Plasma Angiotensin-Converting Enzyme Activity and Carotid Wall Thickening

Claire Bonithon-Kopp, MD; Pierre Ducimetière, PhD; Pierre-Jean Touboul, MD; Jean-Marc Fève, MD; Eliane Billault, PhD; Dominique Courbon; Véronique Héraud, MD

Background. Mechanisms underlying the previously reported association between a deletion polymorphism in the gene encoding for angiotensin-converting enzyme (ACE) and the risk of myocardial infarction in low-risk subjects are unclear. The purpose of this case-control study was to examine the relation of plasma ACE activity to intimal-medial thickness of the carotid wall measured ultrasonographically in an apparently healthy population.

Methods and Results. We determined plasma ACE activity in 80 pairs of subjects without any history of ischemic heart disease or any treatment of hypertension and diabetes. Cases and control subjects were defined on the basis of intimal-medial thickness measured in the common carotid arteries by B-mode ultrasound and were matched for sex, sonograph, and the presence of atheromatous plaques. Subjects were selected from a sample of 434 men and 602 women between 60 and 69 years old participating in an ongoing study on vascular aging (EVA). Subjects with intimal-medial thickening (cases) showed a slight but not significant increase in plasma ACE activity in comparison with control subjects (P<.16). However, after exclusion of subjects receiving lipid-lowering drugs, the mean plasma ACE activity became significantly higher in cases than in control subjects (29.9±7.7 U/L versus 27.5±8.0 U/L; n=54 pairs, P<.03). The mean case-control difference in plasma ACE activity was further increased when analysis was restricted to pairs without carotid atheromatous plaques (n=42 pairs). After adjustment for body mass index, smoking, and systolic blood pressure, the odds ratio for having carotid wall thickening based on 1 SD difference in log ACE was 2.29 (95% confidence interval, 1.16 to 4.52; P<.02).

Conclusions. The results of the study suggest that chronic exposure to high levels of plasma ACE could be involved in structural changes of the arterial wall. (Circulation. 1994;89:952-954.)

Key Words: • angiotensin-converting enzyme • ultrasound • carotid arteries

In a recent report from a large European case-control study on genetic factors of myocardial infarction (ECTIM), Cambien et al showed that a deletion polymorphism in the gene encoding for angiotensin-converting enzyme (ACE) was related to the risk of myocardial infarction. This association was particularly strong in subjects otherwise considered to be at low risk of coronary heart disease. Whether acute or chronic pathophysiological mechanisms underlie the association between the polymorphism ACE-ID and the risk of coronary events is still unclear. The polymorphism ACE-ID strongly determines the levels of circulating enzyme, which, in turn, are little influenced by hormonal and environmental parameters. ACE may play an important role in vascular homeostasis by promoting conversion of angiotensin I into angiotensin II and by inactivating bradykinin. According to experimental studies, these two peptide hormones have opposite effects on vascular tone, growth of vascular smooth muscle cells, and production of extracellular matrix proteins, suggesting that chronic exposure to high levels of plasma ACE might result in vascular wall thickening.

The opportunity to test the hypothesis that increased plasma ACE activity is associated with common carotid wall thickening was offered to us in an ongoing population-based ultrasonographic study on vascular aging. We considered that a case-control design was the most straightforward way to verify this hypothesis.

Methods. The EVA study is a longitudinal study on cognitive and vascular aging performed in volunteers aged 60 to 69 years recruited from the electoral rolls of the city of Nantes (western France). The study protocol was approved by the Comité d’éthique du Centre Hospitalier Universitaire de Kremlin-Bicêtre, and written informed consent was obtained from all participants. Between June 1991 and January 1993, 1036 subjects (434 men and 602 women) were examined. Information about demographic background, occupation, personal and family history, drug use, and cigarette smoking was obtained by a standardized questionnaire. Only subjects (n=717) who did not report any history of myocardial infarction and angina pectoris or any treatment for hypertension or diabetes constituted the population from which cases and control subjects were selected. However, subjects receiving lipid-lowering drugs (18.7% of this population) were not excluded.

High-resolution B-mode ultrasound examination of the carotid arteries was performed by four sonographers with a 7.5-MHz transducer (SSD-650, Aloka). Acquisition, processing, and storage of B-mode images were computer assisted with specially designed software. The protocol involved scanning of the common carotid arteries, the carotid bifurcations, and the origin (first 2 cm) of the internal carotid arteries. On a longitudinal B-mode image of the common carotid artery, the far wall is displayed as two parallel bright lines separated by a hypoechoic space. The first line on the far wall

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arises from the lumen-intima interface, whereas the second line arises from the media-adventitia interface. Thus, the distance between the media-adventitia interface and the lumen-intima interface represents the intimal-medial thickness (IMT).13 When an optimal longitudinal image of the common carotid artery was obtained, it was frozen and stored on an optical disk. Measurement of IMT was taken automatically from significant changes in density on a section perpendicular to the vessel wall from the lumen toward subadventitial structures. At the time of scanning, two measurements of IMT were completed on the far wall of both the right and left common carotid arteries on an arterial segment free of any atheromatous plaques. A localized echo structure encroaching into the vessel lumen was considered to be a plaque if the distance between the media-adventitia interface and the internal side of the lesion was ≥ 1 mm. The mean IMT based on four measurements was 0.67 mm, with a standard deviation of 0.13 mm in the population under study (n=717). Repeated measurements of IMT were performed by a second reader in a random subsample of 64 longitudinal images of the common carotid arteries. The mean absolute difference in IMT between the first and the second reading was 0.06 mm (SD: 0.05 mm), and the Spearman's correlation coefficient between them was 0.82.

Each subject with a mean IMT > 0.80 mm was defined as a case (n=86). This cut-point was chosen because it corresponds to 1 SD above the mean of the population. Control subjects were selected from participants with a mean IMT < 0.80 mm who met the matching criteria of sex, B-mode sonographer, and number of atheromatous plaques (none, one, two, or more). When several potential control subjects for one case were available, the closest in age was chosen. Because of insufficient blood collection, plasma samples were not available in two cases who were excluded. Moreover, no adequate control subjects could be found for four cases who had atheromatous plaques. Thus, 80 case-control pairs (46 male and 34 female pairs) were formed.

Plasma samples were drawn between 8 and 9 AM and stored at −80°C until assay. Plasma ACE activity was determined spectrophotometrically by quantifying the hydrolysis of the synthetic substrate p-benzoyl-glycyl-L-histidyl-L-leucine according to a modification of the method of Cushman and Cheung.14,15

The significance of any differences in means or proportions between the case and control groups was tested with Wilcoxon's matched-pairs signed-rank test or the McNemar test, respectively. Adjustment for potential confounders then was made with a multiple conditional logistic regression and, because of the skewed distribution of the plasma ACE activity, a logarithmic transformation was done.

### Results

Among the 80 case-control pairs, the mean IMT (standard deviation) was 0.94 mm (0.12) and 0.58 mm (0.08) in cases and control subjects, respectively. Although cases had slightly higher plasma ACE activity than control subjects, the mean difference between them did not reach the significance level (1.3±10.6 U/L, P<.16). We further examined whether the association between plasma ACE activity and increased IMT was more pronounced in subjects not receiving lipid-lowering drugs (exclusion of 12 cases and 17 control subjects corresponding to 26 pairs). The Figure shows the distribution of plasma ACE activity among the 54 remaining pairs. There was an excess of cases among subjects with intermediate plasma ACE levels (between 28 and 40 U/L), an excess of control subjects among those with low plasma ACE levels, and a roughly equal frequency of cases and control subjects among those with high plasma ACE levels. As shown in Table 1, the mean plasma ACE activity was significantly higher in cases than in control subjects (P<.03). Among pairs without carotid atheromatous plaques (n=42), the mean case-control difference in plasma ACE activity was further increased and became highly significant (P<.005). Conversely, among pairs with plaques (n=12), cases tended to show lower plasma ACE activity than did control subjects. Age was comparable in both groups,

### Table 1. Plasma ACE Activity and Risk Factors in Subjects With Carotid Wall Thickening and Matched Control Subjects*

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Control Subjects</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal-medial thickness, mm</td>
<td>0.93±0.09†</td>
<td>0.56±0.08</td>
<td>[blanks]</td>
</tr>
<tr>
<td>Plasma ACE activity, U/L</td>
<td>All subjects</td>
<td>29.9±7.7</td>
<td>27.5±8.0</td>
</tr>
<tr>
<td></td>
<td>Pairs without plaques (n=42)</td>
<td>30.6±7.5</td>
<td>26.9±8.6</td>
</tr>
<tr>
<td></td>
<td>Pairs with plaques (n=12)</td>
<td>27.2±7.8</td>
<td>29.4±5.4</td>
</tr>
<tr>
<td>Age, y</td>
<td>65.2±2.7</td>
<td>65.2±3.1</td>
<td>.96</td>
</tr>
<tr>
<td>Ever-smokers, %</td>
<td>59.4</td>
<td>37.0</td>
<td>.01</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.4±3.1</td>
<td>24.2±2.7</td>
<td>.03</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>134.1±15.3</td>
<td>127.6±16.4</td>
<td>.04</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80.5±12.5</td>
<td>78.8±10.9</td>
<td>.47</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme. Values are mean±SD unless otherwise stated.

*Subjects receiving lipid-lowering drugs were excluded.
†Wilcoxon's matched-pairs signed-rank test or χ² test.
Table 2. Multivariate Relations of Plasma ACE and Risk Factors to Carotid Wall Thickening*

<table>
<thead>
<tr>
<th></th>
<th>b (SE)</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log ACE, U/L</td>
<td>2.766</td>
<td>2.29 (1.16-4.52)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>0.011 (0.018)</td>
<td>1.20 (0.66-2.18)</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.241 (0.104)</td>
<td>1.92 (1.10-3.33)</td>
</tr>
<tr>
<td>Smoking (0-1)</td>
<td>2.169 (0.898)</td>
<td>8.75 (1.50-50.90)</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin-converting enzyme; SE, standard error; OR, odds ratio; and CI, confidence interval.

*Subjects receiving lipid-lowering drugs were excluded.
†Conditional logistic regression coefficient (SE).
‡OR associated with 1 SD of the explanatory variable (95% CI).

...whereas smoking (ever-smokers versus never-smokers), body mass index (weight in kilograms divided by height in meters squared), and systolic blood pressure were significantly associated with increased IMT. As shown in Table 2, plasma ACE activity remained significantly related to increased IMT (P<.02) after adjustment for these three variables. Similar results were obtained when the analysis was restricted to pairs without atheromatous plaques.

Discussion

To our knowledge, the present study is the first to relate plasma ACE activity to intimal-medial thickening of the common carotid arteries. The significance of a diffuse intimal-medial thickening is still debated. This is partly due to the inability of B-mode ultrasonography to differentiate the intima from the media layer. Diffuse intimal-medial thickening may represent an early phase of the atherosclerotic process, since it has been related to enhanced levels of cardiovascular risk factors and to an increased risk of myocardial infarction. It also may be viewed as an adaptive nonatherosclerotic response to mechanical stress or may reflect age-related changes in the matrix components of the arterial wall. The finding of an independent association between increased plasma ACE activity and a thickened carotid wall in subjects without treated hyperlipidemia suggests that intimal-medial thickening may develop in the absence of classic risk factors of atherosclerosis. This association was due to a high proportion of cases with intermediate values of plasma ACE activity in comparison with control subjects rather than to an excess of cases with high levels of plasma ACE. Thus, heterozygotes for the polymorphism ACE/ID probably constitute the major part of the case group. Selection biases may be responsible for this feature. In accordance with the findings from the ECTIM study, it could be hypothesized that cases having the DD genotype and risk factors for atherosclerosis are underrepresented in our relatively aged population either because they developed clinical manifestations of cardiovascular disease or because they died prematurely. The lack of association between plasma ACE activity and carotid wall thickening in subjects with carotid atheromatous plaques supports such an explanation.

Conclusions

This study suggests that chronic exposure to high levels of plasma ACE may be involved in structural changes of the arterial wall independent of classic risk factors for atherosclerosis. Further investigations are required to assess the clinical relevance of this finding.

Acknowledgments

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