Favorable effects of Hemodialysis using high-performance membrane (BK-F) on renal anemia

Yoshifumi Kawano *1, et al

Introduction

Anemia associated with chronic renal failure is attributed mainly to decreased endogenous erythropoietin production. The clinical use of recombinant human erythropoietin has minimized occurrences of severe anemia that require repeated blood transfusions. However, morbidities of chronic renal failure are rather complicated. Existence of other factors that deteriorate anemia than impaired EPO production has been reported 1), 2), 3). Some cases need bloodlettings during dialyses. We have encountered many cases of renal anemic patients that suggest existence of other erythropoiesis-relating factors than EPO. Better control of these potential renal anemia deteriorating factors has emerged as another key to management of renal anemic patients.

Erythropoiesis-relating factors may interact with the granulocytic series and the lymphocytic series, suggesting relationships among these factors, the hematopoietic system, and the immune system. We consider anemia as a good index to monitor a patient with chronic renal failure, to know how well the immunological function work and to determine how to administer the patient.

Last year, Kobayashi et al reported KR-4 as a substance gained through dialysis using the protein-permeable membrane (BK-F). This study investigated effects of KR-4 on human erythropoiesis by focusing on its responsiveness to EPO through in vivo and in vitro tests.

Patients and methods

Patients

Twelve patients who have experienced four-hour hemodialysis three times a week since five or more years ago due to chronic renal failure and have been given EPO since two or more years ago due to renal anemia were evaluated. The twelve patients were male. The youngest patient was forty-seven years old and the eldest was seventy-seven years old. The median age was sixty-two. We screened the patients into two groups according to the quantity of fractionations of proteins in the dialysis fluid gained through one dialysis using BK-F: the group with the larger quantity (hereafter referred as the L Group) and the group with the smaller quantity (S Group). Note that each patient has experienced hemodialyses using the same type of a dialyzer and with the same dose of EPO in the period of three months immediately before the beginning of this study.

We measured hemoglobin (Hgb) levels and hematocrit (Ht) levels immediately before each dialysis using BK-F. Serum iron (Fe) levels and ferritin levels were measured at least once a month. Fe levels were carefully monitored so that they would not fall below the level of 50 μg/ml. Patients whose Hgb levels or Ht levels continued to increase by 5 % for at least a week received EPO at a dose that decreased by 1,500 U. Some patients received EPO at the same dose even after their Ht levels increased because of their requests. The twelve patients experienced dialysis using BK-F for three months. The Hgb levels, Ht levels and required doses of EPO before introduction of BK-F in dialysis were compared with their counterparts after use of BK-F in dialysis. At the end of the three-month test, a case with at least by 50 % decrease in the dose of EPO or at least by 10 % increase in the Ht level was considered effective case that proved favorable effects of the BK-F dialysis on renal anemia.

*1 Department of Pediatrics, School of Medicine, University of Tokushima, Japan

The fractionation of proteins (KR4) gained in the dialysis fluid

We fractionated the substances by using gel-permeation method, that were removed from blood through BK-F using the method reported by Kobayashi et al 4) and identified the peaks as KR4-0, KR4-1 (IgG as the major), and KR4-2 (albumin as the major) in the increasing order of elution time. The six patients in the L Group offered samples for the in vitro test: 30 l of dialysis fluid was gained in the first BK-F dialysis per patient and used to get KR4 fractionations. Each fractionation was lyophilized, dissolved in Iskof modified Dulbecco medium (IMDM), passed through a 0.45 μ m filter, and applied to cultures described blow.

The method of culturing hemopoietic precusor cells

Erythroid hemopoietic precusor cells (BFU-E, CFU-E) were cultured with the methylcellulose method, using normal human bone marrow monocytes as target cells. In order to culture CFU-E, samples containing 1.0 % methylcellulose, 20 % fetal calf serum, deionized 1 % bovine serum albumin, 450 μ g/ml transferrin, and a certain concentration of EPO were incubated at 37 ℃, 100 % relative humidity, 5 % oxygen, and 5 % carbon dioxide for 7 days. In order to culture BFU-E, samples containing 1.0 % methylcellulose, 20 % fetal calf serum, deionized 1 % bovine serum albumin, 450 μ g/ml transferrin, a certain concentration of EPO, 20 ng/ml interleukin-3 (IL-3), and 20 ng/ml stem cell factor (SCF) were incubated at 37 ℃, 100 % relative humidity, 5 % oxygen, and 5 % carbon dioxide for 14 days. Note that IL-3 and SCF were added as colony-stimulating factors and provided by Kirin Brewery Co., Ltd. Colony counts in the incubated samples were determined using invert microscopes. Granule precursor cells (CFU-GM), resulted from addition of IL-3 and SCF, in the BFU-E samples were also counted.

Results

1. Clinical effects

After the test period of three months in which dialyses using BK-F were conducted, five of the six cases in the L Group turned out to be effective. In the other one case, the test was suspended because of the patients request when one and a half months had elapsed since its beginning. Table 1 and Figure 1 (Appendix 1) show that in the two cases the dose of EPO decreased from 9,000 IU per week to 1,500 IU per week while the haemoglobin level increased by 0.7 g/dl. In the S Group, one of the five cases turned out to be effective. In this case, the dose of EPO did not change while the hematorict level and the hemoglobin level increased by about 20 %. The use of BK-F seemed to bring no change clearly identified in other factors than anemia.

2. Results of in vitro tests

Table 2 (Appendix 1) shows how much KR4-0, KR4-1, and KR 4-2 were separated from 30 l of dialysis fluid of each patient of the six cases. The measurements were scattered in a broader range, from 0 to 17.5 mg. Each separation of KR4-0 was added to methylcellulose media in order to observe its effects on hemopoietic precusor cells. KR4-0 gained from the five patients whose clinical tests turned to be effective inhibited the growth of CFU-E derived from normal human bone marrow monocytes (Figure 2 in Appendix 2). BFU-E incubations with lower concentrations of EPO showed subtle inhibitions. No effect on growth of granule precursor cells (CFU-GM) was found.

Though KR4-1 and KR4-2 samples that had the same concentrations as KR4-0 were applied to CFU-E and BFU-E incubations, no sign that would suggest differentiations or growths are inhibited was found (Figure 3 in Appendix 2).

Discussion

The purpose of the dialysis therapy applied to patients with chronic renal failure is to eliminate water retained in the body longer than usual and uremic toxins including urea nitrogen and creatinine. Even if this purpose is achieved partially, a patient with chronic renal failure would face another problem of possible various complications, as he ore she has to be dialyzed at a regular basis in a rather long period. To prevent or alleviate these complications, considerable efforts had been devoted to improvements in dialysis methods and membranes. The complicacy of morbidities and the diversity of patients characteristics make it a challenge to develop one common approach to be effective against chronic renal failure. A dialysis membrane that acts virtually as the glomerular basement membrane would help the quality of life to improve.

Renal anemia is one of various clinical conditions of a patient with renal failure and has been attributed mainly to a deficiency of erythropoietin secreted chiefly by the kidney. The clinical use of recombinant human erythropoietin has made most of anemia curable, helping the quality of life to improve. However, some patients who receive EPO still suffer from anemia while some patients enjoy considerable alleviation of anemia through only the dialysis therapy. We have experienced that patients respond separately to a certain dose of EPO. These suggest existence of other causes of anemia than impaired EPO production. We reported impaired burst promoting activity (BPA) secretory function of lymphocyte, disorders encountered in the differentiation process in which EPO interacts with CFU-E, and increased macrophage colony-stimulating factors (M-CSF) secreted by activated cells in monocyte series as factors that deteriorate renal anemia 5), 6).

Substances in serums of patients with renal failure whose molecular weights ranging within 10,000 were reported as blood production inhibiting factors 7). Dialyses using existing membranes help anemia to reduce to some extent. We studied protein substances filtered out through hemodialysis using large-pore-size membrane that removes higher molecular weight substances: with in vitro test we observed how blood production is inhibited and in clinical cases we monitored how the severity of anemia is reduced. Our findings indicate that patients with more protein substances of higher molecular weights in their dialysis fluid enjoy greater reduction in the severity of anemia. This suggests that there may be anemia deteriorating factors, substances with higher molecular weights, in patients who still suffer from renal anemia even after dialyses using exsiting membranes that remove lower molecular weight substances. Our findings also point out that a clinical use of BK-F would considerably reduce a dose of EPO. After our three-month observation, some of the test cases turned out to be ineffective cases. Longer-period observations should be conducted in order to identify favorable effects of dialysis using BK-F on renal anemia.

The ability of KR4-0 to inhibit growth of hemopoietic precusor cells worked better in CFU-E than in BFU-E. This suggests that KR4-0 may select the final stage of the maturation division process of erythroid cells as a target to be blocked. We did not gain findings regarding a relationship between KR4-0 separation levels and reduction in anemia severities. We will commit ourselves to conduct more tests and observations in order to find relationships among KR4-0 separations, reduction in anemia severities, and types of membranes used before tests.

References

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Appendix 1

Table 1

Levels of blood components and EPO doses before and after the three-month BK-F dialysis

Case	Age	Sex	Before the test			After the three-month test			Effective
			Hgb (g/dl)	Ht (%)	Epo (IU/W)	Hgb (g/dl)	Ht (%)	Epo (IU/W)	
1	62	M	9.3	27	4,500	10.2	30	1,500	(+)
2	67	M	10.1	30	9,000	10.8	31	1,500	(+)
3	59	M	11.0	33	9,000	11.7	35	1,500	(+)
4	62	M	9.8	28	4,500	10.1	29	1,500	(+)
5	47	M	10.8	32	4,500		N/A		
6	77	M	9.2	27	6,000	11.2	32	6,000	(+)
7	65	M	9.0	26	9,000	8.6	26	9,000	(−)
8	70	M	8.8	26	9,000	8.6	25	6,000	(−)
9	60	M	8.6	24	4,500	10.3	30	4,500	(+)
10	55	M	11.5	34	6,000	8.6	26	4,500	(−)
11	70	M	9.7	29	3,000	9.5	28	3,000	(−)

Cases 1 through 6 belong to the L Group, while cases 7 through 11 are in the S Group.

Table 2

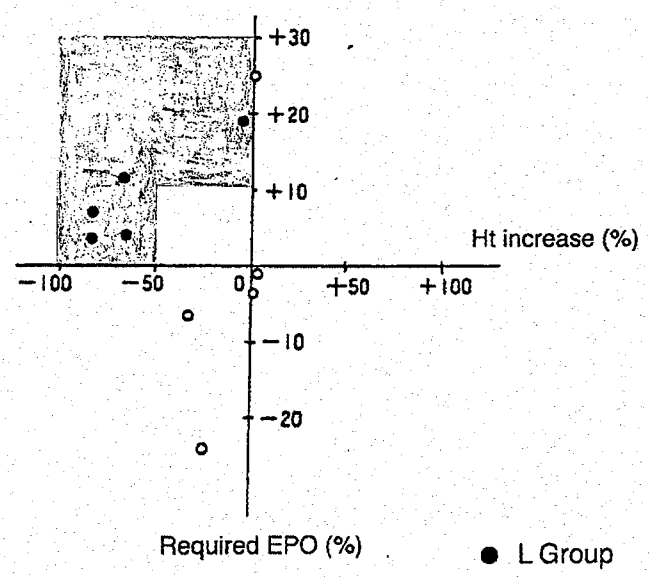
KR4 separated from BK-F dialysis fluid (mg)

Case	KR4-0	KR4-1	KR4-2
1	5.6	143.0	841
2	1.3	73.9	1,055
3	4.0	96.3	1,397
4	17.5	304.5	1,265
5	6.8	15.6	389
6	0	32.0	522

Each patient provided 30 l of dialysis fluid as a KR4 source.

Figure 1

Favorable effects of the three-month BK-F dialysis on anemia or EPO dose

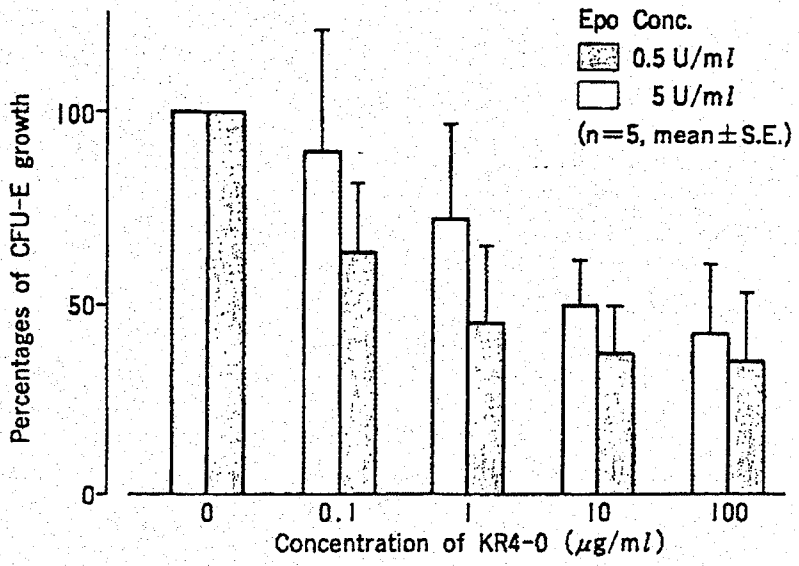


The solid black circles and the white circle in the shadowed area represent cases that are considered as "effective" due to 10 % or more increase in Ht or 50 % or more decrease in EPO. Five out of the ten cases are "effective."

Appendix 2

Figure 2

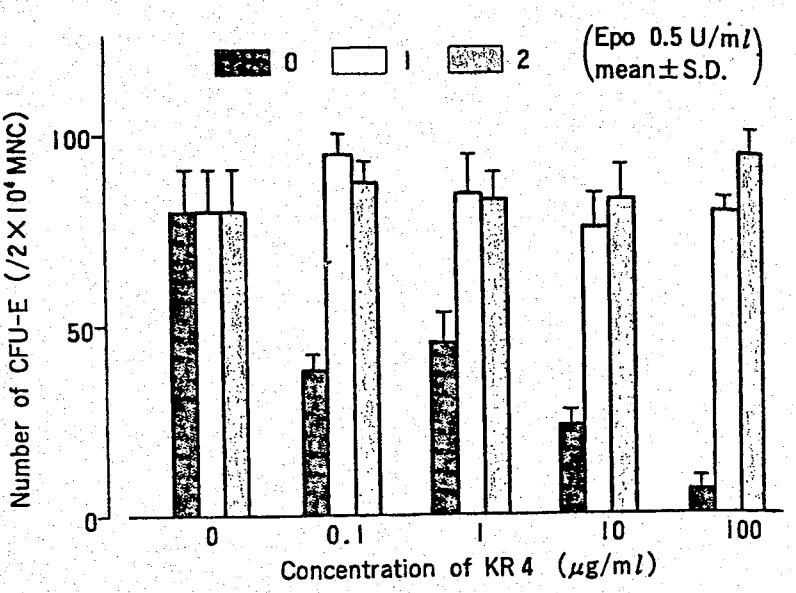
Inhibitory effect of KR4-0 on the growth of CFU-E



KR4-0 with concentrations at least 10 μ g/ml prevented counts of CFU-E in normal human bone marrow monocytes from exceeding 50 % of a KR4-0-free count.

Figure 3

Inhibitory effects of KR4-0, -1 and -2 on CFU-E



Inhibiting effects was only found in KR4-0 samples.