Original Article

Blood Pressure Response to Erythropoietin Injection in Hemodialysis and Predialysis Patients

Kazuhisa MIYASHITA, Akihiro TOJO*, Kenjiro KIMURA*, Atsuo GOTO*, Masao OMATA*, Keisuke NISHIYAMA, and Toshiro FUJITA*

Recombinant human erythropoietin (rHuEPO) has been reported to induce hypertension. We investigated the effect of a single injection of rHuEPO on blood pressure in patients receiving hemodialysis (HD) and in patients with predialysis chronic renal failure (CRF). Forty-one patients receiving HD and 36 patients with predialysis CRF received an intravenous injection of rHuEPO, and blood pressure and plasma endothelin-1 were measured before and 30 min after the injection. Mean blood pressure was increased significantly in HD patients, but not in CRF patients (HD: 103±5 to 105±6 mmHg, $p<0.05$; CRF: 103±4 to 103±6, NS). The percentage of patients with increased mean blood pressure of more than 10 mmHg after rHuEPO injection was significantly larger in the HD than in the CRF group (27.0% *vs*. 5.5%, $p<0.01$). A positive correlation was found between changes in endothelin-1 level and mean blood pressure in the HD ($r=0.43$, $p<0.01$) but not in predialysis chronic renal failure. In conclusion, a single injection of rHuEPO increased blood pressure with a positive correlation with endothelin-1 release in hemodialysis patients, but not in predialysis chronic renal failure patients. (*Hypertens Res* 2004; 27: 79−84)

***Key Words*: erythropoietin, hypertension, endothelin, hemodialysis, chronic renal failure**

Introduction

Recombinant human erythropoietin (rHuEPO) markedly improves anemia in patients receiving hemodialysis (HD); however, hypertension is one of the major adverse effects of rHuEPO (*1*−*3*). Major putative mechanisms for the increase in blood pressure by repeated rHuEPO administration are expansion of blood volume, increased blood viscosity (*4*), and reversal of hypoxic vasodilatation (*5*). A direct vasoconstrictive effect of rHuEPO was also inferred from the results of an *in vitro* animal study in which vascular smooth muscle cells constricted in response to high concentrations of rHuEPO (10−200 U/ml) (*6*). We have demonstrated by *in vivo* study that a single injection of rHuEPO significantly increases blood pressure *via* an increased release of endothelin-1 (ET-1) (*7*). However, it is not well established whether rHuEPO increases blood pressure *via* ET-1 in patients on hemodialysis.

Patients with chronic renal failure (CRF) and renal anemia are also treated with rHuEPO before the initiation of hemodialysis. In chronic renal failure patients, blood pressure often increases with the progression of renal failure *via* salt retention. Even though it has been reported that about 20% of predialysis CRF patients show an increase in blood pressure with rHuEPO therapy (*8*, *9*), it is difficult to distinguish the erythropoietin-induced hypertension from the blood pressure increase associated with the progression of renal failure. In this study, we investigated the effect of a single injection of rHuEPO on blood pressure and ET-1 in patients receiving hemodialysis and in predialysis chronic renal failure patients.

From the Division of Nephrology, the Japanese Red Cross Medical Center, Tokyo, Japan, and *Department of Internal Medicine, University of Tokyo, Tokyo, Japan.

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Address for Reprints: Akihiro Tojo, M.D., Division of Nephrology and Endocrinology, University of Tokyo, 7−3−1, Bunkyo-ku, Tokyo 113−8655, Japan. E-mail: tojyo-2im@h.u-tokyo.ac.jp

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Methods

Study Population

Forty-one HD patients (18 males and 23 females) without cardiovascular complications, and 36 predialysis CRF patients (11 males and 25 females) with serum creatinine of more than 2.0 mg/dl and hemoglobin of less than 10 g/dl participated in this study. The underlying diseases were chronic glomerulonephritis in the HD patients, and chronic glomerulonephritis (20 patients), diabetic nephropathy (9), hypertensive nephrosclerosis (6), and tubulointerstitial nephritis (1) in the CRF patients. The family history of hypertension was analyzed and scored as follows: 1 point, presence of hypertension in a sibling or grandparent; 2 points, presence of hypertension in a parent or child. These studies were performed from 1995 to 1998. They were approved by the ethical committee and conducted according to the principles expressed in the Declaration of Helsinki. All patients provided oral informed consent prior to enrollment.

A Single Injection of rHuEPO and Blood Pressure Monitoring

Early in the morning under a fasting condition, the patients received a single intravenous injection of rHuEPO (Epoetin-beta; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) in the median cubital vein at a dose of 9,000 U (178±5 U/kg body weight (BW)) in HD and 6,000 U (117±3 U/kg BW) in CRF in a 2 ml volume. Patients were resting in bed during the experiments, and drugs, including antihypertensive drugs, were withdrawn on the day of examination. The experiments were performed on the day off hemodialysis in the HD group. The doses of rHuEPO administered in the HD and CRF group were the maximal therapeutic doses per week recommended by the Japanese National Health Insurance guidelines. The average number of previous rHuEPO injections in the CRF group was 2.7, and half of the patients were receiving rHuEPO injection for the first time. Thirteen CRF patients also received the same dose of rHuEPO as the HD patients (9,000 U; 165±5 U/kg BW). To exclude the possibility of blood pressure response due to intravenous injection *per se*, 10 HD patients were injected with 2 ml saline and underwent blood pressure monitoring as controls.

Blood pressure and pulse rate were measured in the contralateral arm of rHuEPO injection using an automatic sphygmomanometer with patients in the supine position before and 15 and 30 min after the administration of rHuEPO. Blood pressure was measured twice at each time point and the average values were used. Blood samples were collected *via* an indwelling intravenous catheter in the forearm vein in the same arm receiving rHuEPO injection, and the plasma concentration of endothelin-1 (ET-1) was determined using an ET-1 enzyme immunoassay kit (Wako Pure Chem. Ind., Osaka, Japan) before and 30 min after the administration of rHuEPO.

Statistical Analysis

Data were expressed as the mean±SEM. The differences among the groups were analyzed by repeated-measures analysis of variance followed by paired Student's *t*-test for blood pressure. The χ^2 test was used for the prevalence of higher mean blood pressure (MBP) response, existence of hypertension, and antihypertensive drug usage. For the physiological data, analysis of variance followed by Dunnett's *post-hoc* analysis was used, and when the values did not show normal distribution, a non-parametric Mann-Whitney *U* test was applied. Values of $p<0.05$ were considered to indicate statistical significance.

Results

Effect of rHuEPO in the Whole Study Population

A single injection of rHuEPO significantly increased systolic blood pressure (SBP) and MBP in the HD patients (SBP: 141±5 to 145±8 mmHg, $p<0.01$; MBP: 103±5 to 105±6 mmHg, 0 to 30 min, $p<0.05$; Fig. 1). The blood pressure elevation persisted for 60 min after rHuEPO injection (MBP 105±5 mmHg, $p<0.01$ *vs*. 0 min). Thus, we examined the blood pressure response at 30 min after rHuEPO injection. However, a single injection of rHuEPO in CRF patients did not result in a significant change (SBP: 147±5 to 148±9 mmHg, NS; MBP: 103±4 to 103±6 mmHg, 0 to 30 min, NS; Fig. 1). In the CRF group, we applied a dose of 6,000 U of rHuEPO, which is the maximal therapeutic dose per week recommended for predialysis patients in Japan. We also tested a higher dose of 9,000 U of rHuEPO (165±5 U/kg BW) in 13 CRF patients, but there was no significant change in blood pressure (SBP: 144±12 to 142±15 mmHg, NS; MBP: 102±9 to 101±12 mmHg, 0 to 30 min, NS), and thus we used only 6,000 U of rHuEPO for the CRF patients. As a control, a single injection of 2 ml of saline was administered in the HD patients, and it did not change MBP significantly (102±8 to 103±9 mmHg).

Clinical Background of Patients with rHuEPO-Induced Hypertension

Patients were then stratified into categories at intervals of 5 mmHg (0−4, 5−9, 10−14 mmHg, *etc*.) according to their maximal change in MBP over the 30 min after rHuEPO injection (Fig. 2). Eighteen HD patients (44%) and 11 CRF patients (31%) showed an increase in MBP of more than 5 mmHg within 30 min after rHuEPO injection. Because this percentage was similar to those in previous reports (*1*–*3*), we defined these patients as responders, and the remaining patients who showed an increase in MBP of less than 4 mmHg

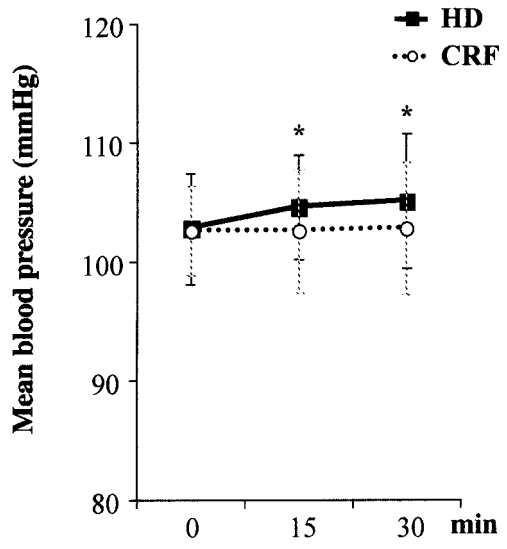

Fig. 1. *The change in mean blood pressure after an injection of erythropoietin (rHuEPO) in hemodialysis (HD) and predialysis chronic renal failure (CRF) patients.* ■*, HD patients (*n=*41),* ○*, CRF patients (*n=*36).* * p<*0.05* vs. *values before rHuEPO injection.*

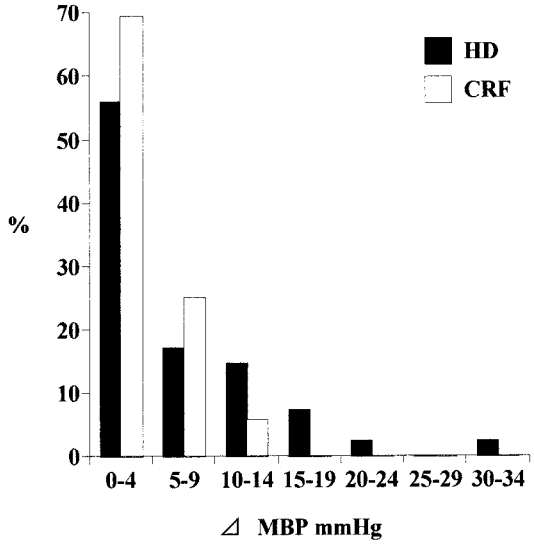

Fig. 2. *Percentage of hemodialysis (HD) and predialysis chronic renal failure (CRF) patients showing changes in mean blood pressure (MBP) of 0−35 mmHg, stratified at 5 mmHg intervals, after erythropoietin-injection.* ■*, HD patients (*n=*41);* □*, CRF patients (*n=*36).*

Table 1. Clinical Background of Hemodialysis and Predialysis Chronic Renal Failure Patients

	Hemodialysis patients			Predialysis patients		
	Responder (*n*=18)	Non-responder (*n*=23)	*p*	Responder (*n*=11)	Non-responder (*n*=25)	*p*
Sex M/F	7/11	11/12	NS	6/5	12/13	NS
Age (year)	46±3	44±2	NS	61±5	67±2	NS
Body weight (kg)	52±2	52±2	NS	56±3	51±2	NS
Creatinine (mg/dl)	——	——	——	5.9±0.8	5.8±0.4	NS
Hematocrit (%)	29±1	28±1	NS	24±2	25±1	NS
Serum albumin (g/dl)	——	——	——	3.2±0.1	3.3±0.1	NS
CTR (%)	50±2	47±1	NS	47±2	50±1	NS
HD history (year)	7.9±1.8	7.0±1.5	NS	——	——	——
Score of FH	1.5±0.4	1.0±0.3	NS	1.2±0.6	1.4±0.3	NS
Hypertension (%)	72	74	NS	72	92	NS
Antihypertensives (%)	56	61	NS	46	84	<0.05
Ca channel blocker	56	61	NS	36	72	0.06
α blocker	11	26	NS	0	8	NS
β blocker	11	17	NS	0	12	NS
ACE inhibitor	11	13	NS	0	0	NS
Diuretics	0	0	NS	27	36	NS
rHuEPO (U/kg BW)	176±7	179±7	NS	111±6	120±4	NS

M/F, male/female; CTR, cardiothoracic ratio; HD, hemodyalysis; FH, family history of hypertension; ACE, angiotensin converting enzyme; rHuEPO, recombinant human erythropoietin; BW, body weight; NS, not significant.

as non-responders. The percentage of patients showing an MBP increase of more than 10 mmHg was significantly higher in the HD than in the CRF group (27.0% *vs*. 5.5%, $p<0.01$). The age, body weight, serum creatinine, hematocrit, serum albumin, family history of hypertension, pre-existence of hypertension, and dose of rHuEPO injection were not different between responders and non-responders in either the HD or CRF group (Table 1). All HD patients had been receiving rHuEPO during the previous HD, but the period under HD was not different between responders and

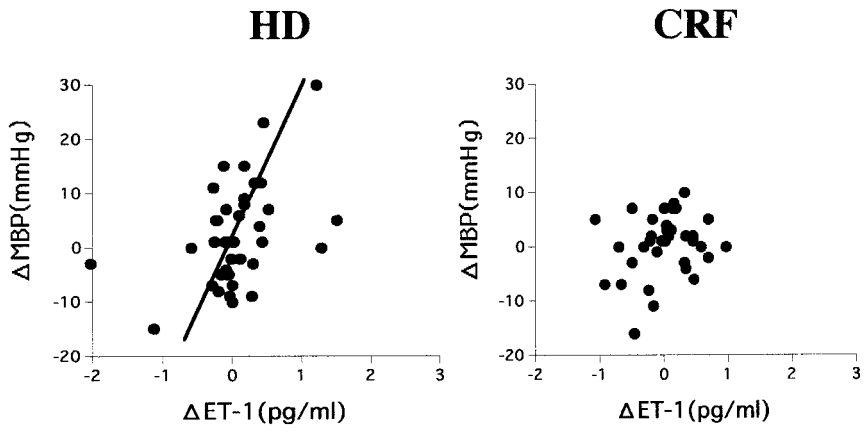

Fig. 3. *The correlation between changes in ET-1 and mean blood pressure (MBP) from 0 to 30 min after rHuEPO injection in hemodialysis (HD) and predialysis chronic renal failure (CRF) patients. There was a significant correlation between changes in ET-1 and MBP in HD patients (*r*=0.43, p<0.01), but not in CRF patients (*r*=0.22, p=0.2).*

non-responders. In CRF patients, the percentage of patients taking antihypertensive drugs was higher in the non-responders than in the responders (84% *vs*. 46%, $p<0.05$; Table 1). There was no difference between CRF responders and non-responders in the use of α_1 blockers, angiotensin converting enzyme (ACE) inhibitors, β blockers, or diuretics; however, treatment with a calcium channel blocker was more common in the non-responder than in the responder CRF patients (72% *vs*. 36%, $p=0.06$). In HD patients, no differences were observed between responders and non-responders in the type of antihypertensive drugs used.

Effects of rHuEPO on ET-1

To investigate the mechanism of blood pressure increase after rHuEPO injection, we evaluated the effect of rHuEPO on ET-1. In HD patients, ET-1 in the responders increased significantly at 30 min after rHuEPO injection (1.0 ± 0.1 to 1.3 ± 0.2 pg/ml, $p<0.05$), but not in the non-responders (1.4 ± 0.3 to 1.2 ± 0.2). Changes in ET-1 level had a positive correlation with changes in MBP at 30 min after injection ($r=0.43$, $p<0.01$; Fig. 3).

In CRF patients, ET-1 did not change after rHuEPO injection and there was no correlation between changes in ET-1 and MBP ($r=0.22$, $p=0.2$).

Discussion

In the present study, 44% of HD patients and 31% of CRF patients showed an increase in MBP of ≥ 5 mmHg within 30 min after a single rHuEPO injection. In addition, a greater percentage of HD patients than CRF patients (27.0% *vs*. 5.5%, $p<0.01$) exhibited a significant MBP increase of ≥ 10 mmHg following rHuEPO injection. These percentages are consistent with those in previous reports in which 20−30% of HD patients receiving rHuEPO therapy required initiation of antihypertensive drug therapy, or an increase in its dosage, particularly during the first 4 months of the rHuEPO therapy (*2*, *3*). Multicenter studies have also demonstrated that rHuEPO therapy induces hypertension in about 20% of predialysis CRF patients (*8*, *9*). In the present study, we demonstrated that a single injection of rHuEPO increased blood pressure in both HD and predialysis CRF patients.

Our clinical observation of an rHuEPO-induced blood pressure elevation was consistent with the results of animal experiments. Heidenreich *et al*. (*6*) demonstrated a marked and instantaneous isometric contraction in renal and mesenteric resistance vessels exposed to rHuEPO at a concentration ranging from 10 to 200 U/ml. And we previously demonstrated that a single injection of rHuEPO increased blood pressure in spontaneously hypertensive rats (*7*).

The proposed mechanisms of erythropoietin-induced hypertension in HD patients treated with chronic repeated injections of rHuEPO are increment of blood viscosity (*6*) and a loss of hypoxic vasodilatation (*7*). In our present study we showed that hypertension can be induced in HD patients by only a single injection of erythropoietin, raising the possibility that some other mechanism may be involved.

There are several possible mechanisms for the vasoconstriction after a single injection of rHuEPO. First, erythropoietin receptor exists in endothelial cells (*10*), and rHuEPO increases the release of ET-1 from endothelial cells (*11*, *12*) as well as the expression of ET-1 mRNA in the endothelial cells (*13*). However, other investigators did not show the increase in ET-1 by erythropoietin (*14*). We have previously reported that a single injection of rHuEPO increased the plasma ET-1 level in spontaneously hypertensive rats, and that ET_A receptor blocker inhibited the rHuEPO-induced blood pressure elevation in this model (*7*). Moreover, Kang *et al.* (*15*) demonstrated that the pressor effect of a single injection of rHuEPO (100 U/kg, i.v.) in HD patients lasts for 3 h with increase in the ET-1 levels. In the present study, the

plasma level of ET-1 was increased at 30 min after rHuEPO injection in the HD patients, and the changes in MBP showed a positive correlation with changes in ET-1 after rHuEPO injection. Thus, erythropoietin-induced hypertension may be mediated in part by an increased release of ET-1. Second, rHuEPO can directly constrict the vascular smooth muscle cells of arteries without endothelium (*6*). Erythropoietin receptor exists in vascular smooth muscle cells (*16*), where it has been shown to induce Ca^{2+} mobilization and cellular constriction (*17*). Third, rHuEPO elevates mRNA of angiotensin II receptor in cultured vascular smooth muscle cells (*18*). Recently, Kuriyama *et al*. (*19*) demonstrated that angiotensinogen gene polymorphism at codon T235 may be responsible for the development of hypertension in CRF patients after repeated injections of erythropoietin. In our study, because we investigated an acute effect of rHuEPO, the effect of rHuEPO in the mRNA transcriptional activation of the renin-angiotensin system can be excluded. Among these possible effects of rHuEPO, we showed that ET-1 release could in part explain the blood pressure elevation after rHuEPO injection.

There are some reports that did not detect a rapid effect of rHuEPO injection on blood pressure in HD patients (*20*, *21*). In these reports the doses of rHuEPO (50−75 U/kg) were lower than those in our study. In a previous animal study, we reported that the effect of rHuEPO on blood pressure was dose-dependent (*7*). Thus, we think that the difference can be explained by the dose of rHuEPO.

It is interesting that we observed a more prominent effect of rHuEPO on blood pressure in the HD patients than in predialysis patients. That is, the number of patients who showed an increase in MBP of more than 10 mmHg was significantly less in the predialysis CRF than in the HD patient group. Kuriyama *et al*. (*22*) reported that rHuEPO at a therapeutic dose (6,000 U, i.v.) once a week for 4 months did not induce hypertension in predialysis patients. Even when using a higher dose of 9,000 U rHuEPO, we did not observe a significant elevation of blood pressure in CRF patients. It remains uncertain why our predialysis patients showed a lower magnitude of rHuEPO-induced blood pressure response. This finding may have been related to fact that, in the CRF group, there were more non-responders than responders receiving antihypertensive drugs, while in the HD group, an equal number of responders and non-responders received antihypertensive drugs. Calcium channel blockers, including the long-acting amlodipine, were used more frequently in the non-responder group of CRF patients, and this may have influenced the blood pressure response to rHuEPO in the CRF patients. The anuric HD patients probably had greater extracellular volume expansion than the CRF patients, who were nonanuric, even though their cardiothoracic ratio (CTR) values were not significantly different. This may also have been responsible for the higher MBP elevation after erythropoietin injection in HD patients than in CRF patients. Whereas the HD group consisted of relatively younger patients with chronic glomerulonephritis, the CRF group included older patients who had been diagnosed with chronic glomerulonephritis, diabetes nephropathy, hypertensive nephrosclerosis, or tubulointerstitial nephritis, and these differences in age and underlying disease may have been related to the different vascular responsiveness to rHuEPO injection. In addition to these experimental background differences, it is possible to speculate that the number of erythropoietin receptors in the endothelial cells or in the vascular smooth muscle cells might have been lower in CRF patients, many of whom were receiving rHuEPO injection for the first time, than in HD patients, who had repeatedly received rHuEPO injections before the study. Further studies will be needed to elucidate the mechanism of the differential response to rHuEPO between HD and CRF patients; however, our data showed that the usual therapeutic dose of rHuEPO injection in predialysis CRF patients was safe and did not cause a severe blood pressure elevation.

In conclusion, a single injection of rHuEPO increased blood pressure with a positive correlation with enhanced ET-1 release in HD patients, but blood pressure changes by rHuEPO injection were less pronounced in predialysis CRF patients.

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