The Role of Endothelin and Prostacyclin on Recombinant Erythropoietin-α Induced Hypertension in Hemodialysis Patients

Hemodiyaliz Hastalarında Rekombinan İnsan Eritropoetin-α ile İlişkili Hipertansiyonda Endotelinin ve Prostasiklinin Rolü

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ABSTRACT

OBJECTIVE: Recombinant human erythropoietin (r-HuEPO) was found to be associated with increased blood pressure in hemodialysis (HD) patients. Plasma endothelin-1(ET-1) was reported to be increased during treatment with r-huEPO in HD patients. Prostacyclin (PGI2) was found to be associated with the reduction in vascular resistance. We aimed to determine the role of ET-1 and PGI2 on r-huEPO-induced hypertension in HD patients.

MATERIAL and METHODS: 43 HD patients (19 females, 24 males) were randomly assigned to groups. Group 1 (n=15) consisted of patients receiving r-HuEPO-α subcutaneously after each dialysis session for 6 to 42 months. Group 2 (n=15, control group) consisted of patients who did not receive any r-huEPO and group 3 (n=13) consisted of patients who received r-huEPO-α subcutaneously after each dialysis session for 12 weeks. Plasma ET-1,2 and 6-keto-PGF1-α were measured.

RESULTS: There were no differences in terms of ET-1,2 and 6-keto-PGF1-α, systolic and diastolic BP and hematocrit levels in group 1 and group 2 patients. However, in group 3 patients, hematocrit, plasma ET1,2 and 6-keto PGF1-α levels were significantly elevated after 12 weeks of rHuEPO treatment.

CONCLUSION: We showed that ET-1.2 and 6-keto-PGF1-α levels were found to be significantly increased with 12 weeks of rHu-EPO therapy in HD patients.

KEY WORDS: Hemodialysis, Erythropoietin, Endothelin, Prostacyclin, Hypertension

ÖZ

AMAÇ: Hemodiyaliz (HD) hastalarında renal aneminin tedavisinde kullanılan rekombinan insan eritropoetin (rHuEPO) tedavisi artmış kan basıncı ile ilişkili bulunmuştur. Plazma endotelin 1 (ET1) konsantrasyonlarının hemodiyaliz hastalarında rHuEPO tedavisi esnasında yükseldiği tespit edilmiştir. Prostasiklin (PGI2) önemli bir vasodilatör madde olup vasküler dirençte azalma ile ilişkili bulunmuştur. Hemodiyaliz hastalarında rHuEPO ile ilişkili hipertansiyonda ET-1 ve PGI2 etkisini araştırmayı hedefledik.

GEREÇ ve YÖNTEMLER: 43 HD hastası (19 kadın, 24 erkek) rastgele olarak gruplara ayrıldı. Grup 1'deki hastalar (n=15), 6-42 ay boyunda her diyaliz seansı sonunda subkutan rHuEPO-α kullanan hastalar, grup 2'deki hastalar (n=15, kontrol grubu) hiç r-huEPO almayan hastalar, ve grup 3'teki hastalar (n=13) 12 hafta boyunca her diyaliz sonrasında subkutan rHuEPO-α kullanan hastalardı. Plazmada ET-1,2 ve 6-keto-PGF1-α ölçüldü.

BULGULAR: Grup 1 ve 2'deki hastalarda tedavi öncesinde ve sonrasında hematokrit, ET-1,2 ve 6-keto-PGF1-α düzeyleri ve sistolik ve diyastolik kan basınçları açısından bir fark saptanmadı. Bununla birlikte, grup 3 hastalarda 12 haftalık rHuEPO tedavisi sonrasında hematokrit, ET-1,2 ve 6-keto-PGF1-α düzeyleri anlamlı düzeyde artmış bulundu.

SONUÇ: Çalışmamızda, HD hastalarında 12 haftalık rHu-EPO tedavisi ile ET-1.2 ve 6-keto-PGF1-α'nın anlamlı olarak arttığını saptadık.

ANAHTAR SÖZCÜKLER: Hemodiyaliz, Eritropoetin, Endotelin, Prostasiklin, Hipertansiyon

Received : 20.12.2011
Accepted : 15.06.2012

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INTRODUCTION

Despite the improvements in the pathogenesis and the treatment of hypertension in chronic kidney disease (CKD), elevated blood pressure (BP) remains one of the most important cardiovascular risk factors in end-stage renal disease (ESRD) patients (1). Altered BP measurements are also found to be related with increased morbidity and mortality in this population (2). Treatment of renal anemia with recombinant human erythropoietin (r-HuEPO) was found to be associated with increased blood pressure in hemodialysis (HD) patients (3-4). Endothelin (ET) is one the most potent vasoconstrictive polypeptide which has a fundamental role in the maintenance of BP. Increased levels of ET-1 has been shown in HD patients, especially those with ischemic heart disease (5). Plasma ET-1 concentrations were also reported to be increased during treatment with r-huEPO in HD patients (6). Prostacyclin (PGI_2), an important vasodilator eicosanoid, was found to be associated with the reduction in pre and post glomerular resistance (7). One of the adaptive mechanisms against increased BP in CKD includes increased PGI_2 secretion from the vessel wall (8). Prostacyclin can be measured by its stable metabolite 6-keto PGF1-α (9).The opposite role of ET-1 and PGI_2 may be a part of an important mechanism in r-huEPO-induced hypertension in ESRD patients. Therefore, in the present study, we aimed to determine the role of ET-1 and PGI_2 on r-huEPO-induced hypertension in HD patients.

MATERIAL and METHODS

The study protocol was approved by the Medical Ethics Committee of Selcuk University (Meram School of Medicine, Konya, Turkey). Written informed consent was obtained from all subjects included in the study.

This was a cross-sectional study involving 43 ESRD patients (19 females, 24 males; mean age, 38±14 years) receiving HD in the dialysis unit of Selcuk University. Hemodialysis modality includes conventional 4-h HD three times a week with polysulfone dialysers. A 250 ml/min (range 200-300 ml/min) of mean blood flow rate was obtained during dialysis sessions. Dialysate fluid composition includes 140 mEq/l of sodium, 1-4 mEq/l of potassium, 3 mEq/l of calcium, 1.8 mEq/l of magnesium, and 33 mEq/l of bicarbonate. Demographic data, medications, primary cause of ESRD and dialysis duration were recorded. Systolic and diastolic blood pressures were measured on the non-fistula arm in an upright sitting position after at least 5 min of rest using an Erka sphygmomanometer (PMS Instruments Ltd, Berkshire, UK) of appropriate cuff size. Two readings were recorded for each individual, and the average was defined as the subject's blood pressure. Patients with systolic and diastolic blood pressure above 140 and 90 mmHg, respectively, or those receiving antihypertensive medications were assumed as hypertensive.

Patients with ESRD were randomly assigned to groups according to treatment with 2000-4000IU/week recombinant human erythropoietin alpha (r-huEPO-α). Group 1 (n=15) consisted of patients who received r-huEPO-α subcutaneously after each dialysis session for 6 to 42 months. Group 2 (n=15, control group) consisted of patients who did not receive any r-huEPO and group 3 (n=13) consisted of patients who received r-huEPO-α subcutaneously after each dialysis session for 12 weeks during the study. Blood samples were obtained before each dialysis session before and after 12 weeks of treatment.

Of the 43 patients, 15 had glomerulonephritis, 3 had hypertensive nephrosclerosis, 3 had diabetes, 6 had tubulointerstistial nephritis, 2 had autosomal dominant polycystic kidney disease, 1 had amyloidosis, 1 had ankylosing spondylitis, 1 had congenital urethral stenosis, 1 had renal steosis, 1 had Alport syndrome and the origin was unknown in 9.

Seventeen patients were on treatment with antihypertensive drugs (7 of them on angiotensin-converting enzyme (ACE) inhibitors, 10 of them on calcium channel blockers).

Measurements of plasma Endothelin (ET)-1,2 and 6-keto Prostaglandin (PG) F1-α:

Blood samples were drawn into two tubes; one for ET-1,2, containing aprotinin (500 kallikrein inhibitory unit/mL of blood from Bayer, Leverkusen, Germany) and 1 mg EDTA/mL of blood and the other one for 6-keto PGF1-α containing EDTA and indomethacin. Mixing EDTA solution (2 g disodium EDTA and 0.8 g NaCl adjusted to pH 7.4 with NaOH and made to final volume of 100 ml in distilled water) with 0.05 ml of a 0.04M indomethacin solution (50 mg indomethacin dissolved in 3.5 ml absolute ethanol) is recommended. The plasma was separated and stored at-20^0C.

Endothelin was measured by the Endothelin1,2 (I^{125}) assay system with the Amerlex-M™ magnetic separation method (Amersham Int. Plc., Amersham UK). The cross-reaction with ET-1, ET-2 and ET-3, big ET-1 and ANP was 100%, 204%, 0.0024%, 37.9%, and 0.024%, respectively. PGF1-α was measured by 6-keto-prostaglandin F1- α (I^{125}) assay system with Amerlex-M™ magnetic separation method (Amersham Int. Plc., Amersham UK). The cross-reaction with 6- keto-prostaglandin F1-α, PGF2-α, PGE_2 was 100%, 8%, 5%, respectively.

Statistical Analysis

The statistical analysis was carried out by the Statistical Package for Social Sciences for Windows ver. 15.0 (SPSS Inc., Chicago, IL, USA). Data were expressed as the mean ± SD, with a significance level of $P < 0.05$. For dichotomous variables, the frequency of positive occurrences was given along with their corresponding percentages. Statistical comparisons of individual groups were based on independent sample *t*-test for continuous variables whereas the correlations between groups were evaluated by the Kruskal-Wallis test.

RESULTS

Clinical features of the patients are shown in Table I. Biochemical parameters of group 1 HD patients receiving r-HuEPO for 6-42 months, group 2 HD patients (control group) and group 3 HD patients who are started r-HuEPO with the beginning of the study are depicted in Table II, III and IV, respectively.

There were no differences between initial values and the values at the end of 12 weeks in terms of ET-1,2 and 6-keto-PGF1-α, systolic and diastolic BP and hematocrit levels in group 1 HD patients (Table II) (p>0.05 for all). In group 2 HD (control group) patients, there were also no differences between initial values and the values at the end of 12 weeks in terms of ET-1,2 and 6-keto-PGF1-α, systolic and diastolic BP and hematocrit levels (Table III) (p>0.05 for all). However, in group 3 patients, plasma ET1,2 and 6-keto PGF1-α levels were significantly elevated after 12 weeks of rHuEPO treatment (40.5±21.2 fmol/mL vs. 96.3±80.7 fmol/ml, p=0.031; 94.2±58.2 (pg/mL) vs. 162±99.1(pg/mL), p=0.047, respectively) (Table IV). The mean hematocrit levels were also increased significantly in group 3 HD patients (21.96± 2.98 % vs. 27.02±4.5%, p<0.003).

When we separated HD patients according to hypertensive status, the mean plasma ET1,2 levels of 17 hypertensive patients

Table I: Demographic features of ESRD patients.

Parameters	Group 1 HD Patients (n=15) (mean±SD)	Group 2 HD patients (n=15) (mean±SD)	Group 3 HD Patients (n=13) (mean±SD)	p value
Gender (M/F)	8/7	8/7	8/5	0.89
Age (years)	36.7±14.3	40.7±13.1	35.3±11.9	<0.0001
Dialysis Vintage (months)	32±19	39.7±25.5	27.8±24.5	0.64
Hypertensive/Normotensive	6/9	5/10	6/7	0.46
Systolic BP (mmHg)	140.7±26	132.7±22.8	131.9±22.9	0.71
Diastolic BP (mmHg)	83.3±13.5	80.0±13.1	79.6±13	0.44
Calcium (mg/dL)	9.5±0.6	9.8±1.2	9.5±1.1	0.51
Phosphorus (mg/dL)	5.1±1.35	5.2±1.8	5.2±1.5	0.98
PTH (pg/mL)	352±529	395±390	483±553	0.48
Albumin (g/dL)	4.1±0.3	4.2±0.3	4.1±0.4	0.78
Triglyceride (mg/dL)	166±70	160±94	203±95	0.27
Total Cholesterol (mg/dL)	188±20	180±73	205±54	0.28
Hematocrit (%)	29.53±5.08	27.92±2.69	21.96±2.98	0.04

BP; Blood pressure, **PTH;** Parathormone, **SD;** standard deviation

Table II: Biochemical parameters of group 1 HD patients.

Parameters	Initial values (Mean±SD)	Values at the end of the study (Mean±SD)	p value
ET 1,2 (fmol/mL)	53.5±22.5	64±44.3	0.41
6-keto PGF1-α (pg/mL)	114.7±93.2	126±116	0.76
Systolic BP(mmHg)	140.7±26	141.7±25.2	0.92
Diastolic BP(mmHg)	83.3±13.5	85±8.6	0.69
Hematocrit (%)	29.5±5.0	30.4±4.0	0.61

BP; Blood pressure, **SD;** standard deviation

Table III: Biochemical parameters of group 2 HD (Control group) patients.

Parameters	Initial values (Mean±SD)	Values at the end of the study (Mean±SD)	p value
ET 1,2(fmol/mL)	49.2±14.8	61±27.0	0.14
6-keto PGF1-α (pg/mL)	166±103	168±118	0.96
Systolic BP (mmHg)	132.7±22.8	137.3±21.2	0.57
Diastolic BP (mmHg)	80±13.1	86±12.8	0.29
Hematocrit (%)	27.42±5.5	26.9±5.0	0.80

BP; Blood pressure, **SD;** standard deviation

Table IV: Biochemical parameters of group 3 HD patients.

Parameters	Before treatment (Mean±SD)	After treatment (Mean±SD)	p value
ET 1,2(fmol/mL)	40.5±21.2	96.3±80.7	0.031
6-keto PGF1-α (pg/mL)	94.2±58.2	162±99.1	0.047
Systolic BP (mmHg)	131.9±22.9	150±32	0.01
Diastolic BP (mmHg)	79.6±13	93.1±17	0.003
Hematocrit (%)	21.96±2.98	27.02±4.5	0.003

BP; Blood pressure, **SD;** standard deviation

were found to be higher than those of normotensive patients (70.2±18.3 fmol/mL vs. 40.5±16.3, p<0.005). However, there were no significant changes between 6-keto PGF1-α levels of hypertensive and of normotensive patients. Of 13 patients in group 3, 7 had increased systolic BP and diastolic BP.

DISCUSSION

The main findings of the present study are as follows; i) the mean ET-1.2 and 6-keto-PGF1-α levels of group 3 HD patients who were initiated rHu-EPO at the beginning of the study and received this therapy for 12 weeks were found to be significantly increased with 12 weeks of rHu-EPO therapy, ii) the systolic and diastolic blood pressure measurements of Group 3 HD patients were found to be significantly increased, iii) when HD patients were separated according to hypertensive status, the mean plasma ET1,2 levels of 17 hypertensive patients were found to be higher than those of normotensive patients, however, there were no significant changes between 6-keto PGF1-α levels of hypertensive and of normotensive patients, iv) There were no significant changes in terms of ET-1,2 and 6-keto-PGF1-α, systolic and diastolic BP and hematocrit levels in group 1 and group 2 HD patients.

Recombinant human erythropoietin (rHuEpo) corrects the anemia of ESRD. However, hypertension has been observed as an adverse effect of increasing red cell mass. Therefore, development or exacerbation of hypertension is one the expected complications of r-HuEPO therapy in patients with ESRD (3-4, 10-12). Buckner et al. (13) demonstrated that 44 of 63 patients (70%) treated with rHuEpo had an increase in mean arterial pressure greater than 10 mm Hg or required new or additional hypertensive medications. According to results of this study, they concluded that rHuEpo-induced hypertension can be controlled with routine medication, however, hypertensive encephalopathy may occur if the blood pressure increases rapidly when the hematocrit increases with rHuEpo therapy.

Takahashi et al. (6) reported that mean BP measurements were found to be significantly increased over 10 mmHg and plasma immunoreactive ET levels with 12 weeks of intravenous r-HuEPO treatment in 19 hemodialysis patients (37%). There was no significant change in immunoreactive ET concentrations in normotensive HD patients.

Miyashita et al. (11) found that a single injection of rHuEPO increased blood pressure with a positive correlation with ET-1 release in hemodialysis patients, but not in predialysis chronic renal failure patients. Also Carlini et al. (14) compared the patients having IV and subcutaneous (SC) rHuEPO in terms of increase in BP and plasma ET-1 levels. They found that mean

BP and plasma ET-1 levels were higher in patients receiving IV rHuEPO than SC rHuEPO. They also showed that rHUEPO stimulated the release of ET-1 in vascular endothelial cell cultures. In the present study, our results are in accord with those studies mentioned above as plasma ET-1,2, and systolic and diastolic BP were significantly increased with 12 weeks rHuEPO treatment in group 3 HD patients.

Raising the hematocrit level from 20% to 30% leads to an increase in peripheral resistance by approximately 25% (15). An increase in red blood cell mass during or after correction of anemia leads to an increase in whole blood viscosity and thereby increases cardiac afterload (15). In the study of Lebel et al. (16) systemic vascular resistance increased by 28% but was not adequately counterbalanced by the decrease of only 6% in cardiac output, resulting in an increase of predialysis and postdialysis systolic and diastolic blood pressure. Red blood cell mass increased significantly during correction of renal anemia, but plasma volume, extracellular fluid volume, and exchangeable sodium decreased significantly. Therefore, the authors suggest that volume-independent mechanisms contribute to rHuEPO-induced blood pressure increase. Sunder-Plassmann et al.(17) also described the mechanisms contributing to the increase in peripheral resistance associated with rHuEPO therapy. Increase in whole blood viscosity, vascular endothelial dysfunction, endothelin release, inhibition of nitric oxide synthesis, activation of various neurohumoral systems, low baseline hematocrit, too rapid correction of anemia are the other factors of rHuEPO-induced hypertension. In our study, both group 1 and group 3 patients are receiving maintenance HD and group 3 patients had more profound anemia than group 1. Low baseline hematocrit and rapid correction of anemia in the group 3 patients might explain the etiology of rHuEPO-induced hypertension.

In the present study, we could not find any significant change in plasma ET-1,2 and 6-keto PGF1-α levels as well as systolic and diastolic BP in group 1 and group 2 HD patients. However, plasma ET-1,2 and 6-keto PGF1-α levels and systolic and diastolic BP were significantly increased with 12 weeks rHuEPO treatment in group 3 HD patients. These results were in accord with previous studies and one might suggest that rHuEPO might induce the release of ET-1 by vascular endothelium in a direct or indirect manner.

Our study has some limitations as the sample size was relatively small. Since this is not a prospective controlled study we can not draw cause and effect relations from our findings.

In conclusion, we showed that the mean ET-1.2 and 6-keto-PGF1-α levels of group 3 HD patients who were initiated rHu-EPO at the beginning of the study and received this therapy for 12 weeks were found to be significantly increased with 12 weeks of rHu-EPO therapy. Further experimental and clinical studies are needed to define exact relationship between rHUEPO-induced hypertension and ET in this population.

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