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Association of I/D angiotensin-converting enzyme genotype with erythropoietin stimulation in kidney failure

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Abstract: Angiotensin-converting enzyme (ACE)-gene polymorphism is a possible predisposing factor of erythropoietin response under hypoxic conditions. However, it is not completely clear whether the ACE insertion/deletion (I/D) genotype has an impact on anemia in patients with permanent kidney failure. A 9-month prospective trial was conducted on 53 patients on hemodialysis aimed at determining the beneficial effect of oral vs intravenous iron in anemia management with recombinant human erythropoietin (rHuEpo), and identifying a possible association of the ACE gene I/D polymorphism with the response to rHuEpo. Patients were randomly allocated to receive 50-100 mg daily of ferrous gluconate orally (N=26) or intravenously every two weeks (N=27), together with rHuEpo-beta (200 IU/kg) subcutaneously, to achieve a hemoglobin increase to 105 g/L; subsequently the rHuEpo dose was adjusted at one or two week intervals. In 34 patients who regularly received ACE-inhibitor (ACEi) medication, genotyping for ACE-gene I/D polymorphism was performed using PCR, gel analysis and appropriate restriction digestion. After prolonged rHuEpo treatment, 24.5% of patients attained the targeted 9th-month hemoglobin concentration (105 g/L). Of these, 6/26 of patients received elemental iron orally and 7/27 received it intravenously. We observed an association between homozygous DD (deletion) of the ACE gene and a remarkable early increase in blood hemoglobin (p=0.028), erythrocyte count (p=0.020) and hematocrit (p=0.043) after reduction of the dose of rHuEpo (F=3.95; p=0.029), irrespective of the iron repletion mode (p=0.960). This is the first report on DD genotype as a linkage marker for the optimization of rHuEpo dose for anemia management in hemodialysis patients.

Key words: hemodialysis; hemoglobin; oral iron; IV iron; epoetin beta; ACE I/D genotype

INTRODUCTION

Angiotensin II is a regulator of the erythropoietin basal level (under physiological conditions) [1]. Angiotensin II acts as a growth factor that directly stimulates the proliferation of erythroid progenitors in the bone marrow [2]. A worsening of chronic kidney disease (CKD)-associated anemia by treatment with ACE inhibitors and angiotensin II type 1 receptor blockers is well documented [3,4]. Angiotensin-converting enzyme (ACE)-gene polymorphism is a possible predisposing factor of erythropoietin response under hypoxic conditions. Jeong et al. [5] discovered an association between the presence of DD-ACE genotype and a lower resistance to recombinant human erythropoietin (rHuEpo) treatment in CKD patients on peritoneal dialysis. It is not well understood whether the ACE I/D genotype has an impact on anemia management with rHuEpo and iron repletion in patients with permanent kidney failure on regular hemodialysis (HD).

The majority of HD patients are currently administered intravenous (IV) iron to optimize rHuEpo treatment according to the KDIGO (Kidney Disease: Improving Global Outcome) protocol recommendations for anemia management. This allows excellent control of the regular administration of iron [6]. However, up to one third of patients do not experience a satisfactory level of anemia control and the prevailing tide of current opinion in iron supplementation

in rHuEpo control of renal anemia is the benefit of continuous iron administration via the dialysate fluid escaping the intestinal iron transport during the hemodialysis procedure [7].

Progress in the HD procedure has significantly improved gastrointestinal tolerance to oral iron supplements, allowing daily iron repletion for the period of rHuEpo treatment. Oral iron supplementation regulates iron metabolism in a more physiological manner than intermittent supplementation with a high dose of IV ferrous. As regards control of hemoglobin concentrations in renal anemia, there are no data related to the possible concerns about the route of iron supplementation and the potential impact of ACE I/D polymorphism on rHuEpo treatment. The aim of our study was to compare the efficiency of oral vs intravenous iron supplementation in CKD patients undergoing hemodialysis, and the inherited factor ACE I/D genotype.

MATERIALS AND METHODS

Subjects

Patients suffering from CKD-related anemia (blood hemoglobin <95 g/L) that were on a regular hemodialysis program with dose adequacy assessed by Kt/V (urea)>1.2, were enrolled in the study. The main inclusion criteria were an iron-binding capacity (transferrin saturation (TSAT)), calculated as sFe/TIBCx100>20% that was confirmed after a month-long monitoring, and whether a prior intravenous (IV) iron treatment was required. The patients were randomly allocated into two treatment groups: one group received rHuEpo-beta and 50-100 mg daily of ferrous gluconate orally (Tot Hema, Innotech), the other group received rHuEpo and intravenous ferrous gluconate (Ferrlecit, Sinofi) of 100 mg every two weeks, according to standard KDIGO recommendation for anemia management in CKD patients [6]. All patients received the rHuEpo-beta (Recormon, Roche) in a starting dose of 200 IU/kg, administered subcutaneously three times per week, to achieve a hemoglobin increase up to 105 g/L, which was subsequently adjusted at intervals of one or two weeks.

Additional inclusion criteria were: ≥18 years of age, dialysis vintage of at least 3 months, hemodialysis adequacy according to Kt/V(urea)>1.2, absence of hepatitis virus and HIV infection, malignancy, other active infection or elevated inflammation parameters. Exclusion criteria were: non-CKD-related anemia, a history of hepatitis B or C, pregnant or nursing women, blood transfusion within the previous 3 months, sustained ferritin ≥800 ng/mL for longer than 3 months, new malignancy, iron overload (hemochromatosis) or serious disturbances in the utilization of iron (allergies to iron preparations, chronic vomiting), decompensated liver cirrhosis or active hepatitis, active acute or chronic infections, frequent need of blood transfusions, untreated vitamin B12 or folate deficiency.

Study protocol

Epoetin beta (200 IU/kg) was administered subcutaneously at the end of the HD procedure three times per week until an increment of 1 g/dL of hemoglobin was achieved. Administration was continued, but with a 25% reduction in the dose of epoetin in order to maintain a therapeutic target hemoglobin maximum of 110 g/L, which was monitored over the period of one month. Iron supplementation with an oral iron preparation containing ferrous gluconate (quantity equivalent to 50 mg iron) was prescribed 1-2 times daily, while IV iron supplementation with a sodium ferric gluconate complex of 100 mg was applied once in two weeks after the hemodialysis. This study protocol of iron repletion and erythropoietin stimulation and in association with ACE-I/D genotype was approved the Ethical Committee of the Belgrade School of Medicine, University of Belgrade, and the Ethical Committee of the General hospitals of Novi Pazar and Kraljevo. The informed consent from all the examined patients was obtained.

Biochemical investigation

Venous blood samples were collected in vacutainer tubes (BD Vacutainer Systems, Franklin Lakes, New Jersey), with potassium EDTA as anticoagulant, for determination of a complete blood count. All samples were tested on an ADVIA 2120i hematology analyzer (Siemens Healthcare Diagnostics, Eschborn, Germany). Serum iron, total iron binding capacity (TIBC), ferritin and transferrin were measured using the commercial assay on a Roche Cobas 6000 automated analyzer (Roche Diagnostics, Mannheim, Germany).

Treatment regimen efficiency was evaluated by monitoring the respective hematological and iron metabolism parameters that were regularly measured in a three-month period. Trial safety parameters included blood leukocytes, platelet count, serum urea and potassium, together with systolic/diastolic pressure.

ACE I/D genotyping

Blood samples were obtained in EDTA tubes and DNA was isolated by the salting-out method [8]. The I/D polymorphism of ACE, an accepted marker for renin-angiotensin system (RAS) activity, was determined using the polymerase chain reaction (PCR) with primers for detection of ACE deletion (D) insertion/(I) polymorphisms, as follows: forward: 5'- CTG GAC ACC ACT CCC ATC CTT TCT -3', reverse: 5'- GAT GTG GCC ATC ACA TTC GTC AGA T-3' (Metabion). Half a µl of each primer was solved in a final volume of 25 µl, containing 25 mM MgCl2, 10mM of each dNTP, 10xB (8500 mM KCl, 100 mM TRIS HCl, pH 8.3, 15 mM $MgCl_2$, 0.01% gelatin) and 1 unit of Taq polymerase. PCR was performed with 5 min of initial denaturation at 95°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing at 63°C for 1 min, extension at 72°C for 1 min and final extension for 10 min at 72°C, using 2720 Thermal cycler (Applied Biosystems). After the PCR, the samples were separated by 8% polyacrylamide gel electrophoresis (Sigma), stained with SYBR Safe (Invitrogene), and photographed. Gel analysis showed an amplification band of 477 bp in the presence of the insertion in samples with the II genotype, bands of 477 and 190 bp in samples with ID genotype and a band of 190 bp in the absence of the insertion in samples with the DD genotype**.** To exclude incorrect DD genotyping in samples of ACE DD genotype, the results were confirmed with repeated PCR analysis.

Statistical analysis

Statistical analysis was performed using SPSS 18.0. The patients were randomly separated into two treatment groups as described above. The respective variables were presented as the means±SD, with $p<0.05$ considered as significant. The iron treatment efficacy was evaluated by time-course changes of hematological and iron metabolism parameters based on the comparison of variable measurements conducted at 3-month check points during a 9-month trial period for both iron treatment groups (oral vs. IV) and ACE-gene I/D polymorphism in a general linear model used for analysis of variance (ANOVA) by repeated measures' calculation. To examine the mean differences in related variables with normal distribution between groups, a 2-sample t-test was applied.

Table 1. Summary of the study protocol.

		Oral iron N=26	**Intravenous iron N=27**
Gender		10f / 16m	14f / 13m
Age (years)		50.9±13.2	50.9±14.8
Cardiac comorbidities (%)		6 (23.1)	7 (23.9)
HD vintage (months)		53.7±53.9	46.7±549.4
iron (mg)	1-3rd month	4600.0±0	875.0±0
	4-6th	4534.0±318.0	375.0±0
	7-9th	4450.0±0	375.0±0
rHuEpo beta (IU)	1-3rd	25461±17704	29407± 14270
	4-6th	20231±12031	20444±11771
	7-9th	25153 ±13424	31333± 16371

Parameters are expressed as means±SD; f – female; m – male; HD – hemodialysis; rHuEpo – recombinant human erythropoietin

RESULTS

The treatment protocol and general characteristics of 53 patients enrolled in the prospective trial of rHuEpo in the management of anemia associated with chronic hemodialysis, and randomly allocated to oral vs. IV iron supplementation, are shown in Table 1. Patients entered the study with blood transferrin saturation (TSAT) >20%, to determine the effect of iron repletion on the response to rHuEpo and to identify the ACE-gene I/D contribution to rHuEpo dose optimization. Baseline hematological characteristics along with iron metabolism parameters were at similar levels in the two defined cohorts, with the exception of the serum iron value, which was lower in patients selected for IV ferrous gluconate administration ($p=0.021$) (Table 2). Patient response implied the equal efficiency and safety of the oral vs. IV route of iron application for prolonged rHuEpo control of renal anemia (Table 2). Approximately half of the patients in both iron supplemental groups maintained a hemoglobin concentration >95 g/L, with 24.5% of patients able to reach the target 9th-month outcome hemoglobin concentration in the blood of 105 g/L, i.e. 6/26 (23.1%) of patients

Table 2. Baseline values of parameters and treatment efficacy and safety.

	Baseline level (1st month)			**Time course of changes**				
	Parameters	iron 26/27	1st month	1-9th month		3rd month	6th month	9th month
				F	p			
Iron treatment efficacy	Hemoglobin (g/L)	oral	100.1±2.8	2.36	0.073	98.7±2.4	93.4±2.9	95.3±2.9
		intravenous	93.4±2.1			**98.5±2.8***	94.6±2.1	95.3±2.7
	Erythrocyte (x 10.e6/L)	oral	3.2±0.1	0.99	0.400	3.0±0.1	3.0±0.1	3.1±0.1
		intravenous	3.1±0.1			3.1±0.1	3.1±0.1	3.2±0.1
	Hematocrit (%)	oral	29.7±1.1	2.39	0.070	**27.6±0.6***	26.6±1.0	28.5±1.0
		intravenous	27.3±0.6			27.6±0.8	27.1±0.6	28.4±0.8
	Serum iron (mcmol/L)	oral	14.8±0.9	2.63	0.052	**11.9±0.8***	11.4±0.9	13.8±1.0
		intravenous	12.0±0.7			12.4±0.5	11.6±0.6	12.8±0.7
	TSAT (%)	oral	35.5±2.8	2.35	0.074	**28.7±1.9****	30.1±2.5	30.8±2.4
		intravenous	30.1±2.2			32.8±1.8	29.8±1.9	29.8±1.6
	Ferritin (ng/mL)	oral	321.1±78.6	1.21	0.306	242.4±55.5	249.3±49.9	225.0±56.0
		intravenous	361.4±58.8			362.1±47.6	335.6±47.5	272.2±37.2
Treatment safety	Potassium (mmol/L)	oral	5.60 ±0.15	0.48	0.699	5.29 ±0.15	4.90 ±0.15	5.10 ±0.17
		intravenous	5.64 ±0.15			5.24 ±0.18	5.09 ±0.12	5.00 ±0.16
	Urea (mmol/L)	oral	26.4±1.2	2,60	0.054	25.2±1.1	23.3±0.9	22.1±0.8
		intravenous	22.9±1.0			22.3±1.2	21.5±0.9	22.3±1.1
	Leukocytes (x 10e9/L)	oral	6.2±2.1	1.30	0.276	5.8±1.7	5.9±2.1	5.1±1.6
		intravenous	6.5±2.1			6.5±2.5	6.2±2.2	6.1±2.5
	Platelets (x 10e9/L)	oral	169.3±9.1	0.51	0.661	183.1±6.7	188.2±10.5	205.4±11.5
		intravenous	204.8±10.6			227.7±11.1	231.8±10.7	233.6±10.0
	Mean arterial pressure (mmHg)	oral	96.0±2.1	2.26	0.084	97.9±1.7	94.9±2.3	94.9±2.2
		intravenous	97.5±1.9			101.3±2.7	92.6±2.1	90.7±2.6

Parameters are expressed as means±SE; asterisks represent significance levels: *p<0.05, **p<0.005 of the difference between prior and subsequent checkpoint values over time

administered oral elemental iron, and 7/27 (25.9%) administered IV iron. Monitoring of the hematological status revealed proportionate response patterns to both iron regimens after the 3rd month, which were mirrored by the time-course changes in the hemoglobin levels (Table 2, Fig. 1).

Significant benefits of iron supplementation to the hematological status and rHuEpo efficacy in renal anemia management were observed during the first 3 months of the treatment (starting from the time of randomization), while the hemoglobin level was augmented relative to the iron supply. The concentration of hemoglobin increased with IV iron administration (p=0.026), while the serum iron and TSAT remained stable. During the same period, in the group undergoing the oral iron administration regimen, the hemoglobin concentration did not change, while the serum iron (p=0.018), TSAT (p=0.009) and storage iron ferritin declined considerably (Table 2, Fig. 1). During the first 3 months, the amount of rHuEpo was proportionately reduced in both iron administration groups (Table 1, Fig. 2). The validated early discrepancy in the hemoglobin response to iron supply can be explained by the presence of other factors that triggered and supported erythropoiesis.

Patients did not show serious adverse reactions to ferrous treatment and regularly used medications. Gender, diabetes, cardiac comorbidities (coronary disease and cardiac insufficiency) or underlying kidney disease did not elicit a response to rHuEpo. Regarding the well-known suppressor effect of antihypertensive ACE inhibitors on hematopoiesis and the lower hemoglobin level observed in our patients on ACE-inhibitor medication at 3rd month (97.2±13.2, and 106.2±12.2 g/l, respectively, p=0.077), subsequent analysis included subgroup of 34 patients on standard ACE-inhibitor treatment during the entire study period (with 17 patients each receiving iron either orally or IV) to deter-

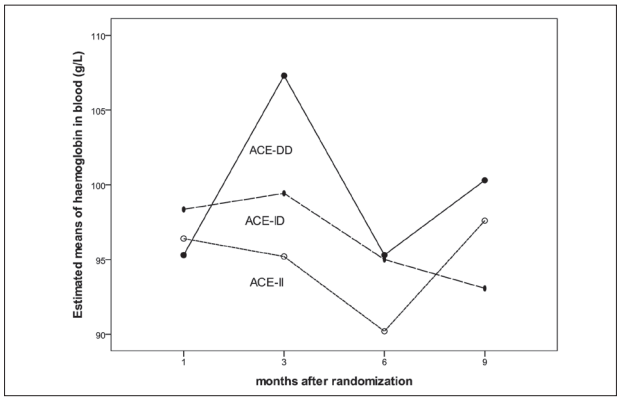

Fig. 1. Time-course changes of blood hemoglobin concentration with respect to the I/D-ACE genotype. The curves illustrate the estimated hemoglobin concentration at each time point relative to angiotensin-converting enzyme (ACE) I/D genotype carriers, by the general linear model for repeated measures analysis. Curve II – genotype carriers hemoglobin means±SE (g/L) at 1st month = 96.4±4.74; 3rd month=95.2±4.6; 6th month=90.2±4.8; 9th month= 97.6±5.0; Curve ID – genotype carriers – hemoglobin means±SE (g/L) 1st month = 98.4±4.0; 3rd month=99.4±3.9; 6th month=95. 1±4.8; 9th month= 93.1±4.2; Curve DD – genotype carriers – hemoglobin means±SE (g/L): 1st month = 95.3±4.7; 3rd month=107.3±4.6; 6th month=95.3±4.8; 9th month= 100.3±5.0.

mine the possible impact of ACE genotype on an early hemoglobin increase induced by rHuEpo stimulation. The remaining 19 patients (10 on IV iron and 9 on oral iron) out of the whole group included in the study received either temporarily ACE inhibitor or started with medication after the 3rd month (5 patients received ACEi between the 3rd and the 6th months, and 3 patients were supplied with ACEi medication after the 5th month of the study), as required to maintain the mean arterial pressure within a normal range, and 11 out of 53 patients with the mean arterial pressure below 105 mmHg did not receive antihypertensive drugs over the study period. By analyzing the possible impact of ACE genotype DD, DI or II on early hemoglobin response to rHuEpo, an association was observed between homozygous DD (deletion) of the ACE gene and a remarkable early increase in blood hemoglobin concentration (p=0.028), erythrocyte count (p=0.020) and hematocrit (p=0.043). Carriers of the ID or II genotypes maintained the hematological parameters irrespective of the mode of iron repletion ($F_{\text{iron x ACEgenotype}}$=0.003; p=0.960) (Table 3A, Figs. 1 and 3A,B). There was a significant reduction in the dose of rHuEpo in patients with the DD genotype in the 2nd month of the study ($F_{\text{1st vs. 2nd month}}$ = 3.95; p=0.029) (Table 3B, Fig. 2).

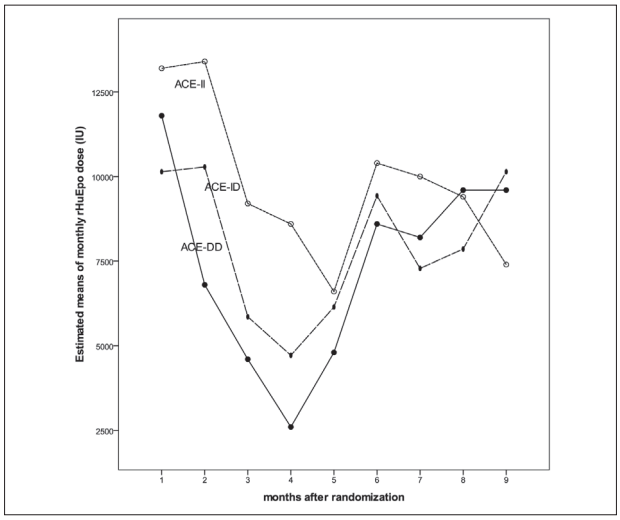

Fig. 2. Time-course of changes in monthly dosage of rHuEpo with respect to the I/D-ACE genotype. The curves show the estimated mean dose of recombinant human erythropoietin (rHuEpo) (UI) at each month relative to ACE I/D genotype carriers.

During the next three months, the supplementation with rHuEpo was significantly reduced (Fig. 2); the hemoglobin concentration was significantly decreased only in patients with the DD-ACE genotype. In these patients, the mean arterial systolic and diastolic pressure decreased. The patients with either ID or II genotype were not as receptive to rapid changes in rHuEpo dosage and their hemoglobin levels remained much more stable during the same time (Figs. 1 and 2).

DISCUSSION

Herein are presented the results of a randomized 9-month trial examining the hematological response to rHuEpo (beta) administration with respect to the route of iron supplementation (oral vs. intravenous), and I/D-ACE gene polymorphism. It was demonstrated that about one-fifth of the 53 hemodialysis patients enrolled attained the set blood hemoglobin concentration of 105 g/L with comparable success of both oral elemental iron and intravenous (IV) iron repletion. The elevation of iron transport parameters (serum iron and total iron binding capacity (TIBC)) was associated with a proportional increase in hematological parameters following the 3rd month of treatment. The significant increase in the first 3 months in hemoglobin level in patients on intravenous iron and in ACE-

Table 3A. ACE I/D genotype and hematological parameters.

Baseline levels			Time course of changes				
Parameters	**I/D-ACE genotype (n)**	**1st month**	**1→9th month**		**3rd month**	**6th month**	**9th month**
			F	**p**			
Hemoglobin (g/L)	II (10)	96.4±4.6	1.92	0.086	95.2±4.7	90.2±3.8	97.6±5.6
	ID (14)	98.4±4.0			99.4±4.3	95.0±4.0	93.1±3.9
	DD (10)	95.3±4.9			**107.3±3.5****	95.3±5.8	100.3±4.7
Erythrocyte (x 10e6/L)	II	3.2 ±0.2	2.13	0.057	3.0 ±0.1	3.0 ±0.2	3.3 ±0.2
	ID	3.2 ±0.1			3.1 ±0.1	3.0 ±0.1	3.0 ±0.1
	DD	3.1 ±0.2			**3.4 ±0.1***	3.1 ±0.2	3.3 ±0.2
Hematocrit (%)	II	29.2±2.3	1.75	0.118	26.8±1.2	26.1±1.2	29.2±1.9
	ID	28.9±41.5			27.6±1.8	26.5±1.4	27.2±1.4
	DD	28.1±1.4			**30.7±1.0***	27.3±1.9	29.9±1.5

Parameters are expressed as means ± SE; asterisks represent significance levels: *p<0.05, **p<0.005 of the difference between prior and subsequent checkpoint values over the study period

Table 3B. Monthly dosage of rHuEpo with respect to the I/D-ACE genotype.

Month	II	ID	DD
1	13200±2254	10143±1561	**11800±2723**
2	13400±2130	10286±1814	**6800±1665***
3	9200±1982	5857±1266	4600±1462
4	8600±2171	4714±1179	2600±1400
5	6600±1431	6143±1248	4800±1794
6	10400±1627	9429±1670	8600±2023
7	10000±1032	7286±1477	8200±1698
8	9400±1863	7857±1747	9600±2544
9	7400±2212	10143±2272	9600±2344

Monthly rHuEpo doses (IU) are expressed as means±SE; asterisk represents significance level p=0.029 of the difference between first and second month doses of rHuEpo (IU) in DD carriers

DD genotype carriers (p=0.026, and p=0.028, respectively) can be considered as two independent events. It seems that the DD carriers were much prone to an oscillation in hemoglobin levels with rapid changes in rHuEpo stimulation dosage than DI or DD carriers.

Several trials have previously reported the improved effect of oral administration on iron levels in dialysis patients [9,10]. In accordance with our results, forty-six patients treated with epoetin, and grouped randomly to receive different oral iron preparations, maintained the target hematocrit during a 6-month period [11], with the hemoglobin concentrations and iron indices maintained, rather than increased.

Impaired dietary iron absorption, in addition to impaired iron release from body storage and lack of regular daily intake of iron, are established limiting factors in oral iron supplementation [12]. The high adequacy of hemodialysis with kT/V (urea)>1.2, which is the standard measure of optimal hemodialysis in the last decade [6], enables satisfactory clearance of uremic toxins and substantially improved gastrointestinal tolerance to iron supplements. This was not the case in the past when an attempt to introduce oral iron in hemodialysis failed, and oral iron repletion was applied to CKD patients prior to chronic dialysis [6,13]. Although IV administration of iron provides rapid iron repletion and is superior to oral iron administration in many circumstances, long-term IV treatment, intermittently applied at high dosage, may contribute to increased mortality in the hemodialysis population, due to deleterious side effects and disruption of the physiological pattern of iron metabolism. Intravenous iron may cause oxidative stress and endothelial injury due to high single and cumulative ferrous doses. rHuEpo treatment *per se* induces many serious adverse events, such as elevated systolic blood pressure. Optimization of the rHuEpo and iron repletion dose is of crucial importance for renal anemia management and attenuation of the risk of chronic hemodialysis. ACE I/D genotyping provides important information about the inherent potency of the hematological response to rHuEpo. We determined that the DD genotype requires a lower amount of rHuEpo in the first months of application, although treatment with an ACE inhibitor can limit this effect.

There are no literature data evaluating the association between ACE genotypes and iron replacement therapy in the control of anemia by rHuEpo in patients on regular hemodialysis. It was recognized more than two decades ago that ACE gene polymorphism on chromosome 23(17q), characterized by the deletion/

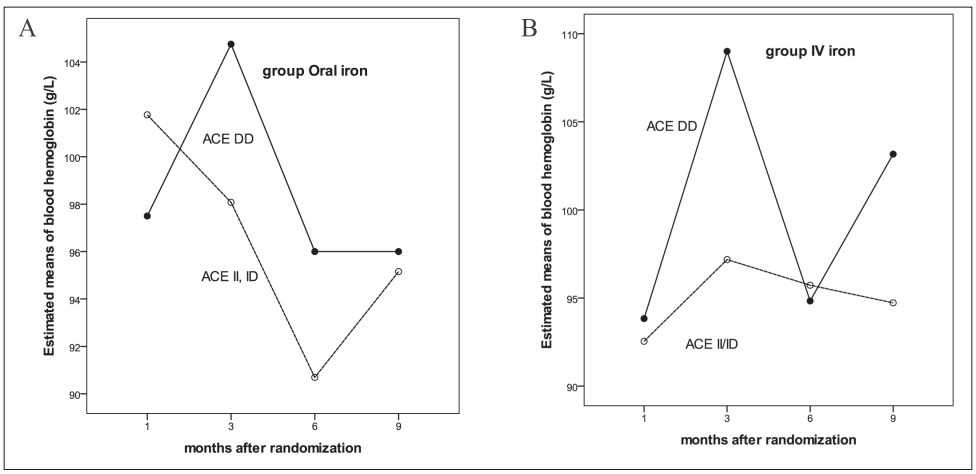

Fig. 3. Time-course of changes in blood hemoglobin concentration with respect to the I/D-ACE genotype iron supplementation. A. Changes after oral iron supplementation. The curves illustrate the estimated mean hemoglobin level at each time point relative to angiotensin-converting enzyme (ACE) I/D genotype carriers in patients on oral iron supplementation during rHuEpo stimulation, by the general linear model for repeated measures analysis over the study period. The curve II/ ID genotype carriers hemoglobin means±SE (g/L) at the 1st month = 101.8±4.5; the 3rd month=98.1±3.9; the 6th month=90.7±4.5; the 9th month= 95.1±5.1; The curve DD genotype carriers hemoglobin means±SE (g/L) at the 1st month = 97.5±9.7; the 3rd month=104.7±6.3; the 6th month=96.0±11.7; the 9th month= 96.0±6.4. B. Changes after intravenous iron supplementation. The curves illustrate the estimated mean hemoglobin level at each time point relative to angiotensin-converting enzyme (ACE) I/D genotype carriers in patients on intravenous iron supplementation during rHuEpo stimulation, by the general linear model for repeated measures analysis over the study period. The II/ ID carriers – hemoglobin mean±SE (g/L) at the 1st month = 92.6±3.3; the 3rd month=97.2±5.3; the 6th month=95.7±3.0; and the 9th month= 94.7±3.9. The DD carriers – hemoglobin means±SE (g/L) at the 1st month = 93.8±5.7; the 3rd month=109.0±4.4; the 6th month=94.8±6.7; the 9th month= 103.2±7.6.

insertion of 287-base pairs, could have an important impact on ACE activity. ACE gene polymorphism is a functional polymorphism that is linked to several other important polymorphisms in the ACE gene [14]. The ACE I/D genotype is now an accepted marker for renin-angiotensin system (RAS) activity. ACE and its novel homolog, angiotensin converting enzyme 2 (ACE2), are two key enzymes involved in the synthesis of bioactive components of the RAS [15]. Individuals with the DD genotype produce increased angiotensin II and present higher angiotensin II activity. Angiotensin II concentration may be around 1000 times higher in the kidney than in the peripheral blood outside the kidney in CKD. High RAS activity is a mechanism for the progression of CKD in the advanced stage, followed by low erythropoietin production and serious anemia [15,16]. Individuals from the general population with the DD genotype are at high risk of permanent loss of kidney function [17]; DD poses a risk of CKD progression in transplant kidneys, as well [18].

Increased activity of ACE and angiotensin II may have a beneficial effect on erythropoiesis, in a direct manner. The addition of angiotensin II to cultured kidney peritubular fibroblasts induces the production of endogenous epoetin, as has been reported in patients on hemodialysis [19]. Angiotensin II is an important additional stimulator of erythropoiesis, and as a growth factor it can assist in epoetin augmentation of the erythroid pool [4]. Two clinical trials have revealed an increased requirement for epoetin at the end stage of kidney failure in patients with II or ID genotypes on peritoneal dialysis [20,21]; one trial included hemodialysis patients [5]. We observed that the DD-ACE genotype is more receptive to rapid decrease of rHuEpo dose than individuals with ID and II genotypes, as a result of a very rapid decline in hemoglobin concentration. Consequently, a decrease in the dosage of epoetin in D allele carriers will subsequently require additional supplementation with rHuEpo and iron. Recently, in a cross-sectional study on a large group of patients, Kiss

at al. [22] reported that hemodialysis patients with DD genotype on ACE-inhibitor therapy had lower hemoglobin concentrations and a higher erythropoietin resistance index than patients with II genotype.

To conclude, although small numbers of patients were included in the randomized study of iron repletion and ACE-I/D genotype association with erythropoietin stimulation, an important impact of ACE-I/D genotype on hematological regulation was observed in patients on hemodialysis. Our investigation suggests that the genetic ACE typing can provide valuable information regarding the optimal dosage of rHuEpo and iron supplementation via oral or intravenous administration.

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