INTRODUCTION

Clinical trials of rhEPO began in 1985 to 1986, and replacement therapy with rhEPO quickly became the standard therapy for anemia caused by chronic renal failure.\textsuperscript{1-3} In ESRD patients, the rhEPO dose needed for target hematocrits ranges from 25 to 450 U/Kg/week.\textsuperscript{4} Thus, weekly needs of rhEPO vary by ten times in different groups of patients. Efforts to optimize the use of rhEPO have focused on administration efficiency and addressing possible causes of resistance to rhEPO, such as iron deficiency, hyperparathyroidism, and aluminum overload.\textsuperscript{5-8}

In clinical practice, some HD patients with iron
overload or functional iron deficiency respond poorly to rhEPO owing to inadequate iron mobilization and defective iron utilization. For this group of patients, iron therapy is potentially hazardous, leading to iron overload and consequent hemosiderosis. Therefore, choosing an effective adjuvant therapy to treat these iron-overloaded patients is an important and emergent issue. In 1995, a preliminary study by Gastaldello et al. demonstrated that intravenous (IV) administration of ascorbic acid could overcome rhEPO resistance in four iron-overloaded HD patients. Three years later, Dr Tarrg et al. conducted a parallel, comparative study to further examine the effects of IV iron supplementation and ascorbic acid (IVAA) in rhEPO hyporesponsive, iron overloaded HD patients. They concluded that IVAA therapy could be a potential adjuvant therapy for anemic HD patients with iron overload. Therefore, IVAA therapy appears to provide a promising method of improving iron mobilization from tissue stores and increasing iron utilization.

The dose of IVAA in Gastaldello’s study was 500 mg (one to three times weekly), while that in Tarrg’s study was 300 mg thrice weekly. However, concerns exist that high dose of ascorbic acid could cause oxalate accumulation. The efficacy of vitamin C at doses less than 900 mg/week has never been studied. By clinical observation, lower doses may be both safe and effective. This work on EPO-hyporesponsive anemia in hemodialysis patients examined the effect of low dose IVAA in improving anemia and erythropoiesis-related parameters.

MATERIALS AND METHODS

Patients

Patient enrollment was based on the following criteria: (1) receiving hemodialysis for more than six months in a stable condition, (2) stable hematocrit value below 30% for four consecutive weeks, (3) ferritin > 300 ng/ml, (4) iPTH < 300 pg/ml, (5) aluminum < 5 μg/dl, (6) KT/V > 1.2, (7) no blood transfusion during the last three months. During the study period, patients were excluded if they experienced of the followings: (1) blood transfusion, (2) gastrointestinal bleeding, (3) uncontrolled hypertension, (4) treatment with ACEI or theophyllin, (5) hospitalization for any medical problems. Most patients routinely received multivitamin supplements, but vitamin C was not included. Patients were dialyzed for 4 hours three times per week, blood flow of 250-300 ml/min, and dialysate flow of 500 ml/min. A total of 42 patients participated in the study.

Study design

The study comprised two phases. In phase I, all patients were treated with fixed rhEPO dosage, namely 2000 U twice weekly, administered subcutaneously at the end of hemodialysis. Observation lasted 12 weeks. If hematocrit values failed to reach the target level (above 30%) during the final four weeks, the patients were defined as poor responders. Meanwhile, good responders were defined as those with hematocrit values exceeding 30% at the 10th and 12th weeks. Only the poor responders were enrolled in phase II of the study. The same policy of rhEPO therapy and iron supplementation was applied to the poor responders during the two different phase periods. Patients who participated in the phase II study were administered 100 mg ascorbic acid intravenously (IVAA) at the end of hemodialysis thrice weekly for 8 weeks. Moreover, the observation period extended for a further four more weeks without IVAA therapy. During the study period, if ferritin level fell below 300 ng/ml, a total of 480 mg of ferric chloride was administered intravenously in four successive hemodialysis sessions, 120 mg in each session.

Laboratory measurements

The erythropoiesis-related parameters were measured before hemodialysis. Hemoglobin and hematocrit were determined every two weeks using standard automated counter methods. Iron indices, including serum iron, total iron binding capacity (TIBC) and ferritin were measured before the study and every four weeks during the study period. Serum iron and TIBC were measured by a colorimetric method (Katayam, Olympus AU550, Tokyo, Japan). Transferrin saturation (TSAT) was calculated by dividing serum iron by TIBC×100. Serum ferritin (ng/ml) was measured with a two-site immunoradiometric assay (IRMA-mat® Ferritin, ByK-Sangtec Diagnostica GmbH & Co. KG, Dietzenbach-Germany). To identify factors resistant to rhEPO treatment, serum albumin, aluminum, iPTH, Kt/V were measured before starting the study. Serum albumin values were measured by Bromcresol purple (Beckman Coulter Synchron Lx system, USA). Serum aluminum concentrations were assessed by inductively couple plasma spectrometer (Elan 6100 Perkin Elmer, USA). Serum intact PTH levels were measured by a radioimmunoassay (Incstar, MN, USA). Before the study, KT/V was measured once based on pre-dialysis and immediate postdialysis blood urea nitrogen levels using a formal single-compartment model of dialysis urea kinetics. The calculating formula was -Ln[(C2/C1)-0.03-UF/W] (C1 & C2: predialysis and postdialysis plasma urea concentration, UF: quantity of ultrafiltration, W: postdialysis body weight).

Statistical analysis

The statistical results are expressed as mean ±SD. Statistical analysis was performed using the Student’s
unpaired t-test for good and poor rhEPO responders and the Student’s paired t-test to compare differences between the variable during the two phases of longitudinal part of the study. Additionally, the differences between the two groups, were analysed with the Mann-Whitney ranked sum test using the mean changes in laboratory values for each individual patient. Furthermore differences in categorical variables were examined using the chi-square test with Yate’s correction. A P value less than 0.05 for two-side tests was considered significant. The major calculations were performed on a personal computer using StatView 5.0 (Abacus Concept Inc., Berkeley, CA, USA).

RESULTS

A total of 37 patients completed phase I of the study, while five patients dropped out. The reasons for withdrawal included shunt failures in two patients, uncontrolled hypertension in one, and two hospital admissions owing to nonketotic hyperosmolar syndrome and pneumonia, respectively. Twenty-one patients responded well to low dose (2000 U biw) rhEPO therapy. The HCT values of these patients reached 30% or more at the 8th week. Sixteen patients displaying no obvious resistance to rhEPO therapy, except for functional iron deficiency, were unable to reach the target HCT level during the 12-week study period. Table I summarizes the demographic characteristics of the 21 good responders and 16 poor responders. Comparing the good and poor responders revealed no differences in age, sex, H/D duration, serum albumin, serum aluminum, serum iPTH and K/V. At the end of phase I, mean HCT level was significantly higher in the good responders than in the poor responders (33.0±2.2 vs 27.7±1.7%, P<0.01) (Fig. 1). The two groups also displayed significant differences in iron indices. Before enrollment, TSAT was significantly higher among the good responders than the poor responders (33.0±2.2 vs 27.7±1.7%, P<0.01) (Fig. 1). Moreover, baseline data of mean serum ferritin level was significantly lower among the good responders than the poor responders (635.0±176.6 vs 837.3±221.9, P<0.05) (Table I). During the phase I period, 13 patients received parenteral iron therapy, 11 in the good responder group and 2 in the poor responder group (11/21 vs 2/16, P=0.03).

The poor responders (n=16) entered the phase II study receiving IVAA 100 mg thrice weekly for 8 weeks. The mean HCT value for this group was 27.7±1.7% initially, then 28.1±2.4% at the 4th and significantly increasing to 29.5±2.2% at the 8th week (27.7±1.7 vs 29.5±2.2%, P<0.05). After ceasing IVAA therapy, the mean HCT value declined to 27.8±2.3% at the 12th week. Fig. 2 illustrates the series HCT changes of the poor responders during the phases I and II periods. The mean HCT values of phases I and II differ significantly at the 8th weeks.

During phase II, ferritin levels at 0, 4, 8, and 12 weeks were 823±171.4, 780.4±201.3, 650.7±165.1 and 743.6±164.2 ng/ml (Fig. 3). Notably, mean ferritin level was significantly reduced from its initial value by the 8th week (823.4±171.4 vs 650.7±165.1, P<0.05). Four patients received 480 mg ferric chloride therapy after the 8th week of phase II owing to ferritin values below 300 ng/ml. Fig. 3 displays the evolution of the ferritin values of the poor responders during the phase I and II periods.

Fig. 4 illustrates series TSAT changes of poor responders during the phases I and II. During phase I, the values of TSAT did not differ significantly at 0, 4, 8 and 12 weeks. However, during phase II, mean TSAT values were significantly higher at the 4th and 8th weeks than initially recorded (23.2±5.2 vs 31.9±4.9; 27.0±7.8%, P<0.05).

After 8 weeks of low dose IVAA therapy, patients displayed an 8% increase in mean HCT level (27.7±1.7 to 29.5±2.2%); a 21% decrease of mean ferritin level (823.4±171.4 to 650.4±165.1 ng/ml) and 18% increase of TSAT (27.0±7.8 to 31.9±4.9%).

DISCUSSION

Phase I of this study demonstrated that good responders to rhEPO therapy tended to have lower ferritin levels and higher TSAT than poor responders. According to the report from The Health Care Financing Administration’s ESRD Core Indicators Project, higher TSAT percentage was associated with high hematocrit value. This association implies that reduced serum ferritin level in good responders to rhEPO therapy results from higher utilization of iron, compared to poor responders, and it therefore should be reflected by the upward trends in TSAT. Eleven of 21 patients in the group of good responders and two of 16 patients in the group of poor responders required I.V. iron supply. Thus, similarly to rhEPO therapy inducing iron deficiency status, we can infer that the good responders achieve better iron mobilization and utilization compared to those with poor rhEPO responses. The observations of the present study are consistent with those of El-Reshaid et al. and Ali et al. Their investigations demonstrated that iron-overloaded patients have higher rhEPO requirement and depleted marrow iron store. Thus, iron-overloaded patients tend to exhibit functional iron deficiency with inadequate iron mobilization and defective iron utilization.

As it is well known, it is difficult to theorize any
beneficial effects for intravenous iron therapy, and conventional theory even suggests that such therapy could be detrimental in iron-overloaded patients leading to consequent hemosiderosis. Consequently, prescribing adjuvant therapy for rhEPO poor responders in HD patients has become a critical issue. So far, vitamin C has been used as an adjuvant therapy of rhEPO in HD patients with functional iron deficiency in several reports and vitamin C also has been proven to be able to facilitate iron release from inert storage sites and increase iron availability. Gastaldello et al and Tarng et al have already demonstrated the effectiveness of IVAA therapy. Moreover, two subsequent studies by Francescol et al, and Sezer et al further confirmed the effectiveness of IVAA treatment as a potential adjuvant therapy for erythropoietin-resistant, iron-overloaded, anemic hemodialysis patients. All the above studies appear to use higher dosages of Vitamin C to document the effect of IVAA therapy in improving iron utilization. However, the efficacy of IVAA therapy at doses below 900 mg/week has not been tested. This study investigated the above question by testing the efficacy of low dose of IVAA in treating rhEPO-hyporesponsive anemia in uremic patients. Since ascorbic acid is known to increase the risk of secondary oxalosis in hemodialysis patients, this work focused on the

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Good responders (n=21)</th>
<th>Poor responders (n=16)</th>
<th>P value</th>
</tr>
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<tr>
<td>Age (years)</td>
<td>51.9±13.6</td>
<td>55.3±13.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>7/14</td>
<td>6/10</td>
<td>NS</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.5±12.5</td>
<td>55.4±10.5</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of dialysis (years)</td>
<td>3.5±2.3</td>
<td>3.4±2.0</td>
<td>NS</td>
</tr>
<tr>
<td>HCT (baseline)</td>
<td>27.5±1.4</td>
<td>26.4±1.5</td>
<td>NS</td>
</tr>
<tr>
<td>HCT (12th week)</td>
<td>33.0±2.2</td>
<td>27.7±1.7</td>
<td>&lt;0.01</td>
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<tr>
<td>TSAT (%)</td>
<td>37.9±8.5</td>
<td>26.4±7.1</td>
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<tr>
<td>Serum Ferritin (ng/ml)</td>
<td>635.0±176.6</td>
<td>837.3±221.9</td>
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<td>Serum aluminum (ug/dl)</td>
<td>1.58±1.05</td>
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<td>Serum albumin (g/dl)</td>
<td>3.57±0.37</td>
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<td>NS</td>
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<tr>
<td>Serum iPTH (g/dl)</td>
<td>111.25±109.40</td>
<td>84.83±92.71</td>
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<tr>
<td>KT/V</td>
<td>1.80±0.37</td>
<td>1.92±0.41</td>
<td>NS</td>
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</table>

Fig. 1. The hematocrit changes of the good and poor responders during phase I. Symbols are: (□) good responders, (△) poor responders. Analysis of variance was used for between group comparison * P<0.05.

Fig. 2. The hematocrit changes of the poor responders during phase I and II. Symbols are: (□) phase I, (△) phase II. Analysis of variance was used for inside group comparison * P<0.05 as compared to 8th of phase I; # P<0.05 as compared to 0th week of phase II.
effect of low dose vitamin C therapy. The dose of IVAA (300 mg weekly) used in this study was less than the recommended regimen. In the phase II, 16 iron-overloaded hemodialysis patients (mean ferritin 823.4±171.4 ng/ml) were treated with 8 weeks of IVAA, involving 100 mg thrice weekly. Mean HCT was significantly higher at the 8th week than the baseline HCT value (29.5±2.2 v.s. 27.7±1.7, p<0.05). This study showed that low dose IVAA administration significantly raised HCT values after 8 weeks. Despite a smaller increase in HCT values, the present finding mirrors those of Gastaldello et al (mean HCT 26.5±0.7 to 32.7±0.4%)\(^{11}\) and Tarng et al (mean HCT 25.8±0.5 to 30.6±0.6 %).\(^{16}\) After the cessation of IVAA treatment, mean HCT value decreased to 27.8±2.3% at the 12th week. We couldn’t find a sustained erythropoiesis effect after stopping IVAA therapy and that was also not in contrast to the observations by Gastaldello et al and Tarng et al.

Transferrin saturation (TSAT) is a well-known marker of iron availability. Functional iron deficiency is characterized by low TSAT with normal or elevated iron stores. The rationale for using ascorbic acid as an adjuvant therapy to rhEPO is that it acts as a mediator facilitating iron release from the inert deposit site. Theoretically, IVAA therapy can correct functional iron deficiency and increase TSAT level. According to the study by Tarng et al,\(^{19}\) TSAT less than 25% and E-ZZP more than 105 µmol/mol heme had the superior positive predictive values for predicting a response to IVAA treatment. We did not check E-ZZP, but patients enrolled into phase II had lower TSAT levels. Compared to Tarng’s study,\(^{16}\) the TSAT increased from 27±3% to 48±6% after 8 weeks’ IVAA therapy (300 mg TIW); in Gastaldello’s study,\(^{11}\) the TSAT increased from 27±8% to 54±12% under IVAA therapy (500 mg once to three times a week); and in our study 8 weeks of low dose IVAA therapy increased mean TSAT levels from 27.0±7.8 to 31.9±4.9% (P<0.05). Although with a lesser degree of elevation in TSAT level, from our results, low dose IVAA therapy appears to be able to increase iron mobilization and availability significantly.

Many investigations have shown that serum ferritin is in equilibrium with tissue ferritin.\(^{22}\) Serum ferritin, thus can be a good indicator of iron store. In HD patients, Anastassiades et al consider ferritin level > 500 ng/ml,\(^{23}\) and Macdougall et al consider ferritin level > 800 ng/ml\(^{24}\) to indicate iron overload. Importantly, iron overload not only increases the cardiovascular events and infection risk in HD patients but also can cause relative resistance to rhEPO therapy. Interestingly, mean ferritin level at the beginning of phase II was significantly higher compared to the mean ferritin of patients that had undergone 8 weeks of low dose IVAA therapy (823.4±171.4 vs 650.7±165.1 ng/ml. P<0.05). Notably, these findings imply that decreasing in ferritin level represents increased iron utilization. Thus, IVAA provides additional benefits for iron-overload HD patients.
In conclusion, low dose of IVAA (300 mg weekly) used in this study is a worthy recommendation regimen for treating rhEPO-hyporesponsive anemia in iron-overloaded HD patients.

REFERENCE


