the present study, renal function, blood pressure, and the total amount of daily Tm excretion remained unchanged throughout the study period. This evidence unequivocally excludes the possibility that increased renal excretory capacity reduced the circulating Tm, making it reasonable to suggest that this was due to an im-

FIGURE 3. Changes in hematocrit (Ht) and hemoglobin concentration (Hb) in response to rHuEPO therapy in predialysis patients. Values at 12 weeks significant at P < .05 and values at 16 weeks significant at P < .01, compared to week 0.

FIGURE 4. Effect of rHuEPO therapy on serum thrombomodulin concentration (Tm) in predialysis patients. *P < .005, **P < .001 compared to pre by paired t test. Values in right graph are ×10⁻¹².
provement in endothelial function. One must be aware, in addition, of the possibility that a decrease in circulating Tm could also be due to enhanced non-renal elimination by the liver or, less likely, due to increased catabolism by kidney parenchyma. Taken together, our data strongly suggest the beneficial effect of rHuEPO therapy on endothelial cell dysfunction in predialysis chronic renal failure patients. An improved hematocrit may renew the endothelial cell environment by increasing its oxygen supply.

Direct evidences for rHuEPO receptors in vascular endothelium and for rHuEPO-induced ET-1 release from endothelium have emerged in both human and animal studies, which partially explain the pathogenesis of so-called rHuEPO-induced hypertension. We found that the ET-1 in predialysis patients was higher than that in the normal subjects, but this was not associated with the elevation of blood pressure. Further, rHuEPO therapy did not influence ET-1, per se. The present study also confirms previous work showing no direct effect of rHuEPO on circulating ET-1 levels. These findings suggest that the contribution of circulating ET-1 in the regulation of blood pressure in predialysis patients is small.

There are risks of increased blood pressure in a considerable proportion of rHuEPO treated patients, on one hand. However, amelioration of renal anemia by rHuEPO does not cause an increase in blood pressure. This discrepancy could be explained by the fact that a rapid rise in Ht is associated with a rise in blood pressure. A rise in Ht is more than 0.6%/week in the former reports, and is less than that in the latter. The rate of increase in Ht in our patients is 0.5%/week, thus possibly resulting in no change in blood pressure.

Little is known regarding the effect of rHuEPO on blood pressure regulating hormones such as PRA, h-ANP and catecholamines. Our results show no changes in these parameters, along with no changes in blood pressure after the treatment with rHuEPO (see Table 1). This is in accordance with the previous finding showing that rHuEPO therapy does not affect PRA, angiotensin II, and noradrenaline concentration in patients with chronic renal failure. The absence of rHuEPO effect on these parameters indicates that none of these substances are involved in the regulation of blood pressure during rHuEPO therapy.

In summary, rHuEPO therapy reverses abnormally elevated concentrations of the endothelial derived hormone, Tm, in predialysis patients with progressive anemia, suggesting the beneficial effect of rHuEPO on endothelial dysfunction.

TABLE 1. CHANGES IN VARIOUS PARAMETERS IN RESPONSE TO rHuEPO THERAPY IN PREDIALYSIS PATIENTS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before</th>
<th>After</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>137 ± 19</td>
<td>136 ± 12</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>79 ± 8</td>
<td>76 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>UN (mg/dL)</td>
<td>52 ± 15</td>
<td>55 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>3.8 ± 0.01</td>
<td>4.4 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>PRA (ng/mL/hr)</td>
<td>2.75 ± 2.62</td>
<td>2.48 ± 1.84</td>
<td>NS</td>
</tr>
<tr>
<td>h-ANP (pg/mL)</td>
<td>37 ± 27</td>
<td>44 ± 21</td>
<td>NS</td>
</tr>
<tr>
<td>ADN (pg/mL)</td>
<td>19 ± 10</td>
<td>20 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>NADN (pg/mL)</td>
<td>707 ± 331</td>
<td>559 ± 492</td>
<td>NS</td>
</tr>
<tr>
<td>DPN (pg/mL)</td>
<td>38 ± 39</td>
<td>27 ± 20</td>
<td>NS</td>
</tr>
<tr>
<td>Ccr (mL/min/1.73 m²)</td>
<td>14.2 ± 5.7</td>
<td>15.1 ± 11.8</td>
<td>NS</td>
</tr>
<tr>
<td>U-alb (g/day)</td>
<td>1515 ± 1326</td>
<td>1169 ± 468</td>
<td>NS</td>
</tr>
<tr>
<td>U-prot (g/day)</td>
<td>3.0 ± 2.6</td>
<td>2.0 ± 1.5</td>
<td>NS</td>
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<tr>
<td>U-Tm (mg/mL)</td>
<td>2.2 ± 1.2</td>
<td>2.2 ± 1.0</td>
<td>NS</td>
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<tr>
<td>U-Tm * Vol (ng)</td>
<td>4826 ± 2486</td>
<td>4331 ± 2011</td>
<td>NS</td>
</tr>
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</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure; PRA, plasma renin activity; h-ANP, α-human atrial natriuretic peptide; ADN, plasma adrenaline concentration; NADN, plasma noradrenaline concentration; DPN, plasma dopamine concentration; U-alb, daily excretion of albumin; U-prot, daily excretion of urinary protein; U-Tm, urinary thrombomodulin concentration; U-Tm * Vol, daily excretion of thrombomodulin; Ccr, clearance over creatinine clearance.

REFERENCES
