HEMODYNAMICS, AUTONOMIC FUNCTIONS, AND PLATELET CYTOSOLIC CALCIUM FOLLOWING ERYTHROPOIETIN TREATMENT OF HEMODIALYSIS PATIENTS


Background: Although recombinant human erythropoietin (rh-EPO) is a major therapeutic advance in the treatment of uremic anemia, its systemic effects in hemodialysis (HD) patients have not been well documented.

Methods: Twelve HD patients with anemia (6 men and 6 women) were investigated. The initial dose of rh-EPO was 25-50 μg/kg intravenously three times a week. The dose was then adjusted to maintain the predialytic hemoglobin concentration in the range of 11-12 g/dL for 3 months. Measurements were conducted before and after rh-EPO treatment.

Results: rh-EPO treatment significantly increased mean arterial pressure, hematocrit, and total peripheral vascular resistance, and decreased cardiac output. Plasma concentrations of norepinephrine, epinephrine, dopamine, and platelet cytosolic calcium concentrations were also significantly elevated by rh-EPO therapy. Autonomic function was improved by rh-EPO therapy. Mean arterial pressure was positively correlated with total peripheral resistance, orthostasis systolic pressure, norepinephrine, and platelet cytosolic calcium concentration.

Conclusions: Erythropoietin corrected anemia, augmented hemodynamics, and improved autonomic functions in HD patients. Increased total peripheral vascular resistance, augmented plasma catecholamine levels, and increased intracellular calcium concentrations all contributing to the elevation in blood pressure following rh-EPO therapy. The effects of long-term use of rh-EPO require further study. (Acta Nephrologica 2006; 20: 109-115)

Key words: erythropoietin, platelet cytosolic calcium, catecholamines, hemodynamics, autonomic function

INTRODUCTION

It is well known that cardiovascular disease is the most important risk factor for long-term dialysis patients, accounting for more than 50% of deaths in these patients. Anemia, hypertension, arterial stiffness, and cardiovascular autonomic dysfunction have been proposed to explain this phenomenon. Renal anemia resulting from restricted production of erythropoietin by the kidneys is usually present in end-stage renal disease (ESRD) patients. The major hemodynamic response to severe anemia is hyperdynamic cardiac output. Left ventricle hypertrophy and afterload reduction (decreased wall resistance) develop as compensatory mechanisms for maintaining oxygen supply to peripheral tissues. Autonomic dysfunction is common in ESRD, but its pathophysiology is unclear. Left ventricular hypertrophy and orthostatic hypotension are improved following partial correction of anemia. Thus, correction of renal anemia is important for patients undergoing hemodialysis (HD).

Erythropoietin (EPO) is the primary regulator of erythropoiesis. Therapy with recombinant human erythropoietin (rh-EPO) is a major therapeutic advance in the treatment of anemia of ESRD. However, several possible complications, including hypertension,
thrombosis of arteriovenous fistulas, flu-like syndrome, and increased plasma potassium concentration have been described. Of these, hypertension is the most pressing concern. Approximately one third of hemodialysis patients who have been treated with rh-EPO have experienced aggravation of pre-existing hypertension or de novo hypertension. Several factors have been implicated in rh-EPO-associated hypertension. These factors include an increase in hematocrit and red blood cell mass, increased blood viscosity, loss of hypoxic vasodilation, change in production of or sensitivity to endogenous vasopressors, a direct vasoconstrictor effect, and an increase in vascular smooth muscle calcium concentration. The pathophysiological mechanism of rh-EPO and the long-term outcome of patients subjected to rh-EPO treatment have not yet been elucidated. We studied hemodynamics, autonomic function, and concentrations of vasoactive hormones, and platelet cytosolic calcium before and after rh-EPO therapy in regular HD patients. Correlations between these parameters were estimated to gain further understanding of the mechanisms of the cardiovascular effects of rh-EPO.

**MATERIALS AND METHODS**

**Patients**

This study was approved by the Ethics Committee on Human Research of the Tri-Service General Hospital, Taipei, Taiwan. All patients gave written informed consent. Twelve HD patients with anemia (6 men and 6 women, 35-68 years of age, mean age 54.2 ± 5.1 years) were enrolled in this study. They had received regular hemodialysis treatment for 4 hours three times a week for a mean period of 5.4 ± 0.8 months. They had not received rh-EPO therapy prior to being admitted to this study. The etiology of uremia included chronic glomerulonephritis in 6 patients, autosomal dominant polycystic kidney disease in 1 patient, chronic interstitial nephritis in 3 patients, and hypertensive nephrosclerosis in 2 patients. Body weight at euvolemia was estimated by trial and error. Patients were normotensive and had stable blood pressure, even during the interdialytic and intradialytic periods. All patients maintained their usual medication for calcium phosphate control and their daily intake of water-soluble vitamins during the trial.

HD dialysate flow was 500 mL/min and blood flow was 200 mL/min. Dialysate temperature was maintained at 36.5°C. HD was performed using the same dialyzer (CA170; Baxter S.A., Deerfield, IL) for all patients. Ultrafiltration was employed to remove excess fluid, the extent of which was estimated from interdialysis weight gain. The dialysate contained 140 mmol/L sodium, 2.0 mmol/L potassium, 1.75 mmol/L calcium, 1.0 mmol/L magnesium, 35 mmol/L bicarbonate, 4 mmol/L acetate, and 200 mg/dl glucose. Heparin was used for anticoagulation according to individual clinical indications. The duration of HD sessions was 4 hours.

Patients were treated with rh-EPO (initial dose = 25-50 μ/kg body weight) intravenously for 3 months. The dose of rh-EPO was adjusted to maintain a predialytic hemoglobin concentration of 11-12 g/dL. All measurements of hemodynamic and autonomic function were done prior to hemodialysis, and before (Before EPO) and after 3 months rh-EPO therapy (After EPO). Plasma concentration of catecholamines and platelet cytosolic calcium were measured prior to beginning the dialytic procedure. These blood samples were obtained between 7:00 and 8:00 A.M. after overnight fasting and 1 hour of recumbency. The needle was inserted at least 30 minutes before the initial sample was drawn.

**Methods**

**Systemic hemodynamic evaluation.** Blood pressure (BP) was measured with a standard cuff and a sphygmomanometer after recumbency for 30 minutes prior to hemodialysis. Korotkoff phase V was used to estimate diastolic blood pressure; the mean of three estimates was used for analysis. Pulse rate was recorded by an electrocardiographic oscilloscope (Hewlett Packard). Mean arterial pressure (MAP) was calculated as the sum of diastolic pressure and one third of pulse pressure. Cardiac output (CO) was assessed by echocardiography (HP Sono 1000). Total peripheral resistance (TPR; dyne×sec×cm⁻⁵) was calculated as MAP × 80 ÷ CO.

**Autonomic function tests.** Patients were not permitted food, coffee, alcohol, or nicotine for 48 hours before the measurements were obtained. Ingestion of anticholinergics (including antidepressants, antihistamines, and over-the-counter cough and cold medications), 9-α-fludrocortisone (Florinef), diuretics, and antihistamines, and over-the-counter cough and cold medications), 9-α-fludrocortisone (Florinef), diuretics, and sympathomimetic or parasympathomimetic agents 48 hours prior to tests was not permitted. Electrocardiography (ECG) electrodes were attached to the left and right interscapular sites just medial to the scapular tips; a ground electrode was attached to the left mid-axillary region. The Valsalva ratio and the 30:15 ratio tests assess parasympathetic and cardiovagal function, respectively. The supine and standing blood pressure tests assess sympathetic function.

**Valsalva ratio.** The rested and recumbent patient was asked to blow into a bugle and maintain a column of mercury at a level of 40 mmHg for 15 seconds. The bugle was equipped with a small air leak to ensure an open glottis. ECG was continuously monitored during
Continuous ECG monitoring was performed at rest and immediately after standing. The 30:15 ratio was estimated as the quotient of the RR interval of the 30th heartbeat divided by that of the 15th heartbeat immediately after standing. The following categories were used: normal, > 1.07; borderline, 1.04-1.06; pathologic, < 1.03.15

Orthostatic test. Following 20 minutes of supine rest, patients were calm, relaxed, and comfortable in a quiet laboratory maintained at 23°C. Patients then stood for 5 minutes. Blood pressure was recorded before standing, immediately after standing, and at 1 minute intervals during orthostasis. The maximum decrease in systolic and diastolic BP was calculated. A normal response was classed as a systolic pressure decrease of < 10 mmHg and a diastolic pressure increase of 5 mmHg.16


catecholamine assay. Noradrenaline (NE), epinephrine (EP), and dopamine (DA) were measured using a high performance liquid chromatograph equipped with an electrochemical detector.17 Briefly, 2 mL of previously separated and frozen plasma samples containing glutathione and EDTA were prepared by centrifugation at 4°C (12,000 rpm, 10 minutes) following the addition of 200 µL of a 2.5 ng/mL solution of 3,4-dihydroxybenzylamine. The supernatant was then introduced into a Bond Elute SCX cation exchange column (Analytichem International, Harbor City, CA) that had been washed with 1 mL absolute methanol followed by 1 mL phosphate buffer (0.2 M, pH 6.5). The sample was eluted with 1 M perchloric acid and alumina was added to the eluate under Tris buffer to adsorb catecholamines. Catecholamines were then eluted with 0.2 M PCA and the eluate was injected into the HPLC-ECD for quantitation. The overall recovery of 3,4-dihydroxybenzylamine was about 60% (CV 10%).

Measurement of platelet intracellular calcium concentration, [Ca++].

ACD-anticoagulated blood was centrifuged for 10 minutes at 160 x g according to the method described by Rink et al.18 and its modification by Ding et al.19 for isolation of platelet-rich plasma (PRP). The PRP was incubated with 2.5 µM Fura-2-AM (Sigma Chemical Co, St Louis, MN, USA) for 40 minutes at 37°C, centrifuged, washed, and suspended in Tyrode’s solution (145 mM sodium chloride, 5 mM potassium chloride, 1 mM magnesium chloride, 5 mM glucose, 10 mM HEPES pH 7.4, 0.5 mM sodium biphosphate, 25 µg/mL apyrase, and 6.4 U/mL heparin). Platelets were counted using a Coulter counter (model ZM) and platelet concentrations were adjusted to 2 x 10^8/mL. Fluorescence (F) of Ca++ was measured using a Jasco CAF-100 analyzer (Japan Spectroscopic Co., Tokyo, Japan) set to an excitation wavelength of 340 nm and an emission wavelength of 490 nm. Maximal fluorescence (Fmax) was measured following the addition of 40 µM digitoxin and minimal fluorescence (Fmin) was measured after the addition of 9 nM EGTA. Intracellular calcium concentration [Ca++i] was calculated using the equation: [Ca++i] (nM) = Kd (F–Fmin) ÷ (Fmax–F), where Kd = 224 nM.

Statistical analysis. Results are expressed as means ± SEM. Statistical comparison was carried out using Student’s t-test for paired or unpaired values. Correlation analysis was made by linear regression. P values less than 0.05 were considered significant.

RESULTS

Hematological and biochemical parameters. As shown in Figure 1, there was a significant increase in hematocrit (Hct) concentration following rh-EPO therapy (Hct, 33.2% ± 2.1% vs. 27.6% ± 1.4%, P < 0.01). rh-EPO therapy had no effect on renal function as assessed by serum urea and creatinine concentrations, nor did it have an effect on serum concentrations of calcium, phosphate, albumin or alkaline phosphatase.

Blood pressure. Mean arterial pressure was increased by rh-EPO therapy (96.8 ± 6.9 mmHg vs. 85.8 ± 5.7 mmHg, P < 0.05; Fig. 2).

Hemodynamics. Total peripheral resistance was increased by rh-EPO therapy (1980 ± 270 dyne×sec×cm⁻² vs. 1570 ± 180 dyne×sec×cm⁻², P < 0.05) and cardiac index was decreased (3.54 ± 0.32 L/min/m² vs. 4.36 ± 0.34 L/min/m², P < 0.05) (Table 1).

Autonomic function. rh-EPO therapy improved autonomic function as evaluated by orthostasis systolic and diastolic pressure (systolic, –8.3 ± 3.0 mmHg vs. –15.3 ± 4.2 mmHg, P < 0.01; diastolic, 0.67 ± 1.1 mmHg vs. –8.5 ± 2.4 mmHg, P < 0.05), Valsalva’s quotient (1.123 ± 0.028 vs. 1.048 ± 0.019, P < 0.01), and 30:15 ratio (1.048 ± 0.016 vs. 1.026 ± 0.010, P < 0.05; Table 1).

Vasoactive hormones. Plasma catecholamine concentrations were augmented following rh-EPO therapy (NE 638.3 ± 58.8 pg/mL vs. 433.3 ± 26.5 pg/mL, P < 0.01; EP 210.6 ± 19.3 pg/mL vs. 155.6 ± 13.3 pg/mL, P < 0.01; DA 216.6 ± 12.3 pg/mL vs. 152.8 ± 13.7 pg/mL, P < 0.01; Table 1).

Platelet cytosolic calcium. Platelet cytosolic calcium concentrations were increased by rh-EPO therapy (90.8 ± 11.4 nM vs. 63.7 ± 9.4 nM, P < 0.01; Fig. 3).
Table 1. Comparison of baseline and post rh-EPO treated plasma catecholamines, autonomic function tests, and hemodynamic changes in regular HD patients

<table>
<thead>
<tr>
<th></th>
<th>Before EPO</th>
<th>After EPO</th>
<th>p value</th>
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<tbody>
<tr>
<td>Norepinephrine (pg/ml)</td>
<td>433.3 ± 26.5</td>
<td>638.3 ± 58.8</td>
<td>&lt; 0.01</td>
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<tr>
<td>Epinephrine (pg/ml)</td>
<td>155.6 ± 13.3</td>
<td>210.6 ± 19.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Dopamine (pg/ml)</td>
<td>152.8 ± 13.7</td>
<td>216.6 ± 12.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Orthostasis systolic (mmHg)</td>
<td>-15.3 ± 4.2</td>
<td>-8.3 ± 3.0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Orthostasis diastolic (mmHg)</td>
<td>-8.5 ± 2.4</td>
<td>0.67 ± 1.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Valsalva’s Quotient</td>
<td>1.048 ± 0.019</td>
<td>1.123 ± 0.028</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>30 :15 ratio</td>
<td>1.026 ± 0.010</td>
<td>1.048 ± 0.016</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Total peripheral resistance (dynsecxcm⁻⁵)</td>
<td>1570 ± 180</td>
<td>1980 ± 270</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Cardiac index (L/min/m²)</td>
<td>4.36 ± 0.34</td>
<td>3.54 ± 0.32</td>
<td>&lt; 0.05</td>
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Before EPO: Measurements were done prior to hemodialysis and before rh-EPO treatment.
After EPO: Measurements were done prior to hemodialysis and after 3 months rh-EPO treatment.

![Fig. 1. Hematocrit before and after 3 months rh-EPO treatment in regular HD patients. ** P < 0.01.](image1)

![Fig. 2. Mean arterial pressure before and after 3 months rh-EPO treatment in regular HD patients. **P < 0.01.](image2)

![Fig. 3. Platelet cytosolic calcium concentrations before and after 3 months rh-EPO treatment in regular HD patients. **P < 0.01.](image3)
Correlations. The rh-EPO-induced increment in MAP was positively correlated with the increment in total peripheral resistance \((r = 0.430, P < 0.05)\), orthostasis systolic pressure \((r = 0.549, P < 0.05)\), norepinephrine \((r = 0.415, P < 0.05)\) and platelet cytosolic calcium concentration \((r = 0.442, P < 0.05)\; \text{Fig. 4}\).

Complications. Complications such as thrombosis of AV fistula, flu-like symptoms, and hyperkalemia were absent during this study. None of the patients required antihypertensive drug therapy during rh-EPO therapy.

DISCUSSION

The results of this study confirm the effectiveness of rh-EPO therapy as a means of increasing hematocrit. Blood pressure, platelet cytosolic calcium concentration, and plasma catecholamine concentration were also increased. Elevated blood pressure was correlated with total peripheral resistance, orthostatic systolic pressure, norepinephrine and platelet cytosolic calcium concentration. Total peripheral vascular resistance was increased and cardiac output was decreased by rh-EPO therapy. Augmented hemodynamics and improved autonomic functions were disclosed following rh-EPO treatment in HD patients.

Severe anemia is one of the risk factors for a reduced lifespan of patients undergoing hemodialysis. Cardiovascular adaptations that take place during anemia to maintain tissue oxygen supply include increased cardiac output, reduced peripheral vascular resistance, and increased oxygen extraction from hemoglobin. High cardiac output accompanied by a hyperdynamic circulatory state and progressive left ventricular hypertrophy is common in uremic patients. Because the main cause of renal anemia in HD patients is insufficient production of EPO, rh-EPO is used in clinical practice to restore hematocrit. However, various complications have been documented, especially EPO-induced hypertension.

We demonstrated that levels of noradrenaline, adrenaline, and dopamine were elevated following rh-EPO treatment. Bode-Boger and Hand et al. reported increased vasoconstrictor responses to norepinephrine after EPO treatment of rabbits and humans, respectively. The pathophysiological role of the autonomic nervous system in the development of arterial hypertension during therapy with rh-EPO is unclear. However, catecholamines enhance the entry of calcium from the extracellular space into the intracellular space through the excitation of alpha-2 adrenoreceptors. The number of alpha-2 adrenoreceptors is increased in rh-EPO-induced hypertension. In patients treated with rh-EPO, high plasma catecholamine concentrations could easily induce hypertensive crises in response to mild stimuli such as exercise or low doses of angiotension II, which normally induce moderate increases in blood pressure. It has been suggested that this is the result of factors such as increased red cell mass, altered sympathetic nervous activity, or direct hormone action. Cytosolic free calcium concentration in vascular smooth muscle cells is a factor common to vascular tone, vascular reactivity, and peripheral vascular resistance. Platelets have been extensively employed as a model of vascular smooth muscle cells because both cell types have calcium-dependent contraction mechanisms and similar surface receptors. Cytosolic calcium levels in platelets are elevated in primary hypertension or hypertension accompanying chronic renal failure. Platelet cytosolic calcium levels and blood pressure are increased after rh-EPO therapy of uremic patients. Because elevation of basal \([\text{Ca}^++]_i\) increases vascular smooth muscle tone, it is not surprising that chronic EPO therapy, which also elevates \([\text{Ca}^++]_i\), causes hypertension. This is the first report of a correlation between hypertension and hemodynamic effects, vasoactive hormones other than noradrenaline, and autonomic function. Our data demonstrate that there is a good correlation between MAP and platelet cytosolic calcium concentration, TPR, orthostatic systolic pressure, and NE before and after rh-EPO therapy. Increased total peripheral resistance after rh-EPO treatment may be the result of increased smooth muscle cytosolic calcium concentrations and increased platelet calcium concentrations. Therefore, these studies demonstrate that increased platelet cytosolic calcium concentrations may reflect elevated cytosolic calcium levels in smooth muscle cells and that this may contribute to the pathogenesis of EPO-induced hypertension.

Autonomic dysfunction is common in dialytic patients, but the cause remains unknown. Metabolic abnormalities induced by endogenous opioids and enhanced nitric oxide production have been proposed,
but this should be verified. Our study shows that autonomic function is improved after rh-EPO therapy, and that it is correlated with the improvement in orthostatic systolic pressure changes and the increment in blood pressure. Hoeldtke and Streeten suggested that erythropoietin treatment improves orthostatic hypotension in patients who have other types of autonomic neuropathy such as diabetes mellitus. We have previously shown impaired autonomic function in uremic patients undergoing regular hemodialysis and a good correlation between intradialytic blood pressure and autonomic function. The improvement may contribute to the elevated pressure following rh-EPO therapy, and, together with increased platelet cytosolic calcium, augmented catecholamine secretion, and increased total peripheral resistance, may reduce the incidence of dialytic hypotension.

In conclusion, rh-EPO treatment corrected anemia, augmented hemodynamics, and improved autonomic functions in regular HD patients. Increased hematocrit, increased total peripheral resistance, augmented plasma catecholamine levels, and increased intracellular calcium all contribute to elevated blood pressure after rh-EPO treatment. Nevertheless, additional prospective studies are warranted to determine the effects after long-term use of rh-EPO.

REFERENCES