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Failure of Aldosterone Suppression Despite Angiotensin-Converting Enzyme (ACE) Inhibitor Administration in Chronic Heart Failure Is Associated With ACE DD Genotype

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OBJECTIVES The objective of this study was to assess whether the angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism influences the adequacy of the neurohormonal response to ACE inhibitors in patients with chronic heart failure (CHF).

BACKGROUND The renin-angiotensin-aldosterone system (RAAS) plays an important role in the pathophysiology of CHF, and aldosterone levels closely relate to outcome in patients with CHF. Angiotensin-converting enzyme inhibitors suppress the RAAS, but a significant proportion of patients exhibit elevated serum levels of aldosterone despite long-term administration of apparently adequate doses of these agents.

METHODS We prospectively studied 132 patients with CHF (ejection fraction <45%) receiving long-term therapy with ACE inhibitors for over six months. Patients taking aldosterone antagonists were excluded from the study. "Aldosterone escape" was defined as being present when plasma aldosterone levels were above the normal range in our laboratory (>42 nmol/L). Patients were then divided into two subgroups according to the presence (group 1) or absence (group 2) of aldosterone escape. Genotype analysis for the ACE I/D polymorphism was performed by polymerase chain reaction.

RESULTS The prevalence of aldosterone escape in our patients was 10% (13/132). The two groups of patients did not differ regarding the dose of ACE inhibitor, diuretics and their renal function. There was a statistically significant different distribution of genotypes between the two groups, with a higher proportion of DD genotype in group 1 compared with group 2 (62% vs. 24%, $p = 0.005$).

CONCLUSIONS Patients with CHF with aldosterone escape have a higher prevalence of DD genotype compared with patients with aldosterone within the normal limits. Angiotensin-converting enzyme gene polymorphism contributes to the modulation and adequacy of the neurohormonal response to long-term ACE-inhibitor administration in CHF. (J Am Coll Cardiol 2001;37:1808–12) © 2001 by the American College of Cardiology

Angiotensin-converting enzyme (ACE) inhibitors reduce both morbidity and mortality in patients with chronic heart failure (CHF) (1–3). Neurohormonal activation in CHF is closely related to the outcome of treated patients with heart failure (4), and the progression of the disease itself has been largely attributed to neurohormonal factors (5). It has been hypothesized, therefore, that the effects of ACE inhibitors on patient survival might be due more to hormone suppression than to hemodynamic improvement (6). Nevertheless, the renin-angiotensin-aldosterone system (RAAS) is not uniformly suppressed during therapy with ACE inhibitors. In fact, it has been reported that up to 20% of patients with CHF have elevated plasma levels of aldosterone despite long-term ACE-inhibitor administration (7–9). This "aldosterone escape" carries important clinical consequences because aldosterone levels are an important prognostic marker in patients with CHF. This observation is further supported by the Randomized Aldactone Evaluation Study (RALES), which showed that aldosterone antagonism with spironolactone strongly reduces mortality in patients with severe CHF (10). Nevertheless, the mechanisms underlying incomplete aldosterone suppression with ACE inhibitors have not yet been adequately investigated. An insertion/deletion polymorphism, consisting of a 287 base pair (bp) sequences in intron 16 of the ACE gene, has been reported to predict approximately half of the variance in serum ACE levels among individuals (11). Homozygotes for the deletion allele (DD) have higher serum (11) and tissue (12) ACE levels and activity compared with heterozygotes (ID) and homozygotes (II). Angiotensin-converting enzyme gene DD polymorphism is also known to be a risk factor for the development of dilated cardiomyopathy and ischemic heart

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Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
CHF = chronic heart failure
D = deletion
I = insertion
LVEF = left ventricular ejection fraction
PCR = polymerase chain reaction
RAAS = renin-angiotensin-aldosterone system

disease (13). However, a possible role of ACEs in determining the neurohormonal response to ACE inhibitors has never been investigated. We hypothesized that different ACE genotypes, because of their variable intrinsic enzymatic activities, might partially account for the different degree of aldosterone suppression observed during long-term ACE-inhibitor therapy. Therefore, we focused our investigation on whether the DD genotype might be associated preferentially with aldosterone escape in patients with CHF who were undergoing long-term ACE-inhibitor treatment.

METHODS

Patients. Between May 1999 and February 2000, we evaluated 175 consecutive patients with CHF as a result of idiopathic dilated cardiomyopathy, ischemic or valvular heart disease. All had been followed by our heart failure outpatient clinic. All patients had a left ventricular ejection fraction (LVEF) <45%, as assessed by echocardiography. Every patient had been in a stable clinical condition for at least three months before the study. Each patient was receiving standard therapy for CHF, including an ACE inhibitor at the highest tolerated dose for at least six months. None of the patients had renal failure (creatinine >150 μmol/L), liver or lung disease, malignant hypertension, inducible ischemia or recent (less than six months) myocardial infarction. Forty patients undergoing therapy with aldosterone antagonists and three with malignancies were excluded from further study. The final study group included 132 patients. Various ACE inhibitors were used. The dose of ACE inhibitor was, therefore, expressed as a percentage of the maximal recommended dosage (14). The dose of diuretics was expressed in equivalent frusemide dosage. The protocol of the study was in accordance with the ethical standards of the Helsinki Declaration and was approved by the Ethics Committee of the Ospedale Civile Maggiore of Verona. Every patient provided written informed consent before being enrolled in the study.

Hormonal measurements. Venous blood samples were drawn after a 30 min supine rest in a fasting state between 8 and 9 AM For plasma aldosterone measurement, 4 mL of blood were collected into an evacuated glass tube containing EDTA (50 μL, 4.9%) and ε-aminocaproic acid (3,000 KIE). After centrifugation, the plasma sample was frozen at −80°C until the analysis was performed (maximum eight months). The concentrations of aldosterone were measured by a sandwich radioimmunoassay (Biochem Immuno System, Verona, Italy) at the Laboratory of Clinical Chemistry of our hospital. "Aldosterone escape" was prospectively defined as the presence of aldosterone plasma concentrations above the upper limit of the reference range (>42 nmol/L). Patients were subsequently subdivided into two groups according to the presence (group 1) or absence (group 2) of aldosterone escape.

ACE genotyping. The polymerase chain reaction (PCR) method used to establish the ACE genotype was previously described (15). The procedure used to prepare DNA for PCR from whole blood was based on the one described by Walsh et al. (16) for forensic material. Putative DD genotypes were further confirmed by using the ACE 2 primer (17), which eliminates mistyping that can occur with a two-primer system (11). The PCR results were scored by two independent investigators unaware of patient identity.

Statistics. All continuous variables are reported as mean ± 1 SD. Comparisons between groups were made by the Student *t* test for unpaired data for continuous variables and by the chi-square test for categorical variables; for the latter, subgroup comparisons were performed by using the Brandt and Snedecor test. A p value <0.05 was considered statistically significant.

RESULTS

Clinical and demographic characteristics of the study patients are summarized in Table 1. The majority of patients were men (n = 113; 86%). The mean age was 62 ± 9 years (range 35 to 77), and the mean LVEF was 34% ± 9%. Most of the patients were in New York Heart Association functional class II (n = 59; 45%) or III (n = 42, 32%) and diagnosed with an ischemic etiology (n = 84; 64%); 46 (35%) had idiopathic dilated cardiomyopathy and two (1%) had primary valvular heart disease.

The frequency of aldosterone escape observed in 13 patients, according to our definition, was 10%. There were no statistically significant differences in the demographics, clinical and laboratory characteristics between the two groups (Table 1). In particular, the two groups had similar renal function, and both frequency and dosage of ACE inhibitors, diuretics and beta-adrenergic blocking agents were comparable.

The genotype was DD in 37 subjects, ID in 72 subjects and II in 23 subjects. The frequencies of D and I alleles were 55% and 45%, respectively. The observed frequencies of the DD, ID and II genotypes did not significantly differ from those predicted by the Hardy-Weinberg equilibrium (28% vs. 31%, 55% vs. 50% and 17% vs. 19%, respectively) and were similar to those of previous reports (18). In group 1, there was a higher frequency of DD genotype compared with group 2 (62% vs. 24%, p = 0.005). In group 1, no patient had II genotype compared with 23 patients (17%) in group 2. Details about genotype distribution in the two groups are given in Figure 1.

Table 1. Clinical Characteristics of 132 Patients With Chronic Heart Failure Divided According to the Presence or Absence of Aldosterone "Escape"

	All Patients (n = 132)	"Escape" (n = 13)	No "Escape" (n = 119)	p Value
Age (yr)	62 ± 9	63 ± 10	62 ± 9	NS
NYHA functional class				
I–II (%)	78 (59)	6 (46)	72 (61)	NS
III–IV (%)	54 (41)	7 (54)	47 (39)	NS
Men (%)	113 (86)	11 (85)	102 (86)	NS
Ischemic heart disease (%)	84 (64)	10 (77)	74 (62)	NS
% ACE inhibitor, maximal dose	50 (25–100)	50 (12.5–100)	50 (25–100)	NS
Beta-blockers (%)	91 (69)	12 (92)	79 (66)	NS
Diuretics (%)	113 (86)	12 (92)	101 (85)	NS
Frusemide equivalent dose (mg)	31 (25–50)	50 (25–100)	25 (25–50)	NS
S-creatinine (μmol/L)	101 ± 27	106 ± 27	100 ± 27	NS
S-potassium (mmol/L)	4.3 ± 0.3	4.3 ± 0.3	4.3 ± 0.4	NS
S-sodium (mmol/L)	140 ± 3	139 ± 3	140 ± 3	NS
LVEF (%)	34 ± 9	33 ± 8	34 ± 9	NS
P-aldosterone (nmol/L)	0.24 (0.19–0.31)	0.50 (0.43–0.60)	0.23 (0.18–0.28)	—

Values are reported as mean ± SD, median (interquartile range) or number (%).
ACE = angiotensin-converting enzyme; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

DISCUSSION

In this study we found that patients with aldosterone escape had a significantly higher prevalence of DD genotype compared with patients without escape. The frequency of DD genotype in patients with aldosterone escape was more than twofold compared with patients with normal aldosterone levels, and every patient with II genotype had adequate aldosterone suppression according to our definition. Our results, therefore, suggest a possible role of ACE gene polymorphism in the modulation of the RAAS after long-term ACE inhibition in patients with CHF.

Aldosterone escape in CHF. Elevated plasma aldosterone levels are associated with a higher mortality rate in patients with CHF as are elevated levels of catecholamines, renin and angiotensin II (1). The Cooperative North Scandinavian Enalapril Survival (CONSENSUS) trial showed that, in patients with severe CHF with above-median plasma aldosterone levels, the six-month mortality rate was significantly higher than it was in patients who had below-median levels (1). Furthermore, when levels of aldosterone were reduced, there was a concomitant reduction in the mortality rate (19). Therefore, it has been hypothesized that the well-established effects of ACE inhibitors on survival in patients with CHF are more due to the neurohormonal suppression than to the hemodynamic effects (5). One of the therapeutic targets during long-term ACE inhibition in patients with CHF should, therefore, be the aldosterone suppression. Nevertheless, it has been described that, in a considerable number of patients with CHF, aldosterone production still occurs despite long-term ACE inhibition (8,20). This evidence provided the background for the RALES study, which showed that the aldosterone antagonist spironolactone, when added to conventional therapy with ACE inhibitors, substantially reduced both morbidity and mortality in patients with severe CHF (10). Moreover, aldosterone has several harmful effects on heart and circulation: it promotes sodium and fluid retention and enhances urinary potassium and magnesium excretion, and it also stimulates myocardial fibrosis, contributes to vasoconstriction both indirectly and by modulating endothelial function; additionally, it contributes to baroreflex depression and potentiates the effects of cathecolamines (21). In general, therefore, because ACE inhibitors do not completely suppress aldosterone production, there are several reasons why this hormone is a potential problem in patients with CHF.

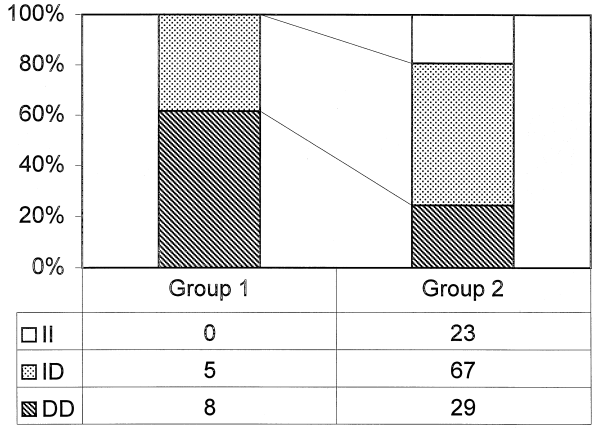

Figure 1. Genotype distribution of 132 chronic heart failure patients with and without aldosterone escape despite long-term treatment with angiotensin-converting enzyme inhibitors. Results are reported as percentages of total (graph) and absolute numbers (table). D = deletion; DD = deletion allele; I = insertion; ID = heterozygotes; II = homozygotes.

ACE gene polymorphism. Recent results suggests that genetic variants in the components of the RAAS may be involved in the pathophysiology of cardiovascular diseases. The observation that approximately 50% of the inter-individual variability of plasma ACE is attributable to a major gene polymorphism (15,22) led to the description of an I/D polymorphism of the ACE gene. This polymorphism consists of the presence or absence of a 287-bp DNA

fragment in intron 16 and is associated with serum (11) and cardiac tissue (23) ACE levels. The mean plasma ACE levels in DD subjects are about twice those of II subjects, with heterozygotes ID having intermediate levels (11). The DD genotype has also been reported to be a risk factor for myocardial infarction (24), to be associated with an increased mortality rate in dilated cardiomyopathy (25) and with the development of left ventricular hypertrophy in normal subjects (26). The ACE genotype has been reported as being a significant predictor of the ACE inhibitor's effect in normotensive subjects (27). In patients with CHF, a significant relation between the pressure response to captopril administration and ACE genotype has been shown, with the greater blood pressure decrease in the II patients (28). Nevertheless, a possible role of ACE gene polymorphism in determining RAAS modulation after ACE-inhibitor therapy in patients with CHF has never been assessed. In this syndrome, the RAAS is activated, and, therefore, the effects of ACE gene polymorphism on the system regulation might be amplified.

Relationship between ACE gene polymorphism and aldosterone escape. We found a significantly higher frequency of DD genotype in patients with aldosterone escape compared with those without escape (62% vs. 24%; $p = 0.005$). Furthermore, no II patient had aldosterone escape, showing that in this group of patients ACE-inhibitor response is adequate. On the other hand, 22% of patients with DD genotype had aldosterone escape. Therefore, we hypothesize that the latter group could particularly benefit from aldosterone antagonist or angiotensin II receptor antagonist therapy. Patients with and without aldosterone escape had otherwise similar clinical characteristics, including renal function, LVEF, dose of ACE inhibitors and diuretics, and the only difference was the distribution of ACE genotype. This observation supports a possible role of ACE gene polymorphism in determining serum aldosterone levels. Consequently, this polymorphism could influence the inhibitory effect of ACE inhibitors on aldosterone production beyond other well-known regulatory mechanisms.

Study limitations. We observed a 10% frequency of aldosterone escape, which is lower than those previously reported. There are two possible explanations for this apparent discrepancy: first, as a cut-off value to define escape we chose the upper limit of the normal range of our laboratory, while other authors used the mean aldosterone level of healthy volunteers (7) or other arbitrary methods (8). Many patients in these studies may have been defined as having aldosterone escape, despite aldosterone levels within normal limits. Conversely, we considered all patients with aldosterone levels below the upper limit of the normal range as not having escape. Secondly, we excluded patients who were being treated with aldosterone antagonists, and, therefore, the true prevalence of the escape phenomenon in our patients with CHF might be underestimated. However, we did not seek to describe the prevalence of escape but rather to identify a selected group of patients suitable for studying the effects of ACE genotype on aldosterone production.

Conclusions. In this study, we found that the frequency of aldosterone escape in patients with CHF receiving long-term ACE-inhibitor treatment is significantly higher in patients having DD genotype compared with those having ID and II genotype. The D/D patients who are already being treated might particularly benefit from drugs like aldosterone antagonists or angiotensin II receptor antagonists or the association of two drugs. Therefore, in this specific subgroup of patients, medical therapy has to be optimized and maybe guided by hormonal measurements. Prospective, randomized studies on larger populations are needed to assess the relative role of each group of drugs according to the ACE gene polymorphism. The challenge for the future might be the "right therapy for the right genotype."

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