Clinical Effect of Recombinant Human Erythropoietin (rhEPO, Espogen™) in Anemia of Chronic Renal Failure Patients on Dialysis.

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<ABSTRACT>

To evaluate the safety and efficacy of recombinant human erythropoietin (Espogen™, LG Chemical Ltd.) in the treatment of anemia of chronic renal failure (CRF) patients on hemodialysis and peritoneal dialysis, multi-center clinical trial was performed in Asan Medical Center, Samsung Medical Center, and Seoul National University Hospital. The patients involved in the trial were end stage renal failure (ESRD) patients who had been on hemodialysis or peritoneal dialysis for more than 3 months. They were renal anemia patients with $\leq$8g/dl of hemoglobin (Hb) and $\geq$100ng/ml of serum ferritin. Initial dose for the patients on hemodialysis was 150 unit/kg weekly and 50 unit/kg of initial dose was administered to the patients on peritoneal dialysis twice a week. Increasing rate of Hb was monitored every other week and the dose was adjusted to maintain the optimum level of Hb. If $\geq$10g/dl of Hb level was reached, the dose of erythropoietin was reduced to maintain Hb level at 10-11g/dl.

Among 64 patients who were enrolled in this trial, final analysis was performed in 54 patients. More than 0.5g/dl of Hb increase was shown in all the patients and 52 of them (96.3%) showed more than 1.0g/dl of Hb increase compared with before treatment. In addition, Hb and hematocrit were improved from 7.11 ± 0.85g/dl and 21.3 ± 2.6% at baseline to 10.42 ± 1.31g/dl and 31.9 ± 3.5% at the end of the treatment (p=0.0001), respectively.

Reticulocyte increased significantly from 0.90 ± 0.74% to 2.45 ± 0.84% on the 2nd week of the treatment. Hypertension, headache, and increase of serum potassium and phosphorus were observed as adverse effects of the test drug.

From the results of this clinical trial, it was demonstrated that domestically developed recombinant human erythropoietin, Espogen™ was efficacious in the treatment of anemia of most of CRF patients and didn’t cause any adverse effects except for those known in the existing preparations.

Key Words : recombinant human erythropoietin (rhEPO), Espogen™, chronic renal failure, dialysis, anemia, hemoglobin

INTRODUCTION

Renal anemia is one of the major complications of CRF and is caused by the deficiency of iron and other nutrients, exsanguination from hemodialysis, aluminum poisoning, gastrointestinal bleeding from hemorrhagic inclination, etc. However, it is known that insufficient secretion of erythropoietin produced in the kidney is a critical reason. Erythropoietin, a
hematopoietic hormone of red blood cell and a glycoprotein with a molecular weight of 34,000 daltons, is mainly produced in the renal tubule of the kidney with the stimulation of hypoxia and secreted into blood stream. In the CRF patients who are depressed in the renal functions, erythropoietin production of the kidney is also seriously depressed and consequently, anemia occurs. If the anemia is neglected, it is continuously deteriorated [3, 4]. To treat this, periodical transfusion and injection of androgen preparations to increase erythropoietin production or to stimulate the bone marrow were mainly used in the past. However, these treatments showed effects only in a part of the patients or the adverse effects of infection, accumulation of iron, and sensitization to tissue adequacy antigen occurred [1, 2, 5, 6]. Because erythropoietin is a hematopoietic factor which is physiologically produced in the body, and shows good effects and a few adverse effects, it is an ideal treatment of anemia of CRF. With rapid progress in genetic engineering, the genetic coding of human erythropoietin became possible, and this made production of clinically sufficient amount of recombinant human erythropoietin possible. According to this, recently, recombinant human erythropoietin is widely used to correct the anemia of end stage renal failure patients [7, 8, 9]. However, until the recent development of Epokine [10], Korea had been dependent upon the recombinant human erythropoietins which were imported.

In this research, multi-center clinical trial was conducted to evaluate the clinical efficacy, safety, and usefulness of another domestically developed recombinant human erythropoietin, Espogen™ (LG Chemical Ltd.) in the treatment of anemia of CRF patients on hemodialysis and peritoneal dialysis.

**PATIENTS AND METHODS**

1. Patients

Multi-center clinical trial involving end stage renal disease (ESRD) patients on hemodialysis and peritoneal dialysis in Asan Medical Center, Samsung Medical Center, and Seoul National University Hospital was performed from Aug. 1997 to May 1998. The patients recruited for the study were ESRD patients who had been on hemodialysis or peritoneal dialysis for more than 3 months and showed renal anemia with \( \leq 8 \) g/dl of hemoglobin and \( \geq 100 \) ng/ml of serum ferritin, and their age range was between 18 and 70. However, those who had \( \geq 110 \) mmHg of mean diastolic blood pressure, experienced convulsion in the past or had active inflammatory disease were excluded from this trial. Those who had an experience of using erythropoietin within recent 2 weeks, or who were transfused within the last one month, accompanied by serious hematological disorder, and taking androgen preparations and immunosuppressants were also excluded.

2. Method

1) Dose and administration method of Espogen™

Espogen™, recombinant human erythropoietin, manufactured by LG Chemical Ltd. was used for the test. Manufacturing process of Espogen™ is as follows: Recombinant human erythropoietin of LG Chemical Ltd. was highly purified and manufactured through dye-affinity chemotherapy, ion-exchange chromatography and gel-filtration from media after recombinant CHO cells transferred by pMI-EP060-BiP-DHFR vector were cultured for several days. As an initial dose for hemodialysis patients, 50 unit/kg was administered three times per week by i.v. or s.c. injection after regular dialysis, and if the patients were dialysed only for two weeks, weekly dose was divided into the number of dialysis making it 150 unit/kg/week. For the patients in peritoneal dialysis, initial 50 unit/kg was administered twice a week. As a maintenance dose, in case more than 1.4g/dl of Hb increase occurred within 2 weeks, the dose was reduced by 25 unit/kg weekly, and if more than 1g/dl of Hb increase was not achieved within first 4 weeks,
50 unit/kg of increase was made to sustain the increasing rate of Hb at more than 0.6g/dl in two weeks and the total amount for administration was controlled not to exceed 225 unit/kg. If Hb reached 10g/dl, the dose of erythropoietin was adjusted to maintain the Hb at 10 – 11g/dl according to the researcher’s judgement. Immunosuppressants and androgen preparations which might affect the results of this trial were prohibited and the patients who had been transfused or showed the view of active inflammation were excluded from the trial.

2) Efficacy evaluation method
Hematological test was performed every two weeks for 12 weeks of treatment period and hematochemical test, coagulation test, and ferritin test were performed every fourth week. The safety of Espogen™ was monitored by examining chest X-ray, electrocardiogram, and antibody against erythropoietin before and after the trial. To evaluate the efficacy of the treatment, the trend of slight change in Hb, hematocrit, and so on, due to the administration of the test drug, was observed and statistically significant changes before and after the trial and at each time of examination were analyzed (paired t-test, Repeated Measures ANOVA). Also, to confirm the clinical significance, the rate of the patients in whom ≥ 2g/dl of Hb increase occurred was obtained and analysis of confidence interval was carried out. To evaluate the safety, the changes between before and after the treatment were analyzed by conducting paired t-test for total measurements.

RESULTS

1. Characteristics of the patients
64 patients were enrolled in this research, and 54 among them were available for evaluation. Among the ten patients excluded from the final evaluation, four didn’t meet the inclusion criteria, and three were transfused due to the severe anemic symptoms two weeks after the treatment. One patient died from the exacerbation of the disease and two were excluded by the outbreak of inflammatory disease affecting hematopoietic metabolism. Among the 64 patients who agreed to this clinical trial, male and female patients were 33 and 31, respectively, the average age was 45.2 ± 14.7 (mean ± SD, hereinafter the same), average body weight 54.8 ± 10.9 kg, and average duration of dialysis 43.6 ± 29.0 months. Among the 54 patients included in the final analysis, male and female patients were 26 and 28, respectively, average age was 44.9 ± 15.1, average body weight 54.0 ± 11.0 kg, and average duration of dialysis 41.9 ± 28.5 months (Table 1).

2. Hematological response
Among the 54 patients in whom the efficacy was evaluated, Hb increase occurred in all of them, compared with before treatment (Fig. 1). Not less than 1.0g/dl of Hb increase occurred in 52 patients (96.3%), and ≥ 2.0 g/dl of Hb increase compared with before treatment, occurred in 47 (87%, 95% CI. 78.1 – 96.0 ) (Fig. 2).
When the cases, in which not less than 2.0 g/dl of Hb increase occurred, were analyzed according to the sex, it occurred in 23 of 26 male patients (88.5%) and 24 of 28 female patients (85.7%), showing no differences between the sexes. By age, not less than 2.0 g/dl of Hb increase was shown in 12 of 13 patients aged under 30 (92.3%), in 15 of 17 aged between 31 and 50 (88.2%), and in 20 of 24 aged between 51 and 70 (83.3%), showing the tendency that efficacy of Espogen™ decreased in the elderly group. Both Hb and hematocrit showed statistically significant increase from 7.11 ± 0.85g/dl and 21.3 ± 2.6% at baseline to 10.42 ± 1.31g/dl and 31.9 ± 3.5% at the end of the trial, respectively (p=0.0001). Statistical significance was shown from the 4th week after the administration and thereafter, gradually increasing phase was continued (Fig. 3, Table 2). Reticulocyte increased with statistical significance from 0.90 ± 0.74% in the beginning of the trial to 1.54 ± 0.77% at the end of the trial (p=0.0001).
From the second week after the administration, reticulocyte significantly increased to 2.45 ± 0.84% and continuously increasing aspect was assumed over the total administration period (Table 2). Erythrocyte and platelet also began to increase right after the administration and increased from 2.31 ± 0.24 × 10⁶/mm³ and 146.2 ± 71.4 × 10³/mm³ at baseline to 3.34 ± 0.43 × 10⁶/mm³ and 160.8 ± 47.0 × 10³/mm³ in the 12th week after the treatment (Table 2).

Table 1. Baseline characteristics of patients (n=54)

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<tr>
<td>Age</td>
<td>44.9 ± 15.2</td>
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<td>Sex</td>
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<td>Male (no. of patients)</td>
<td>26</td>
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<td>Female (no. of patients)</td>
<td>28</td>
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<tr>
<td>Dialysis</td>
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<td>Duration (months)</td>
<td>41.9 ± 28.5</td>
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<td>Hemodialysis (no. of patients)</td>
<td>48</td>
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<td>Peritoneal dialysis (no. of patients)</td>
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3. Change in iron status

Serum ferritin and serum iron decreased significantly from 754.4ng/ml and 118.3㎍/dl in the beginning of the trial to 224.4ng/ml and 58.5㎍/dl in the 12th week after the treatment (p=0.0001), and the binding capacity of total iron increased with statistical significance from 214.3㎍/dl in the beginning of the trial to 224.0㎍/dl in the 12th week after the treatment (p=0.015), demonstrating that the iron stored in the body was actively consumed.

Figure 1. Change of hemoglobin concentration of every patient between baseline and 12th week after Espogen™ administration.

Figure 2. Delta (g/dl) and % change of hemoglobin concentration after Espogen™ treatment at 12 weeks.

Figure 3. Change in hemoglobin (g/dl) and hematocrit (%) levels after EspongenTM treatment (n=54). * p < 0.05 vs 0 week.
4. Change in the safety test items

Blood pressure increased from 148/87 mmHg (±20/10) to 154/90 mmHg (±17/9) - 6 mmHg in systolic blood pressure and 3 mmHg in diastolic blood pressure, and according to the result of paired t-test, the systolic blood pressure in the 12th week of the treatment showed significant increase (p=0.039). The pulse almost didn’t change, 74 times/min. (±12) before the administration of the drug and 71 times/min. (±10) at the end of the treatment. Although in the hematological and coagulation tests, clinically significant changes were not observed through the total test period, serum potassium and phosphate increased with statistical significance (p<0.05). In a good many patients, there was no view of special changes in the chest X-ray examination and electrocardiogram before and after the administration, and the view of new abnormalities was not also observed.

5. Antibody against erythropoietin

In the test of antibody against erythropoietin in the 4th and 12th week after the drug administration, the patients suspected of the production of antibody couldn’t be observed.

6. Abnormal responses

The symptoms observed as abnormal responses of the drug were 10 cases. Hypertension occurred in 5 cases with highest frequency, headache in 1 case, transient elevation of ALT in 1 case and so on. Among these, hypertension and headache are the symptoms which were previously reported in the existing erythropoietin preparations and mostly considered to have relations with the drug. However, the causes of the other symptoms were not grasped and the symptoms vanished naturally as transient phenomena.

**DISCUSSION**

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<th>Table 2. Effect of Espogen™ on hematologic value</th>
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<td>Hemoglobin (g/dL)</td>
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<td>Hematocrit (%)</td>
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<td>RBC (×10⁶/㎣)</td>
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<td>Reticulocyte (%)</td>
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<td>Platelet (×10³/㎣)</td>
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<th>Table 3. Comparison of hematological effect and side effect between different erythropoietin forms</th>
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<td>Baseline Hct</td>
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<td>12 weeks Hct</td>
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<td>Side Effect</td>
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<tr>
<td>Headache</td>
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<td>URI-like symptom</td>
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<td>Hypertension</td>
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<td>LFT abnormality</td>
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<td>Seizure</td>
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various complications may occur. Among those complications, anemia is one of the most critical complications which affect the quality of life. The anemia causes the deterioration of patients’ general ability in motion and general state of the body and if hypoxemia is continued, excessive burdens on cardiovascular system are caused, resulting in the secondary complications, and ultimately, the fatality rate of the patients increases. So, if the anemia is corrected, patients’ general state of the body and general ability in motion get better, subalimentation is improved, and subsequently, the quality of life is elevated. Although there are various reasons for the anemia of CRF patients, the main reason for the renal anemia is the insufficient secretion of erythropoietin resulted from the decrease in the production by impaired kidney \([3, 4]\). Therefore, the administration of erythropoietin is the most effective method for the treatment of this.

It was confirmed that the recombinant human erythropoietin recently developed by LG Chemical Ltd.(brand name – Espogen™) has the identical DNA sequence and physicochemical and biological activity with the imported erythropoietin alpha products. In this research, the clinical trial to verify the efficacy and safety of this preparation was carried out and the results mentioned above were obtained. Considering the effect on improving the anemia, which is the fundamental purpose for the use of erythropoietin, the efficacy of Espogen™ was remarkable. That is, Hb and hematocrit were 7.11g/dl and 21.3% on the average, increased with statistical significance from the 4th week after the administration, and improved to 10.4g/dl and 31.9%, respectively, at the end of the trial. Among 54 patients finally evaluated, not less than 1.0g/dl of Hb increase occurred in 52 (96.3%) and not less than 2g/dl in 47 (87%), showing the high ratio of increase. The effects of erythropoietin preparations reported in the literature are not less than 80% of improvements in anemia with much excellence \([11, 12, 13, 14]\). As demonstrated in this clinical trial, remarkable improvements in the anemia were observed in most of the patients with the administration of erythropoietin. It is needed to pursue the reason regarding the patients who didn’t show responses to erythropoietin. The reasons may be iron deficiency, chronic inflammatory diseases, hyperparathyroidism, vitamin deficiency (B₁₂, folate), and excess of aluminum and the therapeutic method can be drawn from the stepwise investigation on this \([4, 15]\). Practically, even in this clinical trial, it was observed that the effects decreased in a part of the patients due to the iron deficiency. Reticulocyte is premature erythrocyte which will be isolated into the blood stream from the bone marrow, and the indicator reflecting the stimulation degree of erythropoietin on the bone marrow \([13, 16]\). The reticulocyte increased significantly from 0.78% in the beginning of the trial to 2.45% from the 2nd week after the administration and assumed the aspect to increase continuously through the entire administration period. It seems that the slight decrease in the result at the end of the trial was resulted from the deficiency in iron store of many patients, which subsequently causes the decrease in stimulation effect of erythropoietin on the bone marrow. Erythrocyte and platelet also began to increase right after the treatment, and it was known that Espogen™ showed similar effect and action on the improvement of anemia to the results of other erythropoietin preparations at similar dose in foreign research reports (Table 3).

The administration of erythropoietin preparation may cause absolute or relative iron deficiency by increasing the patients’ requirements for iron in the body \([17, 18, 19]\), and even in this trial, serum iron and serum ferritin significantly decreased in a good many patients despite the supplementation of iron. This indicates the iron store in the body is being continuously used because the administration of erythropoietin increases erythrocyte production. Therefore, to increase the efficacy of erythropoietin, it is necessary to supplement iron sufficiently during the administration period of erythropoietin. Among the patients in this research, as the administration period
got longer, many patients showed more continuous decrease in iron stores in the body, which showed the possibility that the degree of improvement in anemia would have been much higher with sufficient supplementation of iron. Antibodies against erythropoietin were never detected in the blood collected in the 4th and 12th week after Espogen administration and it seems that antibodies against the protein produced by genetic recombination were not formed.

Adverse effects occurring after erythropoietin preparations are administered are already well known through the past domestic and foreign clinical researches, and even in this research, the adverse effects with the same aspect were observed. But headache was relatively less observed (Table 3). A good many patients on hemodialysis were accompanied by hypertension, which was mainly of humor dependency occurring when the renal function controlling moisture metabolism is depressed, and however, in a part of the patients, the hypertension was of renin dependency influenced by a hormone raising blood pressure. Although it is well known that existing hypertension may be more exacerbated or hypertension may occur in the patients with normal blood pressure with the administration of erythropoietin preparations, the frequency and degree of each case are various according to the reporters and it is known hypertension occurs or existing hypertension gets exacerbated in around 25% of patients. As a reason for the hypertension induced by erythropoietin preparations, the elevation of blood viscosity resulting from the increases in the peripheral blood vessel resistance and erythrocyte volume are suggested. Although it is thought the decrease in cardiac output resulting from the increase in erythrocyte volume is an important mechanism of the increase in blood vessel resistance, this has not been definitely demonstrated yet [21, 22].

In this clinical trial using Espogen™, rise in the blood pressure was observed in 9% and mainly, systolic blood pressure was elevated compared with before treatment. However, no one newly came to take hypotensors due to the rise in blood pressure during the treatment. During this clinical research, there was a tendency serum potassium and phosphorus increased. This is thought to be due to the increase in food intake with the increase of appetite while the anemia was improved, and similarity has been reported in another research [19, 20, 21]. In addition, although statistically significant changes have been shown in the test for liver function and other hematocomical investigations, clinically significant changes were not observed. Although it has been reported convulsion is one of the complications caused by the administration of erythropoietin preparations and its frequency is about 3% [21, 22], no patient experienced the convulsion during the treatment in this research. Also, there was no patient who experienced occlusion by the thrombus formation of arteriovenous fistula induced by the increase in blood viscosity. And, because the other important complications also didn’t occur, it was confirmed Espogen™ is safe enough compared with existing recombinant erythropoietins in the market. Since most of erythropoietin preparations domestically marketed until recently, were imported from foreign countries and quite expensive, it was limited for the patients who were pressed economically to use sufficient dose of erythropoietin required for the correction of anemia [23].

However, as domestically developed erythropoietin preparation recently began to be marketed, the positive effect of price cutting was displayed. Therefore, it seems that the development of this product will help save foreign currency and offer considerable economic benefits to the patients who will practically use the product.

To sum up the above-mentioned results, recombinant human erythropoietin preparation, Espogen™, domestically developed by genetic recombination, showed similar effect on the improvement of anemia to what was reported in the literature and the other adverse effects except for those previously well
known were not observed (Table 3). Through this research, the safety and efficacy of Espogen™ could be confirmed and it is considered Espogen™ can be used for the treatment of renal anemia.

References

11. Hughes RT, Cotes PM, Pippard MJ, Stevens JM,


