Determinants of increased angiotensin II levels in severe chronic heart failure patients despite ACE inhibition

Ruud M.A. van de Wal
H.W.Thijs Plokker
Dirk J.A. Lok
Frans Boomsma
Frans A.L. van der Horst
Dirk J. van Veldhuisen
Wiek H.van Gilst
Adriaan A. Voors

Department of Cardiology, St. Antonius Hospital, Nieuwegein, The Netherlands
Department of Cardiology, Deventer Hospital, Deventer, The Netherlands
Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands
Department of Clinical Chemistry, St. Antonius Hospital, Nieuwegein, The Netherlands
Department of Cardiology, University Hospital Groningen, Groningen, The Netherlands
Department of Clinical Pharmacology, University of Groningen, Groningen, The Netherlands
Abstract

Introduction The beneficial effects of ACE inhibitors are generally ascribed to blockade of neurohormonal activation. However, especially in chronic heart failure patients plasma angiotensin II and aldosterone levels can be elevated despite ACE inhibition, the so-called ACE escape. In the present study, we aimed to identify the frequency and determinants of ACE escape in CHF patients.

Methods We studied 99 stable chronic heart failure patients (NYHA class III and IV, 66% ischemic etiology) receiving long-term therapy with ACE inhibitors. In all patients, cardiac, renal, and neurohormonal parameters were measured. ACE escape was defined as plasma angiotensin level ≥16 pmol/L.

Results Mean (± SD) left ventricular ejection fraction of our 99 patients (79 men and 20 women, age 69 ± 12 years) was 28 ± 10%. In addition to an ACE inhibitor, 93% of patients received diuretics, 71% a β-blocker, and 49% spironolactone. None of the patients used an angiotensin receptor blocker. In our population, 45% of the patients had an angiotensin II plasma concentration higher than 16 pmol/L (median concentration was 14.1 pmol/L). Spironolactone use was an independent predictor of elevated plasma angiotensin II levels. Furthermore, spironolactone users had significantly higher plasma active renin protein and aldosterone levels. Plasma angiotensin II concentration was positively correlated to active renin, plasma angiotensin I and plasma aldosterone. No correlation was found between plasma angiotensin II levels and serum ACE activity, dose of ACE inhibitor, or duration of use.

Conclusion In a group of severe chronic heart failure patients, 45% had elevated plasma angiotensin II levels independent of serum ACE activity despite long-term ACE inhibitor use. Although a causal link could not be proven, an association was found between spironolactone use and active renin protein, angiotensin II and aldosterone levels, suggesting that escape from ACE is mainly caused by a feedback mechanism.
Introduction

Inhibition of the renin angiotensin system with angiotensin-converting enzyme (ACE) inhibitors proved to be beneficial in patients with chronic heart failure (CHF).\(^1,2\) The clinically favorable outcome of CHF patients using an ACE inhibitor is mainly explained by reduction of angiotensin II formation. However, in patients on chronic ACE inhibition the angiotensin II and aldosterone levels will often rise again, even though plasma ACE levels remain suppressed and the antihypertensive effect does not disappear.\(^3-6\) Escape from ACE inhibition occurs particularly in patients with an activated renin angiotensin system (RAS). Activation of the RAS depends on several factors such as medication, salt intake, physical activity, posture and genetic preposition.\(^7-9\) In addition, activation of the RAS is observed in CHF patients and in these patients the activity is related to the severity of CHF. Plasma angiotensin II levels under ACE inhibition vary from less than 10 pg/ml in mild CHF patients to 70 pg/ml in patients with severe CHF.\(^10,11\) Angiotensin II has been viewed as a primary factor causing target organ damage in the cardiovascular system, and aldosterone exacerbates its tissue-damaging properties.\(^12-13\) Moreover, both elevated angiotensin II concentrations and elevated plasma aldosterone levels are associated with poorer prognosis.\(^14,15\)

However, predictors of ACE and aldosterone escape have not been well described. Therefore, the present study was designed to measure plasma angiotensin II levels and other neurohormones in heart failure patients on chronic ACE inhibitor therapy in a routine clinical setting, and to identify which factors are related to ACE escape.

Methods

Between February 2003 and November 2003, we evaluated 106 patients with congestive heart failure New York Heart Association (NYHA) functional class III or IV, as a result of idiopathic dilated cardiomyopathy, ischemic or valvular heart disease, who presented at the Heart Failure Clinic of St. Antonius Hospital (Nieuwegein), University Hospital Groningen (Groningen), and Deventer Hospital (Deventer). All had been followed by the outpatient clinic of one of the participating hospitals and all subjects were being treated with stable doses of ACE inhibitor for at least three months. Diagnosis had been made on the basis of medical history, ongoing symptoms and physical examination. All patients had a left ventricular ejection fraction (LVEF) <45%, as assessed by echocardiography or radionuclide measurement. Every patient had been in a stable clinical condition for at least three months before the study.
Patients who used an angiotensin II receptor blocker were excluded from this study. Various ACE inhibitors were used and the dose of each ACE inhibitor was expressed as a percentage of the maximum recommended dose (Table 1). The study was approved by each hospital’s ethics committee and written informed consent was obtained from all patients.

Hormonal measurements
Venous blood samples and urine samples were taken at the outpatient clinic while the patient was in an upright position. The blood and urine samples were transported to the local laboratory immediately, and each aliquot was processed and stored according to protocol for later batched analysis. The concentration of aldosterone was measured by a sandwich radioimmunoassay (Diagnostic Products Corporation, Breda, the Netherlands). Active renin protein was measured by an immunoradiometric assay (Nichols Institute Diagnostics, Middlesex, United Kingdom) and serum ACE activity was measured by an enzymatic assay (Bühlmann Laboratory AG, Schünenbuch, Switzerland). Analyses were performed in a routine setting according to the guidelines of the manufacturer. Angiotensin I and II were measured by specific radioimmunoassays after SepPak extraction of plasma as described previously. ACE escape was defined as an angiotensin II plasma concentration of ≥16 pmol/L (≥16.7 pg/ml), which is twice the upper limit of the reference value used in our laboratory, and comparable to the definition used by Roig et al. NT-probrain natriuretic peptide (NT-proBNP) was measured by an Elecsys NT-proBNP immunoassay (Roche Diagnostics, Mannheim, Germany).

<table>
<thead>
<tr>
<th></th>
<th>Angiotensin II &lt; 16 pmol/L (n = 54)</th>
<th>Angiotensin II ≥ 16 pmol/L (n = 45)</th>
<th>Significance (two-tailed) p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captopril (%)</td>
<td>27.8 (94 mg)</td>
<td>15.7 (59 mg)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Enalapril (%)</td>
<td>33.1 (18 mg)</td>
<td>37.8 (13 mg)</td>
<td>0.677*</td>
</tr>
<tr>
<td>Fosinopril (%)</td>
<td>1.9 (10 mg)</td>
<td>6.7 (23 mg)</td>
<td>0.327*</td>
</tr>
<tr>
<td>Lisinopril (%)</td>
<td>27.8 (13 mg)</td>
<td>20.0 (15 mg)</td>
<td>0.481*</td>
</tr>
<tr>
<td>Perinodpril (%)</td>
<td>3.7 (2 mg)</td>
<td>17.8 (3.7 mg)</td>
<td>0.040*</td>
</tr>
<tr>
<td>Quinapril (%)</td>
<td>5.6 (23 mg)</td>
<td>2.2 (30 mg)</td>
<td>0.624*</td>
</tr>
</tbody>
</table>

| Dose ACE-inhibitor (% of recommended daily dose) | 72 ± 37 | 68 ± 37 | 0.568* |
| Duration of ACE-i use (months) | 46 ± 28 | 36 ± 30 | 0.110* |

Table 1. ACE-inhibitor characteristics

* Independent samples t-test, * chi-square test

Abbreviations: MDD, median daily dose
Statistics
Values are expressed as mean values ± SD, and neurohormone levels or activity are expressed as median values (25th-75th percentile). Differences between groups were investigated by using the unpaired t-test for independent samples and the chi-square test, when appropriate. Stepwise multiple regression analysis was performed to identify the independent predictors of increased plasma angiotensin II levels. Neurohormonal data were log-transformed before statistical comparison in order to correct for skewness. Linear and logistic regression analysis were performed to identify relations between variables. A p-value <0.05 was considered statistically significant.

Results
Of the 106 patients in our study, seven patients were excluded because they either failed to give informed consent (n=2), because inadequate venous blood samples were collected (n=3), or because the LVEF was higher than 45% (n=2). Therefore, the study population consisted of 99 patients (age 68.5 ± 11.7 years), 65 of whom had ischemic heart disease, 14 had a dilated cardiomyopathy, and the remaining patients had CHF due to other causes. Ninety-four patients (95%) were in NYHA functional class III and 5 patients were in functional class IV. Mean daily dose of captopril, enalapril, and lisinopril were 82.9 mg, 15.2 mg, and 13.9 mg respectively (Table 1). Detailed patient characteristics are listed in Table 2. Despite chronic ACE inhibitor treatment, 45 patients (45%) had plasma angiotensin II levels ≥16 pmol/L (median 35.4 pmol/L), while levels were within normal range in the remaining 54 patients (median 7.7 pmol/L). The variables in Table 2 with a significance level of p<0.10 in the univariate tests were age, LVEF, spironolactone use, and systolic blood pressure. These values were included into a multivariate model. Multivariate analysis identified only spironolactone use and dose to be related to elevated plasma angiotensin II levels (p= 0.020). Patients on spironolactone had significantly higher levels of angiotensin II (40.5 vs. 16.5 pmol/L, p<0.001, Figure 1) and of aldosterone (755.9 vs. 491.2 pmol/L, p=0.005, Figure 1) compared to patients not using spironolactone. Of the patients on spironolactone 60% had elevated angiotensin II levels (versus 31% in non-users, p=0.005), while 54% had aldosterone >650 pmol/L (versus 17% in non-users, p<0.001). Potassium levels were comparable in both spironolactone users and non-users (4.4 ± 0.4 mmol/L versus 4.5 ± 0.5 mmol/L). Median plasma levels of neurohormones are listed in Table 3. Correlations were found between plasma angiotensin II levels and active renin protein (r=0.574, p<0.001), plasma angiotensin I level (r=0.445, p<0.001), and plasma aldosterone level (r=0.337, p=0.016). No significant
correlation was found with serum ACE activity ($r=-0.036$, $p = 0.755$, Table 4). Furthermore, no significant correlation was found between plasma angiotensin II levels and plasma NT-proBNP levels, or duration of ACE inhibitor use, while a weak association was found with LVEF ($r=-0.243$, $p=0.019$). Subgroup analyses demonstrated that the 22 patients receiving captopril had higher serum ACE activity than the patients using another ACE inhibitor (68.2 U/L vs. 18.7 U/L, $p<0.001$). However, the frequency of ACE escape tended to be lower in these patients (32% versus 49%, $p=0.145$).
Discussion

In the present study, we evaluated 99 patients with severe CHF who used an ACE inhibitor for at least three months. We found that 45 patients (45%) had elevated angiotensin II levels despite the long-term use of an ACE inhibitor. No clinical characteristic was found to be related to ACE escape. The use of a non-selective aldosterone antagonist was the only predictor of elevated angiotensin II levels. Moreover, elevated plasma angiotensin II levels were related to increased active renin protein and plasma angiotensin I levels. A previous study suggested that betablockers reduce the activity of the renin angiotensin system.\textsuperscript{18} Our data support this suggestion (Figure 2).

<table>
<thead>
<tr>
<th></th>
<th>Angiotensin II &lt; 16 pmol/L</th>
<th>Angiotensin II ≥ 16 pmol/L</th>
<th>p-value (two-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II (pmol/L)</td>
<td>7.7 (4.3-11.2)</td>
<td>35.4 (2.5-61.5)</td>
<td>&lt;0.001\textsuperscript{a}</td>
</tr>
<tr>
<td>Angiotensin I (pmol/L)</td>
<td>618 (264-1377)</td>
<td>2358 (881-5137)</td>
<td>&lt;0.001\textsuperscript{a}</td>
</tr>
<tr>
<td>Active renin (µU/mL)</td>
<td>138 (53-299)</td>
<td>770 (260-2400)</td>
<td>&lt;0.001\textsuperscript{a}</td>
</tr>
<tr>
<td>ACE activity (U/L)</td>
<td>15.8 (12.6-22.0)</td>
<td>16.5 (11.6-20.9)</td>
<td>0.627\textsuperscript{v}</td>
</tr>
<tr>
<td>Aldosterone (pmol/L)</td>
<td>385 (200-585)</td>
<td>690 (390-1135)</td>
<td>0.001\textsuperscript{v}</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>111.4 (50.0-236.9)</td>
<td>128.0 (50.5-281.9)</td>
<td>0.624\textsuperscript{v}</td>
</tr>
</tbody>
</table>

Table 3. Median neurohormonal levels (25th-75th percentile)

\textsuperscript{a} independent samples t-test, \textsuperscript{v} captopril users excluded

It is firmly established that ACE inhibitor use does not completely block angiotensin II and aldosterone production, and in some patients, angiotensin II and aldosterone levels remain high.\textsuperscript{3;10,14,19} This might be the result of insufficient suppression of ACE due to an inadequate dose of ACE inhibitor. The most obvious explanation for this ACE escape is the existence of alternative enzymes for the formation of angiotensin II.\textsuperscript{20} Non-ACE mediated pathways (e.g. tissue chymase mediated angiotensin II formation) may become activated when the activity of ACE is reduced by an ACE inhibitor.

Remarkably, in our study ACE escape was not affected by either dose or duration of ACE inhibitor use, or with serum ACE activity. In a previous study it was suggested that during storage captopril dissociates from ACE, which might lead to an overestimation of serum ACE activity, whereas other studies suggested that captopril is not able to suppress the activity of ACE as profoundly as other ACE inhibitors.\textsuperscript{21,22} Therefore, in the present study captopril users were excluded from analyses involving serum ACE activity. Active renin protein and angiotensin I levels were elevated in the patients with elevated angiotensin II levels. It has been well described that renin and angiotensin I accumulate
during short-term and long-term ACE inhibition.\textsuperscript{23-25} Since all our patients had been on a steady dose of ACE inhibitor for at least three months, this does not account for the different hormone levels we found in our population. Particularly in patients that used spironolactone, plasma angiotensin II and aldosterone levels were elevated. In addition, these patients had higher plasma levels of active renin protein and angiotensin I. These results indicate that blockade of the aldosterone receptor on top of ACE inhibition in CHF patients induces further activation of the renin angiotensin system, likely through intensification of the positive renin feedback mechanism (Figure 3). Due to activation of renin and increased availability of angiotensin I, more angiotensin II and aldosterone will be formed. The activity of ACE seems to be of minor importance in this process.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.pdf}
\caption{Plasma concentrations of active renin protein, angiotensin II, and aldosterone in patients using spironolactone versus patients not using spironolactone}
\end{figure}

Our data are consistent with the results of Rousseau et al. who performed a neurohormonal substudy in the RALES population.\textsuperscript{26,27} Fifty-four patients on spironolactone had significantly higher plasma angiotensin II levels and plasma aldosterone levels after three and six months of treatment, than 53 patients who were treated with placebo. In their study 97.2\% of the patients received an ACE inhibitor. After 48 months of follow-up cardiac mortality in the spironolactone group was significantly lower (21\% vs. 38\%, \(p=0.05\)) than in the placebo group. They suggested that the mechanism underlying the Angiotensin II and aldosterone escape reflected activated feedback mechanisms on the renin angiotensin system.\textsuperscript{27} The results of this RALES substudy are in contrast
with the conclusion drawn by Roig et al. They identified a group of patients with increased levels of angiotensin II within a selected group of patients using ACE inhibitors, and compared this group to the patients with normal levels of angiotensin II. Both groups had similar left ventricular ejection fractions and no patients used spironolactone. After 3 years, death or new heart failure episodes occurred in 43% of the patients with increased angiotensin II levels at baseline, compared with 13% of the patients with normal angiotensin II levels (p=0.002). Therefore, Roig et al. stated that beside a marker for the severity of heart failure, an increased plasma angiotensin II level under ACE inhibition is an independent predictor for increased mortality and morbidity in heart failure patients as well. Patients using spironolactone appear to be an exception to this statement.

Recently, beneficial effects were described of angiotensin receptor blockers in combination with ACE inhibitors in CHF patients in the CHARM-trial. Although in CHARM no subgroup analysis was performed, we hypothesize that especially CHF patients with elevated plasma angiotensin II and plasma aldosterone levels despite ACE inhibitor use might benefit from additional treatment aimed at receptor blockade within the RAS. Both angiotensin receptor blockers and aldosterone receptor blockers are candidates for neutralizing the detrimental effects of angiotensin II and aldosterone.
Limitations

This was an observational, hypothesis-generating study in an out-patient clinical setting. Consequently, analysis revealed a wide variety of neurohormonal concentrations. This might be due to different levels of activation of the RAS under the circumstances we used. However, we believe that for revealing mechanisms within the RAS in a population-based study, it is a valuable tool. Secondly, the cut-off values for plasma angiotensin II concentrations were partly arbitrary. The cut-off value of 16 pmol/L is comparable to the value used by Roig et al. Approximately half of their patients escaped from ACE inhibitor therapy and had elevated plasma angiotensin II levels, which is comparable to the percentage (45%) we found. Finally, we did find different daily doses of individual ACE inhibitors in both groups (Table 1). However, when all ACE inhibitors were grouped the mean dose (expressed as percentage of the recommended dose) was not statistically different. Consequently, we cannot draw conclusions on the effect of individual ACE inhibitors on RAS activity.

<table>
<thead>
<tr>
<th>Parameter A</th>
<th>Parameter B</th>
<th>Pearson r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose</td>
<td>Log (ACE-activity)*</td>
<td>0.112</td>
<td>0.277</td>
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<tr>
<td>Log (angiotensin II)</td>
<td>Dose</td>
<td>0.027</td>
<td>0.794</td>
</tr>
<tr>
<td>Log (ACE-activity)*</td>
<td>Log (angiotensin II)</td>
<td>-0.036</td>
<td>0.755</td>
</tr>
</tbody>
</table>

Table 4. Correlation between ACE inhibitor dose, ACE activity and angiotensin II levels
* captopril users excluded

Figure 3. Hypothesis: A: negative renin feedback of angiotensin II in untreated patients. B: positive renin feedback (or lack of negative feedback?) when only ACE is blocked, resulting from lower angiotensin II levels; C: intensified renin feedback when both ACE and aldosterone receptors are blocked.
Conclusion

In a group of severe chronic heart failure patients 45% had elevated plasma angiotensin II levels despite long-term ACE inhibitor use. Elevated levels were associated with the use of spironolactone, and with increased levels of active renin protein and angiotensin I. No association was found between angiotensin II levels and serum ACE activity, or dose or duration of ACE inhibitor use. Therefore, we believe ACE escape is related to a feedback mechanism, leading to increased levels of active renin protein and angiotensin I. This mechanism is intensified when patients use both an ACE inhibitor and an aldosterone receptor antagonist.
References


10. MacFadyen RJ, Lee AF, Morton JJ, Pringle SD, Struthers AD. How often are angiotensin II and aldosterone concentrations raised during chronic ACE inhibitor treatment in cardiac failure? Heart 1999;82:57-61.


