Original Article

# Effects of Recombinant Human Erythropoietin Therapy on Blood Coagulation and Fibrinolysis System

Toshiya Kobayashi, MD,[1] Haruo Makuuchi, MD,[1] Kouki Takahashi, MD,[2] Akira Furuse, MD,[3] Yoichi Shibata, MD,[4] Hirokazu Tsuno, MD,[4] Yutaka Kotsuka, MD,[5] Hiroshi Kubota, MD,[5] Kazuhito Imanaka, MD,[5] Toshiyasu Tsukada, MD,[6] and Mitsuyoshi Nakashima, MD[7]

**This study was designed to examine the effects of recombinant human erythropoietin (rHuEPO) therapy on blood coagulation and fibrinolysis in patients scheduled for elective heart surgery and undergoing preoperative autologous blood donation. Twenty-seven patients were studied, of whom 16 patients received rHuEPO (group E) and 11 patients no rHuEPO therapy (group N). The patients in group E were given 6000 units of rHuEPO intravenously every other day, three times a week, beginning from two weeks prior to the operation. In both groups, 400 ml of blood was collected preoperatively for predeposit once a week for two weeks, and the self-donated blood was returned to the patient intra- and postoperatively. Blood samples were drawn at the beginning of the study, immediately before the operation and two weeks after the operation. They were analyzed to assess blood coagulation, fibrinolysis, platelet function and vascular endothelial cell function, in order to examine the effects of the administration of rHuEPO. No significant difference was observed between the two groups in the degree of changes in these parameters following the operation. As enhancement of blood coagulability and fibrinolytic activity was evident postoperatively in both groups, changes in these parameters during the preoperative autologous blood donation period were also assessed excluding the postoperative data. Again, there was no significant intergroup difference in any of the markers evaluated. It was concluded that the administration of rHuEPO during preoperative autologous blood donation is unlikely to affect coagulation and fibrinolysis. (Ann Thorac Cardiovasc Surg 2001; 7: 273–7)**

**Key words:** erythropoietin, blood coagulation, fibrinolysis, heart surgery, autologous blood donation

## Introduction

In cardiac operations, preoperative autologous blood donation is positively undertaken in recent years to minimize homologous blood transfusion, and the hemopoietic factor erythropoietin (recombinant human erythropoietin; rHuEPO) is now generally used to correct anemia associated with the collection of autologous blood.[1,2] However, there have been reports describing the occurrence of shunt troubles, dialyzer disorders or a slight increase in platelet count in hemodialysis patients receiving rHuEPO.[3,4] There also have been reports describing the development of cerebral infarction or pulmonary infarction in patients having undergone preoperative autologous blood donation with the use of rHuEPO.[5] These reports have raised apprehensions for a potential risk of enhanced blood coagulation due to administration of rHuEPO. Articles dealing with the effects of rHuEPO therapy in patients with renal failure undergoing or prior

*From the Departments of [1]Cardiovascular Surgery and [2]Immunohematology, Toranomon Hospital, [3]JR Tokyo General Hospital, Departments of [4]Transfusion Medicine and [5]Cardiothoracic Surgery, University of Tokyo, [6]Chuo University Health Center, and [7]Hamamatsu University School of Medicine, Japan*

Received January 29, 2001; accepted for publication May 21, 2001.
Address reprint requests to Toshiya Kobayashi, MD: Department of Cardiovascular Surgery, Toranomon Hospital, 2-2-2, Toranomon, Minato-ku, Tokyo 105-0001, Japan.

to the induction of hemodialysis have appeared in the literature,[6,7] but such studies on autologous blood donation are few. This study was performed to assess the effects of rHuEPO therapy on blood coagulation and the fibrinolytic system in patients undergoing autologous blood donation prior to elective cardiac surgery.

## Materials and Methods

The patients scheduled for elective open heart surgery and presumably requiring blood transfusion of 800 to 1200 ml were selected for this investigation. The additional inclusion criteria were as follows; patients aged between 20 and 69 years and weighing from 40 kg up to 80 kg, with no laboratory evidence of iron deficiency (male: serum ferritin ≥15 ng/ml; serum iron ≥60 $\mu$g/dl, and female: serum ferritin ≥10 ng/ml; serum iron ≥40 $\mu$g/dl) and with pre-study hemoglobin concentrations of 12.0 g/dl to 14.0 g/dl. The patients with a history of thromboembolism and those with blood coagulation abnormalities were excluded from this study.

There were 27 patients fulfilling the above inclusion criteria, of whom 15 patients were to receive coronary artery bypass grafting (CABG), seven patients to receive valvular surgery and the remaining five patients to receive other surgical treatments. The nature of the study was fully explained to each patient, written consent was obtained, and the patients were randomly divided into two study groups by means of sealed envelopes. As a result, 16 patients were to receive rHuEPO (group E) and 11 patients to be given no such medication (group N).

Patients in group E were given 6000 units of rHuEPO (epoetin $\beta$; Chugai Pharmaceutical, Tokyo, Japan) intravenously every other day, three times a week, beginning from two weeks prior to the operation. Both groups received an oral iron preparation (200 mg/day). 400 ml of blood was collected for predeposit once a week for two weeks, and the self-donated blood was preserved in liquid form and returned to the patient intra- and postoperatively.

## Subjects Studied

Background characteristics of the patients were compared between the two groups, including gender, age, body weight, hemoglobin concentration at the start of the study, volume of preoperative autologous blood donation, duration of the operation, intra- and postoperative blood losses and proportion of cases with homologous blood transfusion. Blood samples were drawn at the beginning of the study, immediately before the operation and two weeks after the operation. They were subjected to hematological and biochemical tests and to tests of blood coagulation and the fibrinolytic system, in order to assess the effects of the administration of rHuEPO. Test parameters for the assessment of effects on the coagulation and fibrinolytic system included prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, thrombin-antithrombin III complex (TAT), plasmin-$\alpha$2 plasmin inhibitor complex (PIC), tissue plasminogen activator (tPA), fibrin degradation product (FDP) D-dimer, $\beta$-thromboglobulin ($\beta$-TG), platelet factor 4 (PF4), thrombomodulin (TM), von Willebrand factor (VWF), CD62, CD63, antithrombin III (AT III), protein C, blood viscosity, and bleeding time.

Numerical data were expressed as mean ± standard deviation of the mean. Statistical analyses were performed using the Student's t-test, chi-squared test, and two-way repeated measured analysis of variance.

## Results

The background characteristics of the patients are shown in Table 1. There was no significant difference between the two groups in respect gender, age, body weight, hemoglobin concentration at the start of the study, volume of preoperative autologous blood donation, duration of operation, intra- and postoperative blood losses or the number of cases with homologous blood transfusion.

Laboratory data on the clotting and fibrinolytic systems are summarized in Table 2. As for any of the parameters of the coagulation system, such as TAT, AT III and protein C, no significant difference was observed between the two groups with respect to the changes before and after the operation. In both groups, TAT significantly increased in values after the operation (group E, $p<0.001$; group N, $p<0.001$), indicating enhancement of coagulability after the operation. Changes following the operation were also not significantly different between the two groups in respect to the fibrinolytic system parameters, such as PIC, tPA and FDP D-dimer. Both groups showed significantly increased D-dimer concentrations postoperatively (group E, $p<0.001$; group N, $p<0.001$), thus indicating an enhanced fibrinolytic state after the operation. b-TG, PF4, CD62 and CD63 assayed to evaluate platelet aggregation also did not show any signifi-

**Table 1. Background characteristics of the study patient population**

| | Group E (n=16) | Group N (n=11) | p value* |
|---|---|---|---|
| Gender (male : female) | 13 : 3 | 7 : 4 | 0.305 |
| Age (yrs) | 57.8 ± 9.9 | 57.2 ± 13.6 | 0.907 |
| Body weight (kg) | 63.0 ± 11.7 | 58.5 ± 8.1 | 0.244 |
| Pre-therapy Hb (g/dl) | 13.1 ± 0.62 | 13.2 ± 1.01 | 0.772 |
| Autologous blood donation (ml) | 892 ± 185 | 836 ± 120 | 0.350 |
| Duration of operation (min) | 410 ± 130 | 443 ± 51 | 0.452 |
| Blood loss (ml) | 980 ± 570 | 1367 ± 956 | 0.267 |
| Homogenous blood transfusion | 2 cases | 3 cases | 0.271 |

*chi-squared test or Student's t-test

**Table 2. Coagulation and fibrinolytic assay data**

| | | Initial blood sampling | Immediately before operation | 2 weeks after operation | p value* |
|---|---|---|---|---|---|
| TAT (gr. E) | (ng/ml) | 2.9 ± 2.1 | 3.8 ± 1.9 | 15.8 ± 12.7 | 0.837 |
| TAT (gr. N) | | 2.8 ± 1.0 | 2.6 ± 1.8 | 13.5 ± 7.9 | |
| AT III (gr. E) | (mg/dl) | 28.9 ± 6.3 | 25.7 ± 4.2 | 26.1 ± 3.7 | 0.139 |
| AT III (gr. N) | | 26.5 ± 6.3 | 26.6 ± 6.0 | 26.0 ± 5.1 | |
| Protein C (gr. E) | (%) | 109.7 ± 18.8 | 103.1 ± 19.8 | 96.3 ± 29.8 | 0.505 |
| Protein C (gr. N) | | 115.1 ± 28.4 | 119.1 ± 38.2 | 98.8 ± 32.0 | |
| PIC (gr. E) | ($\mu$g/ml) | 1.2 ± 0.4 | 1.2 ± 0.7 | 1.5 ± 0.5 | 0.656 |
| PIC (gr. N) | | 1.1 ± 0.5 | 1.3 ± 0.4 | 1.3 ± 0.5 | |
| tPA (gr. E) | (ng/ml) | 5.7 ± 1.7 | 5.9 ± 3.4 | 5.7 ± 2.6 | 0.857 |
| tPA (gr. N) | | 7.3 ± 2.5 | 7.0 ± 2.9 | 7.1 ± 1.9 | |
| D-dimer (gr. E) | ($\mu$g/ml) | 0.61 ± 0.61 | 2.10 ± 4.83 | 9.15 ± 4.69 | 0.318 |
| D-dimer (gr. N) | | 0.54 ± 0.66 | 0.55 ± 0.62 | 11.01 ± 6.86 | |
| $\beta$-TG (gr. E) | (ng/ml) | 217.3 ± 65.5 | 210.5 ± 64.5 | 242.8 ± 33.8 | 0.494 |
| $\beta$-TG (gr. N) | | 244.3 ± 39.7 | 218.0 ± 58.7 | 231.0 ± 43.4 | |
| PF4 (gr. E) | (ng/ml) | 93.4 ± 35.1 | 92.7 ± 38.7 | 104.3 ± 19.3 | 0.721 |
| PF4 (gr. N) | | 99.0 ± 34.3 | 98.1 ± 29.7 | 95.7 ± 30.0 | |
| CD62 (gr. E) | (mfc) | 3.9 ± 1.8 | 4.2 ± 1.9 | 5.3 ± 1.7 | 0.265 |
| CD62 (gr. N) | | 3.2 ± 0.5 | 7.0 ± 6.3 | 5.6 ± 2.2 | |
| TM (gr. E) | (ng/ml) | 14.3 ± 5.5 | 15.3 ± 7.9 | 14.2 ± 4.7 | 0.176 |
| TM (gr. N) | | 14.0 ± 2.9 | 14.1 ± 3.1 | 16.9 ± 6.2 | |
| VWF (gr. E) | (U/dl) | 75.9 ± 19.0 | 92.5 ± 27.1 | 94.8 ± 28.7 | 0.207 |
| VWF (gr. N) | | 73.8 ± 26.6 | 92.0 ± 4.02 | 116.9 ± 40.8 | |

TAT: thrombin-antithrombin III complex, AT-III: antithrombin III, PIC: plasmin-$\alpha$2 plasmin inhibitor complex, tPA: tissue plasminogen activator, $\beta$-TG: $\beta$-thromboglobulin, PF4: platelet factor 4, TM: thrombomodulin, VWF: von Willebrand factor, *two-way repeated measured analysis of variance.

cant intergroup difference. The parameters measured to assess vascular endothelial cell function, such as thrombomodulin (TM), von Willebrand factor (VWF), did not reveal any significant difference between the groups.

We also compared the degrees of changes between the two groups in these parameters during the preoperative period of autologous blood donation, so as to preclude the substantial influence of surgical intervention reflected in their postoperative values (Table 3). Again, none of the parameters showed any significant intergroup difference.

## Discussion

Blood transfusion was formerly thought to be inevitable

**Table 3. Changes in makers during preoperative blood donation period**

| | | Group E | Group N | p value* |
|---|---|---|---|---|
| TAT | (ng/ml) | 0.9 ± 1.5 | –0.2 ± 2.1 | 0.141 |
| AT III | (mg/dl) | –3.2 ± 3.7 | 0.0 ± 4.2 | 0.050 |
| Protein C | (%) | –6.6 ± 21.9 | 4.0 ± 39.0 | 0.430 |
| PIC | ($\mu$g/ml) | 0.0 ± 0.8 | 0.2 ± 0.5 | 0.567 |
| tPA | (ng/ml) | 0.2 ± 3.5 | –0.3 ± 2.3 | 0.674 |
| D-dimer | ($\mu$g/ml) | 1.5 ± 4.9 | 0.0 ± 0.2 | 0.260 |
| $\beta$-TG | (ng/ml) | –6.8 ± 85.1 | –26.3 ± 65.0 | 0.536 |
| PF4 | (ng/ml) | –0.7 ± 50.3 | –0.9 ± 35.8 | 0.993 |
| CD62 | (mfc) | 0.3 ± 2.9 | 3.8 ± 6.6 | 0.263 |
| CD63 | (mfc) | 0.6 ± 8.1 | 3.6 ± 6.2 | 0.460 |
| TM | (ng/ml) | 1.1 ± 4.9 | 0.1 ± 3.4 | 0.597 |
| VWF | (U/dl) | 16.6 ± 27.1 | 18.2 ± 32.5 | 0.894 |

*Student's t-test

in heart operations because the operation involves the use of a cardio-pulmonary bypass machine. It was then demonstrated however, that operations without homologous blood transfusion were feasible by means of predonated autologous blood transfusion. In order to avoid adverse effects of homologous blood transfusion such as viral transmission or transfusion-associated graft versus host disease (TA-GVHD), preoperative autologous blood donation has recently become more prevalent. With the use of the human hemopoietic hormone, rHuEPO, which has been proven to be quite effective for correcting anemia associated with autologous blood predeposition, it is now possible to substantially reduce the period of preoperative autologous blood collection. It has, therefore become a common practice at present to perform autologous blood donation with the aid of rHuEPO in the field of cardiac operations in Japan. Meanwhile, there have been reports describing occurrence of shunt troubles, dialyzer disorders or a slight increase in platelet count in hemodialysis patients receiving rHuEPO. There also have been reports describing development of cerebral infarction or pulmonary infarction in patients having undergone preoperative autologous blood donation aided by administration of rHuEPO. These reports have raised concern about the potential risk of enhanced blood coagulation due to the administration of rHuEPO. This study was designed to examine the effects of rHuEPO therapy on blood coagulation and fibrinolysis in patients undergoing autologous blood donation prior to heart surgery.

TAT, AT III and protein C were measured as parameters of blood coagulability, PIC, tPA and FDP D-dimer as parameters of fibrinolytic activity, $\beta$-TG, PF4, CD62 and CD63 to assess platelet aggregation, and TM and von Willebrand factor to examine vascular endothelial cell function. The degrees of their changes following the operation were compared between the rHuEPO-treated group and the non-treated group. None of these physiological parameters revealed any significant intergroup difference. Nearly all the reports dealing with the effects of rHuEPO therapy on the coagulation-fibrinolysis system, platelet function or vascular endothelial cell function have pertained to patients with renal failure receiving rHuEPO for the purpose of correcting nephrogenic anemia. Changes observed in various markers of coagulation and fibrinolytic systems in these patients have varied according to those reports and there is no unanimity of views on this problem.[8-10] As nephrogenic anemia is corrected, platelet function also improves with a consequent shortening of bleeding time,[11,12] but many reports suggest that such reaction is not directly attributable to rHuEPO.[13,14] There have been sporadic reports concerning effects of rHuEPO on vascular endothelial cell function,[15,16] yet much remains to be clarified. Although studies regarding those effects in cases of autologous blood donation are few as yet,[17] there appears to be no article to demonstrate any obvious influence of rHuEPO therapy on the coagulation-fibrinolytic systems.

The present data may involve a considerable bearing of surgical intervention itself since postoperative values for the various markers are also taken into account in their analysis. In fact, significant increases in TAT and D-dimer were observed as compared with preoperative values in both groups. This indicated postoperative enhancement of coagulation and fibrinolysis due to the influence of the surgical operation per se irrespective of

whether rHuEPO was administered or not. Degrees of changes in the various markers observed during the preoperative blood donation period were thus compared between the two groups in order to evaluate the effects of rHuEPO therapy on the clotting-fibrinolytic systems alone, and not including the effects of the operation. Again, there was no significant difference between the two groups in any of the parameters assessed.

It was concluded that the administration of rHuEPO during preoperative autologous blood donation is unlikely to affect coagulation and fibrinolysis.

## References


1. Watanabe Y, Fuse K, Konishi T, et al. Autologous blood transfusion with recombinant human erythropoietin in heart operations. *Ann Thorac Surg* 1991; **51**: 767–72.
2. Konishi T, Kaneko T, Ohbayashi T, Ohki T, Watanabe Y, Fuse K. Effectiveness of erythropoietin for cardiovascular surgery to prevent homologous blood transfusions. *Jpn J Thorac Cardiovasc Surg* 1993; **41**: 187–91.
3. Jaar B, Denis A, Viron B, et al. Effects of long-term treatment with recombinant human erythropoietin on physiologic inhibitors of coagulation. *Am J Nephrol* 1997; **17**: 399–405.
4. Krzeslowska J, Rysz J, Cierniewski CS, Luciak M. Expression of fibrinogen receptors and GPIIb molecules on uraemic platelets: effects of recombinant human erythropoietin therapy. *Nephrol Dialysis Transplant* 1995; **10**: 653–6.
5. Hiromatsu T, Higuchi F, Sakai T. Fatal pulmonary embolism occurring after hip joint operation with autologous blood transfusion and concomitant EPO therapy. *J Jpn Autologous Blood Transfusion* 1991; **4**: 97–9.
6. Malyszko J, Malyszko JS, Borawski J, et al. A study of platelet functions, some hemostatic and fibrinolytic parameters in relation to serotonin in hemodialyzed patients under erythropoietin therapy. *Thromb Res* 1995; **77**: 133–43.
7. Borawski J, Rydzewski A, Mazerska M, Kalinowski M, Pawlak K, Mysliwiec M. Inverse relationships between haemoglobin and ristocetin-induced platelet aggregation in haemodialysis patients under erythropoietin therapy. *Nephrol Dialysis Transplant* 1996; **11**: 2444–8.
8. Takayama K. Changes in endothelial vasoactive substances and blood coagulation and fibrinolysis functions under recombinant human erythropoietin therapy in hemodialysis patients. *Jpn J Nephrol* 1993; **35**: 179–88.
9. Taylor JE, Belch JJF, McLaren M, Henderson IS, Stewart WK. Effect of erythropoietin therapy and withdrawal on blood coagulation and fibrinolysis in hemodialysis patients. *Kidney Int* 1993; **44**: 182–90.
10. Clyne N, Lins LE, Egberg N. Long-term effects of erythropoietin treatment on the coagulation system during standardized hemodialysis. *Clin Nephrol* 1995; **43**: 260–7.
11. Vigano G, Benigni A, Mendogni D, Mingardi G, Mecca G, Remuzzi G. Recombinant human erythropoietin to correct uremic bleeding. *Am J Kidney Dis* 1991; **18**: 44–9.
12. Akizawa T, Kinugasa E, Kitaoka T, Koshikawa S. Effects of recombinant human erythropoietin and correction of anemia on platelet function in hemodialysis patients. *Nephron* 1991; **58**: 400–6.
13. Zirkman DM, Zeigler ZR, Fraley DS, Bruns FJ, Adler S. Evaluation of platelet hyperfunction in hemodialysis patients receiving recombinant human erythropoietin. *Am J Kidney Dis* 1993; **21**: 31–7.
14. El-Shahawy MA, Francis R, Akmal M, Massry SG. Recombinant human erythropoietin shortens the bleeding time and corrects the abnormal platelet aggregation in hemodialysis patients. *Clin Nephrol* 1994; **41**: 308–13.
15. Nagai T. Effects of rHuEPO on cellular proliferation and endothelin-1 production in cultured endothelial cells. *Jpn J Nephrol* 1994; **36**: 194–200.
16. Carlini RG, Dusso AS, Obialo CI, Alvarez UM, Rothstein M. Recombinant human erythropoietin (rHuEPO) increases endothelin-1 release by endothelial cells. *Kidney Int* 1993; **43**: 1010–4.
17. Biesma DH, Bronkhorst PJH, de Groot PG, van de Wiel A, Kraaijenhagen RJ, Marx JJM. The effect of recombinant human erythropoietin on hemostasis, fibrinolysis, and blood rheology in autologous blood donors. *J Lab Clin Med* 1994; **124**: 42–7.