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Effect of enalapril on exaggerated erythropoietin response to phlebotomy in erythrocytosic renal transplant patients

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Abstract

Background. Exaggerated erythropoietin (EPO) response to phlebotomy regardless of the baseline EPO levels have been shown in patients with post-transplant erythrocytosis (PTE) and administration of angiotensin-converting enzyme inhibitors (ACE-I) seems to be effective in controlling PTE. However, the mechanism of this ACE-I induced reduction in haematocrit (Hct) is not well known. Although some authors have suggested that ACE-I may reduce EPO secretion, this is still controversial. The aim of the present study was to assess the effect of a single dose ACE-I on exaggerated EPO response to phlebotomy.

Methods. In this study, we compared serum EPO and renin (PRA) levels of 10 PTE patients, 10 non-PTE patients and 10 healthy blood donors before and after phlebotomy. The effects of a single dose of ACE-I (enalapril, 5 mg p.o.) in PTE patients were also evaluated in the second phlebotomy.

Results. While the mean basal serum EPO level was significantly higher in the PTE group than the other two groups ($P<0.01$), the mean basal PRA levels did not differ significantly between these groups. Serum EPO and PRA levels increased significantly after the phlebotomy ($P<0.001$) and exaggerated EPO response to phlebotomy was suppressed by single dose enalapril ($P<0.001$) in the PTE patients.

Conclusion. The present study has shown that the renin–angiotensin system plays an important role in EPO formation and the Hct lowering effect of the ACE-I is through reduction of EPO in PTE patients.

Key words: Angiotensin converting enzyme inhibitors; enalapril; phlebotomy; erythropoietin; post-transplant erythrocytosis

Introduction

Erythrocytosis is a relatively common complication after renal transplantation, affecting 10–15% of allograft recipients with normal renal function and can result in serious thromboembolic complications [1]. In contrast to the well-known clinical features of post-transplant erythrocytosis (PTE), the pathogenesis is still vague. Although some authors suggested that it is due to elevated serum erythropoietin (EPO) levels of either native kidney or allograft origin [2–5], PTE can occur with low or undetectable levels of serum EPO [6,7]. Although baseline plasma EPO levels are highly variable, exaggerated EPO response to phlebotomy, regardless of the baseline EPO levels have been shown in patients with PTE [8].

PTE was originally treated with repeated phlebotomy, later it was found that medical treatment with angiotensin-converting enzyme inhibitors (ACE-I) also had a beneficial effect [9]. Although its mechanism(s) of action are not completely known, it has been suggested that this effect of ACE-I could be related to the known property of angiotensin II to stimulate EPO production, so that, conceivably, ACE-I would depress EPO release [10,11]. On the other hand, effective therapy of PTE with ACE-I has not been accompanied by a decline in serum EPO levels in some patients, while ACE-I also effectively reduced haematocrit (Hct) levels in PTE patients with undetectable EPO levels as in those with higher levels, suggesting that ACE-I may reduce Hct levels by other than through reduction of serum EPO mechanisms [12,13].

Most published studies have shown that serum EPO levels and the relationship between ACE-I and serum EPO levels were highly variable in patients with PTE. However, these studies were cross-sectional and fail to explain all aspects of the effects of ACE-I on serum EPO levels, especially patients with lower and undetectable serum EPO level. On the other hand, exaggerated EPO response to phlebotomy regardless of the baseline serum EPO level has been shown in PTE patients as mentioned before. On the basis of these observations, we designed a dynamic study to investigate the relationship between the ACE-I and erythropoiesis and to characterize the pathogenesis of this interaction. We have therefore purposed to stimulate serum EPO secretion by phlebotomy and to examine the effect of a

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single dose ACE-I on this stimulated EPO response in PTE patients.

Patients and methods

Patients

In this prospective study, we examined 10 PTE patients (all males, mean age 41 ± 13 [range 22–58]) and 10 non-PTE patients (nine males, one female, mean age 43 ± 14 [range 30–58]) from our transplant follow-up clinic, and 10 healthy blood donors subjects (eight males, two females, mean age 42 ± 12 [range 26–62]) served as a control, who had no evidence of primary or secondary systemic disease. PTE was defined as a persistent elevation of the Hct level above 51%. All of the PTE patients had documented erythrocytosis for at least 6 months, with three Hct readings of 51% or higher during that period and all of them had had repeated phlebotomies before to maintain Hct below 51%. Patients with the following criteria were excluded from this study: (i) presence of thrombocytosis, leukocytosis or splenomegaly, suggesting the diagnosis of polycythaemia vera; (ii) abnormal arterial blood gases analysis, spirometry and diffusion capacity study suggestive of an obstructive or restrictive lung disease; (iii) presence of renal artery stenosis, hydronephrosis and tumours in the native kidneys or the liver (evaluated by Duplex-Doppler ultrasound examination); (iv) presence of haemoglobinopathies (abnormal haemoglobin electrophoresis); (v) patients on diuretics, anticoagulant/antiplatelets or ACE-I treatment; and (vi) patients who smoke.

The mean onset time of this study after renal transplantation was 40 months (range 18–72) and the mean onset time of erythrocytosis after renal transplantation was 13 months (range 4–26) in this study. Original disease of PTE group consisted of five cases with chronic glomerulonephritis, two with polycystic kidney disease and three with unknown aetiology and all patients had been transplanted from living related donors. Eight patients were hypertensive and it was controlled with calcium channel blockers (nitrendipine) in five patients and by beta-blockers (atenelol) in two and combination of two drugs (nitrendipine/plus atenolol) in one patient in this group. None of the patients received diuretic nor ACE-I for the antihypertensive treatment. All of the PTE patients had good renal function and rejection-free course after transplantation and eight of the patients were on triple immunosuppression therapy that consisted of prednisone-cyclosporin-azathioprine and two of them were on a prednisone-cyclosporin treatment.

The underlying causes of chronic renal failure in non-PTE group included five patients with chronic glomerulonephritis, two patients with pyelonephritis, one patient with polycystic kidney disease and two patients with unknown aetiology. Eight patients had been transplanted from living related donors and two from cadavers in non-PTE group. Eight patients were hypertensive, five patients had been treated with nitrendipine, one patient by atenolol. The other two patients had been under combination of these two drugs in this group. Three non-PTE patients had acute rejection episodes in the early post-transplant period, however, all patients had good renal function during the study in this group. Nine patients received prednisone-cyclosporin-azathioprine and one patient had been treated with prednisone-cyclosporine in the non-PTE group.

Protocol

Informed consent to participate in the study was obtained from all patients. All of the patients were evaluated in an outpatient setting, medical history and physical examination were recorded and they had routine biochemical and haematological monitoring. Supine blood pressure was measured before and after the phlebotomy. Baseline evaluation of serum EPO levels and plasma renin activity (PRA) were performed in all cases. After the baseline evaluation, phlebotomy was performed in both the PTE patients and the healthy blood donors. One unit of blood (approximately 450 ml) was taken at each phlebotomy. Five hours after the phlebotomy, blood samples for complete blood count (CBC), serum creatinine, serum EPO levels and PRA were reobtained. After 8 months (mean; range 4–10) later from the first phlebotomy, the second phlebotomy was performed in the PTE groups. During this observation period, all of the PTE patients' Hct levels reached over 51%. Thus, all patients had had 51% or greater Hct levels before the second phlebotomy. A single dose of enalapril (5 mg p.o.) was administered 1 h before the second phlebotomy and blood samples were obtained for CBC, serum EPO level and PRA 5 h after the phlebotomy.

Methods

Blood samples were taken into tubes containing EDTA, and centrifuged for 15 min at 3000 r.p.m. The supernatant was stored at $-20^{\circ}C$ until assayed. CBC and serum creatinin level were evaluated by standard laboratory methods. Serum Epo level was assayed by radioimmunoassay (Chemiluminescence Immunoassay, Nicols Institute) and the results were expressed in mU/ml. The normal value in healthy individuals with the mentioned method was 1.5–21 mU/ml. PRA was estimated radioimmunologically (Radioimmunoassay, Sorin), the normal supine range with normal salt intake being 0.2–5.7 ng/ml/h.

Statistical analysis

The differences between the groups for baseline Hct, serum EPO levels and PRA were evaluated by using non-parametric tests, Kruskal–Wallis one-way ANOVA. Changes in serum EPO levels and PRA in the PTE and healthy blood donor groups measured before and after phlebotomy were assessed by Wilcoxon matched-pairs signed-rank and Mann–Whitney U test. The differences between presence and absence of enalapril in the PTE groups for serum EPO levels and PRA were compared by Wilcoxon matched-pairs signed rank test. The data were analysed using SPSS v 6.0 for Windows (SPSS Inc) and expressed as mean $\pm$ standard deviation. A P value lower than 0.05 was considered to be significant.

Results

The demographic and clinic variables were comparable in the PTE group and non-PTE group.

There were no significant differences in sex, age, serum creatinine level, original disease, duration of dialysis, pre-transplant Hb and Hct, degree of HLA matching, mean duration of transplantation, frequency of hypertension and antihypertensive drug regimens,

immunosuppressive drug therapy and graft type between groups (Table 1).

Supine mean arterial blood pressure decreased in both the PTE and healthy blood donor group after the phlebotomy, however, it did not reach statistical significance. Mean Hb, Hct, serum creatinin, serum EPO levels and PRA before and after phlebotomy are outlined in Table 2.

The mean basal serum EPO was significantly higher in the PTE group than the non-PTE and the healthy blood donor group ($P<0.0001$). The mean basal PRA was not statistically different in the three groups ($P>0.05$). After the phlebotomy, mean Hb and Hct values fell significantly in both the PTE and healthy blood donor group ($P<0.001$). Following phlebotomy the mean serum EPO levels increased significantly in both groups, but the increment in the PTE group was more marked. Following phlebotomy PRA increased significantly in both groups too, but the increment did not differ significantly between the PTE and healthy blood donor group ($P>0.05$).

Increased EPO levels in response to phlebotomy were suppressed by single dose enalapril (Table 3, Figure 1) (from 79.60 to 44.67 mU/ml, $P<0.001$), while renin levels were elevated (Table 3, Figure 2) (from 3.51 to 7.44 ng/ml/h, $P<0.001$) in the PTE group.

Table 1. Characteristics of PTE and non-PTE patients

	PTE $n=10$	Non-PTE $n=10$	*P*
Sex ratio M/F	10/0	9/1	NS
Age (years)	41±13	43±14	NS
Serum creatinin (μmol/l)	130±22.5	140±24.6	NS
Original disease:			NS
Glomerulonephritis	5	5	
Polycystic kidney disease	2	1	
Pyelonephritis	0	2	
Unknown	3	2	
Duration of dialysis (months)	32.4±32.6	42±46.8	NS
HLA matches	2.1±1.0	2.4±1.1	NS
Graft type			NS
Living	10	8	
Cadavers	0	2	
Duration of transplantation (months)	36.6±32.5	45.7±42.8	NS
Acute rejection	0	3	NS
Hypertension	8	8	NS
Anti-hypertensive drug regimens			NS
Only calcium channel blockers	5	5	
Only beta-blockers	2	1	
Combination	1	2	
Immunosuppression			NS
Cyclosporin-A	10	10	
Steroids	10	10	
Azathioprine	8	9	

Discussion

Although the pathogenesis of PTE is unclear, it may be a result of excessive production of EPO. Previous studies have shown that PTE has been sometimes but not always associated with increased circulating EPO levels [2–5]. Some authors also reported that the majority of PTE patients have normal serum EPO levels that suggest stimulation of EPO independent of EPO secretion [5,13]. However, the finding of normal EPO levels in PTE patients could be commented as abnormal, suggesting a state of non-suppressibility of EPO secretion. This could be the result of enhanced response to normal EPO levels by modification of receptor number and/or affinity, and their erythrocytosis may therefore be considered to be EPO-dependent. On the other hand, EPO excess or EPO dependent mechanisms do not completely explain the phenomenon of PTE. Gaston *et al.* reported that 40% of patients with PTE had undetectable circulating EPO level in their own series and this finding was confirmed

Table 2. Mean serum creatinin, haemoglobin, haematocrit, plasma renin activity and erythropoietin levels before and after phlebotomy in three groups

Parameters	PTE group		Non-PTE group	Healthy blood donor group	
	Basal	Post-phlebotomy	Basal	Basal	Post-phlebotomy
Creatinin (μmol/l)	110±22.5	108±22.5	120±24.6	95±22.5	97±22.5
Hemoglobin (gr/dl)	17.24±0.53[a]	16.21±0.31[b]	14.01±0.73	15.33±0.46	14.44±0.40[b]
Hematocrit (%)	52.41±1.12[a]	47.75±0.83[b]	44.10±0.70	47.37±0.29	44.04±0.61[b]
Renin (PRA) (ng/ml/h)	1.43±0.88	3.51±1.55[b]	1.32±0.68	1.00±0.41	2.81±1.18[b]
Erythropoietin (mU/ml)	22.75±20.70[a]	[c]79.60±45.21[b]	9.09±3.41	8.07±3.77	[c]12.27±4.41[b]

[a]The mean basal haemoglobin, haematocrit and serum erythropoietin levels are significantly higher in the PTE group than the non-PTE and the healthy blood donor group ($P<0.0001$).
[b]Difference between basal (pre-phlebotomy) and post-phlebotomy values is significant ($P<0.001$).
[c]Following phlebotomy the mean serum erythropoietin levels increased significantly in both groups, but the increment in the PTE group is more marked.
Values are mean±SEM.

Table 3. Mean haemoglobin, haematocrit, renin and erythropoietin levels before and after phlebotomy and phlebotomy + ACE-I (enalapril) in PTE patients

Parameters	Phlebotomy		Phlebotomy + ACE-I	
	Before	After	Before	After
Haemoglobin, (gr/dl)	17.24 ± 0.53	16.21 ± 0.31	17.09 ± 0.35	16.02 ± 0.42
Haematocrit, (%)	52.41 ± 1.12	47.75 ± 0.83	52.05 ± 0.93	48.34 ± 0.78
Renin (PRA), (ng/dl/h)	1.43 ± 0.88	3.51 ± 1.55	1.31 ± 0.91	7.44 ± 5.45[a]
Erythropoietin, (mU/ml)	22.75 ± 20.70	79.60 ± 45.21	19.75 ± 19.70	44.67 ± 34.17[a]

[a]Increased mean erythropoietin level in response to phlebotomy was supressed by single dose enalapril, while plasma renin activity level was elevated ($P < 0.001$).

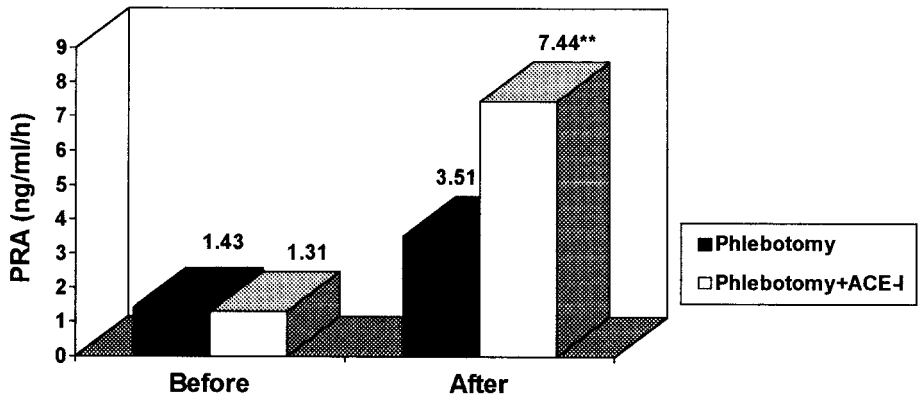

Fig. 1. Mean (±SEM) plasma renin activity (PRA) levels before and after phlebotomy and phlebotomy + ACE-I (enalapril) in PTE patients. Increased PRA level in response to phlebotomy was elevated with single dose enalapril (**$p < 0.001$).

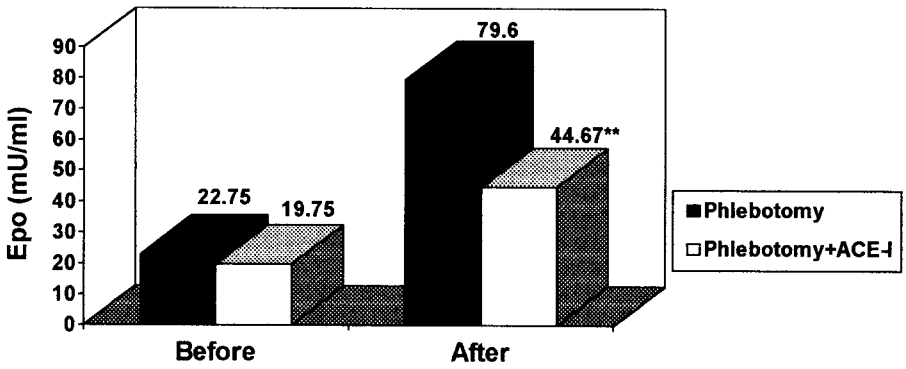

Fig. 2. Mean (±SEM) serum erythropoietin (Epo) levels before and after phlebotomy and phlebotomy + ACE-I (enalapril) in PTE patients. Increased Epo level in response to phlebotomy was supressed by single dose enalapril (**$p < 0.001$).

by at least two other studies [5–7]. Our study showed that PTE patients had higher baseline mean serum EPO levels than non-PTE patients and healthy blood donors. Although mean serum EPO levels were higher than in non-PTE patients and most of the PTE patients had inappropriately high EPO levels, erythrocytosis was associated with undetectable serum EPO levels in one of our PTE patients. These studies and our findings suggested that PTE may be related to inappropriately elevated serum EPO levels, however, other factors including growth factors and cytokines may be involved in the pathogenesis of PTE in addition to circulating EPO.

A negative feedback system, in which tissue oxygenation controls EPO production and EPO controls red blood cell production, provides homeostasis in oxygen delivery to body tissues [14]. As tissue oxygenation is decreased due to hypoxia provoked by phlebotomy, the levels of EPO mRNA increases exponentially [15–17]. In our study, we showed that the mean serum EPO and PRA levels of the PTE patients and healthy blood donors increased significantly after the phlebotomy, but the increment of serum EPO in the PTE group was more prominent. Although earlier cross-sectional studies suggested that EPO dependence of the PTE remains controversial, the finding of more

pronounced exaggerated EPO response to phlebotomy regardless of the baseline EPO status in PTE patients, suggested that PTE may be related to defective feedback regulation of EPO secretion.

Based on the observation that ACE-I caused anaemia in renal transplant recipients, this treatment modality has been used in the management of PTE [18–20]. Recently several studies have demonstrated that administration of ACE-I seems to be effective in controlling PTE, but the mechanism for this association is not well known [10–13,21,22]. Participation of the renin–angiotensin system in erythrocytosis has been suggested by a number of investigators [23–25]. Fried *et al.* [26] showed that a small dose of angiotensin II leads to a rise in plasma EPO level without blood pressure elevation in hypoxic rats. Other investigators have reported that inhibition of ACE caused a reduction in circulating levels of angiotensin II and EPO levels and this could be prevented by the preceding infusion of angiotensin II [27,28]. As renin and angiotensin II can directly regulate erythropoiesis by stimulating EPO production, ACE inhibition may well reduce EPO. During ACE inhibition renal vasodilation will ensue with an increase in renal blood flow which possibly suppresses EPO production. These data suggested that ACE-I might blunt erythropoiesis due to alteration in renal blood flow and oxygen delivery as well as direct or indirect suppression of EPO secretion. Indeed, effective therapy of PTE with ACE-I has been accompanied by a decline in serum EPO levels in some patients [29]. Our study showed that increased EPO level in response to phlebotomy was suppressed by single dose enalapril while renin level was elevated. This observation suggests a direct link between renin–angiotensin system and erythropoiesis. Our results seem to support some other studies published about ACE-I effects in PTE patients. However, our study was remarkable to show that the acute effects of ACE-I on exaggerated EPO response to phlebotomy. As far as we know, this is the first report describing the changes in the phlebotomy-induced EPO response following a single dose ACE-I.

Unfortunately, other data indicate that the relationship between EPO and ACE-I is not so clear in PTE patients. Perazella *et al.* showed that despite enalapril effectively reduced Hct levels, EPO levels did not change [12]. Other studies demonstrated that ACE-I corrects erythrocytosis as effectively in PTE patients with undetectable EPO levels as in those with higher levels [6,7]. Thus, the hypothesis of the Hct-lowering effect of ACE-I due to reduced EPO production does not explain all the observed effects of ACE-I in PTE. Recently, it has been reported that the acute administration of ACE-I to healthy subjects increases the plasma levels of *N*-acetyl-seryl-aspartyl-lysyl-proline (*N*-Ac-SDKP), which is a regulatory factor with myelodepressive activity [30]. This newly recognized effect of ACE-I may be another important mechanism of inhibition of the erythropoiesis in addition to the direct effect of ACE-I on circulating EPO levels.

In conclusion, although mechanisms whereby ACE-I decrease the Hct in patients with PTE are still controversial, our study suggested that the Hct lowering effect of the ACE-I is through reduction of EPO. However, mechanisms other than inhibiting of circulating EPO may be involved in this effect of ACE-I. Additional prospective studies are needed to define the relationship and mechanism by which ACE inhibition and erythropoiesis.

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