ACE Inhibitors Increase Type III Collagen Synthesis: A Potential Explanation for Reduction in Acute Vascular Events by ACE Inhibitors


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Introduction. Large trials have shown that angiotensin converting enzyme inhibitor (ACE-I) therapy reduces the risk of myocardial infarction and stroke. Acute vascular events are thought to be initiated by plaque rupture. Animal models of atherosclerosis show an increase in extra cellular matrix when given ACE-I therapy. ACE-I therapy could influence collagen synthesis, one of the major constituents of the atherosclerotic cap.

Methods. A nested case-control study was performed within the Huntingdon Aneurysm Screening Project. Subjects were assessed for arterial disease, drug history and smoking. Blood samples were taken for a measure of collagen synthesis, the amino-terminal propeptide of type III procollagen (PIIINP), lipid levels, iron metabolism and cotinine levels.

Results. Information was available for 420 subjects. Thirty-five were taking ACE-I therapy and 385 were not. Mean serum PIIINP level was 3.5 μg/l (sd 1.3 μg/l, range: 1.7–16.5 μg/l. There was a marked increase in mean collagen turnover between subjects taking ACE-I therapy compared to those not. Mean PIIINP level for cases and controls was 4.26 μg/l (95% CI: 3.73–4.79 μg/l) versus 3.61 μg/l (95% CI: 3.48–3.75 μg/l). No differences were found for patients taking other antihypertensive drugs. After adjusting for age, weight, height, lipid levels and ferritin, PIIINP levels remained significantly higher in cases than controls: 4.14 μg/l (95% CI: 3.72–4.57 μg/l) versus 3.62 μg/l (95% CI: 3.49–3.75 μg/l) (P-value 0.02).

Discussion. These results suggest that ACE-I therapy up-regulates collagen synthesis, and could improve plaque stabilisation. This may provide an explanation for the decrease in acute vascular events observed in patients on ACE-I therapy.

Key Words: Angiotensin; Collagen; Atherosclerosis.

Introduction

Several large trials have shown that treatment with ACE inhibitors significantly reduces the risk of myocardial infarction and stroke. This effect was demonstrated to be independent of reduction in blood pressure and concomitant medication. It is thought that most acute vascular events begin with rupture of an atherosclerotic plaque. This suggests that ACE inhibitors lower the risk of atherosclerotic plaque rupture.

Unstable plaques, at higher risk of rupture, tend to have a thin, friable fibrous cap. The cap consists of extra cellular matrix, the chief component of which is collagen. Type I and III are the major structural components that confer tensile strength to the fibrous cap. Type III is also the major component of large arteries, muscle and skin. Collagen homeostasis is determined by the opposing metabolic processes of collagen synthesis and collagen degradation. A large amount of research has implicated increased matrix metalloproteinase (MMP) activity as an important factor in increased collagen degradation. However, it is important to consider increased collagen synthesis as well. This study was performed to investigate an effect of ACE inhibitors on collagen type III synthesis.

Methods

A screening programme for asymptomatic abdominal aortic aneurysms in males over the age of 50 started in Huntingdon, England in November 1991. The cohort of screened men over the age of 50 living in the


Huntingdon District served as a basis for this study. All subjects underwent informed consent, and ethical approval was gained locally. All subjects were interviewed about their family history, previous medical history, drug history, and smoking habits. All subjects had a brief medical examination by the same observer. The medical examination included measurement of the ankle brachial pressure index (ABPI) and the aortic diameter by ultrasound. Peripheral arterial occlusive disease (PAOD) was deemed present if the ABPI was less than 0.9. An aneurysm was defined as an infrarenal aortic diameter of 3 cm or more.

A blood sample was taken from all patients for measurement of serum collagen turnover, lipid levels, markers of iron metabolism and cotinine levels. Smoking and cholesterol are standard risk factors for atherosclerotic disease and acute vascular events. Therefore measurements of serum cotinine levels and lipid levels allowed us to adjust for possible differences in the distribution of those risk factors between the groups exposed to ACE inhibitors and those not exposed. Markers of iron metabolism were measured as iron is an important cofactor in collagen metabolism. These markers consisted of serum iron, transferrin, ferritin and antichymotrypsine levels.

Blood samples were taken at the time of the medical examination. They were spun down and the serum frozen at \(-21^\circ C\) on the same day for analysis later. Batches of 74 serum samples were analysed in duplicate on the same day, by the same biochemist from the Department of Chemical Pathology at Hinchingbrooke Hospital. The concentration of the amino-terminal propeptide of type III procollagen (PIIINP) was used as a proxy measure of type III collagen turnover. The amino-terminal propeptide is split from the procollagen molecule during type III collagen turnover. The amino-terminal propeptide of type III procollagen. This was based on highly purified specific human antigen ( Pharmacia & Upjohn Ltd, Diagnostics Division, Milton Keynes, UK) and performed according to the instructions of the manufacturer with the use of duplicate 200 \(\mu l\) aliquots of serum. The assay detects the authentic amino-terminal propeptide of type III collagen, but is not sensitive to the smaller degradation products of the propeptide. The antigens are resistant to repeated thawing and freezing. The benefit of this assay is that standard and human serum samples give parallel inhibition curves allowing more accurate reading of serum levels without the need for repeated dilution and analysis. The intra-assay and inter-assay coefficients of variation of this assay are less than 5% and the sensitivity is 0.2 \(\mu g/l\). The reference range for adults, based on data of Finnish blood donors was 1.7–4.2 \(\mu g/l\), with no differences between males and females. The lipid profile consisting of serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides was determined using an automated procedure on a random blood sample. Blood samples were taken during clinic attendance. Screening clinics were in the evening at 5 pm or on Saturday morning. No instructions about food intake were given prior to the medical examination. Lipid profiles were measured within a few days in the Department of Chemical Pathology at Hinchingbrooke Hospital, as part of their standard routine lipid assays. Total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured using standard enzymatic methods in a fully automated procedure using a Roche Hitachi 717 analyser with Bio Stat reagents. Serum cotinine levels were measured using a standard semi-quantitative microplate enzyme immunoassay (Cozart Biosciences, Abingdon, UK).

### Statistical Analysis

Mean values were compared using a \(t\)-test. Multivariate analysis was performed using linear regression methods with STATA 5.0 for Macintosh. Adjusted mean PIIINP levels were calculated using logistic regression models. Adjusted means were compared using Fisher’s \(F\) test.

### Results

Assessment of collagen turnover in the form of PIIINP levels was available for 456 subjects. Detailed information about medication and PIIINP levels were available for 420 subjects. Of these 420 subjects, 180 had no evidence of PAOD and no aneurysmal disease, 129 had a small aneurysm (30–45 mm diameter) but no evidence of PAOD, 60 patients had both a small aneurysm and PAOD and in 17 patients data about PAOD was missing. They were included in the study. Thirty-five subjects were on ACE inhibitors and 385 were not. Mean serum PIIINP level was 3.5 \(\mu g/l\) (sd 1.3 \(\mu g/l\)). A large variation in PIIINP levels was found, range: 1.7–16.5 \(\mu g/l\). There was a significant increase in mean collagen turnover between subjects on ACE inhibitors compared to those not on ACE inhibitors. Mean PIIINP levels for subject on ACE inhibitors was 3.73–4.79 \(\mu g/l\) (95% CI: 3.73–4.79 \(\mu g/l\), compared to 3.48–3.75 \(\mu g/l\) for subjects not
exposed to ACE inhibitors. Mean PIIINP levels of the 36 subjects with missing data about current medication was 3.78 μg/l (95% CI: 3.30–4.27 μg/l). There were no statistically significant differences in type III collagen turnover between subjects on other antihypertensive drugs such as: calcium channel blockers, diuretics or betablockers (see Table 1). There were no significant differences in PIIINP levels between subjects with a small aneurysm (mean PIIINP: 3.73 μg/l 95% CI 3.53–3.93) and subjects with a normal aorta (mean PIIINP: 3.63 μg/l 95% CI 3.47–3.79) (P-value 0.46). There were no significant differences in PIIINP levels between subjects with evidence of PVD (mean PIIINP: 3.71 95% CI 3.47–3.95) and subjects with no evidence of PAOD (mean PIIINP: 3.65 μg/l 95% CI: 3.50–3.80) (P-value 0.71). The difference in collagen turnover remained significant after adjusting for presence of aneurysmal disease or evidence of PAOD. Adjusted mean PIIINP for subjects on ACE inhibitors was 4.27 μg/l, (95% CI 3.83–4.71 compared to a mean PIIINP of 3.59 μg/l, (95% CI 3.46–3.73 for those not on ACE inhibitors (P-value 0.0046).

Stepwise regression was used to investigate which other variables were associated with PIIINP levels. Variables investigated were age, height, weight, systolic and diastolic blood pressure, diabetic status, smoking status in the form of serum cotinine levels, and the usual standard lipid risk factors such as: total cholesterol levels, HDL cholesterol, LDL cholesterol and triglycerides. Iron is an important cofactor of collagen metabolism. We also investigated the following measurements of iron metabolism: serum iron levels, transferrin levels, ferritin levels and antithrombopypsin. Stepwise regression showed that PIIINP levels were significantly associated with age, height, weight, diastolic blood pressure and ferritin levels. None of these were significantly different between subjects on ACE inhibitors and those not on ACE inhibitors (see Table 2). We calculated adjusted mean PIIINP levels between subjects exposed to ACE inhibitors and those not exposed. The difference in mean PIIINP levels remained significant after adjusting for age, height, weight, HDL and ferritin: 4.14 mcg/l (95% CI: 3.72–4.57 mcg/l) for subjects exposed to ACE inhibitors versus 3.62 mcg/l (95% CI: 3.49–3.75 mcg/l) (P-value 0.02).

### Discussion

A marked increased type III collagen synthesis, as measured by PIIINP levels, was seen in subjects on ACE inhibitors compared to those not exposed to ACE inhibitors. The difference was highly significant and remained significant after adjusting for confounding variables.

Marshall et al. recently demonstrated a reduction in collagen metabolism in lung tissue after administration of intratracheal Ramipril (an ACE Inhibitor) and Losartan (an Angiotensin II type 1 antagonist). This finding was also noted in their control population where intratracheal saline was administered. There was also clear evidence that ACE was not the only controlling factor for Angiotensin II expression in this experiment.

Another in vitro experiment, this time utilising rat cardiac cells, investigated the role of N-Acetyl-Seryl-Aspartyl-Proline (N-SDKP), a natural inhibitor of stem cell entry into S phase (the period for collagen synthesis). This molecule is hydrolysed by ACE-Is. They found it to have a biphasic and dose dependant effect on collagen synthesis. In low doses it had an inhibitory effect on collagen synthesis, but in high dose no significant effect was noted.

The use of ACE inhibitors in other animal models of atherosclerosis has shown a decrease in plaque area with a decrease in macrophage accumulation. An increase in the extracellular matrix was also demonstrated. These phenomena could be explained by the up regulation of collagen synthesis, and the presence of increased quantities of collagen in the fibrous cap.

Reduced collagen content in the fibrous cap may result from decreased synthesis of the extra-cellular matrix by smooth muscle cells (SMCs), increased breakdown, or both. Vascular SMCs synthesize the precursors of type III collagen for the extracellular matrix. The synthesis and degradation of the extracellular matrix proteins are slow in the normal artery. Atherosclerosis and arterial injury lead to increased

<table>
<thead>
<tr>
<th>Drug</th>
<th>Number exposed</th>
<th>Mean PIIINP and 95% CI</th>
<th>Number not exposed</th>
<th>Mean PIIINP and 95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitors</td>
<td>35</td>
<td>4.26 (3.73; 4.79)</td>
<td>378</td>
<td>3.62 (3.49; 3.76)</td>
<td>0.007</td>
</tr>
<tr>
<td>Ca channel</td>
<td>45</td>
<td>3.53 (3.25; 3.82)</td>
<td>368</td>
<td>3.70 (3.56; 3.84)</td>
<td>0.65</td>
</tr>
<tr>
<td>Diuretics</td>
<td>64</td>
<td>3.95 (3.61; 4.30)</td>
<td>349</td>
<td>3.63 (3.49; 3.77)</td>
<td>0.08</td>
</tr>
<tr>
<td>Betablockers</td>
<td>77</td>
<td>3.80 (3.52; 4.08)</td>
<td>336</td>
<td>3.65 (3.51; 3.80)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table 1. Collagen turnover as measured by the serum PIIINP concentration in mcg/l and 95% confidence intervals of the mean between subjects exposed to classes of antihypertensive drugs and those not exposed. P-values were calculated by applying the t-test to the log transformation of the mean PIIINP.
Table 2. Confounding variables associated with PIINP levels and the differences between subjects on ACE inhibitors and those not

<table>
<thead>
<tr>
<th>Variable</th>
<th>On ACE inhibitors</th>
<th>Not on ACE inhibitors</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>70.7 (68.5–73.1)</td>
<td>70.8 (70.1–71.6)</td>
<td>0.95</td>
</tr>
<tr>
<td>Height in cm</td>
<td>172.1 (170.1–174.1)</td>
<td>173.5 (172.8–174.2)</td>
<td>0.24</td>
</tr>
<tr>
<td>Weight in kg</td>
<td>81.6 (76.9–86.3)</td>
<td>80.1 (79.0–81.3)</td>
<td>0.49</td>
</tr>
<tr>
<td>Ferritin mcg/l</td>
<td>136.9 (101.1–172.8)</td>
<td>118.4 (108.4–128.3)</td>
<td>0.29</td>
</tr>
<tr>
<td>HDL mmol/l</td>
<td>1.05 (0.95–1.14)</td>
<td>1.16 (1.11–1.21)</td>
<td></td>
</tr>
</tbody>
</table>

The synthesis of many matrix components including collagen type III, presumably as part of the response to the insult. These results suggest that ACE-I therapy may increase collagen synthesis, and thus plaque stabilisation. This may provide an alternative explanation for the decrease in acute vascular events observed in patients on ACE-I therapy.

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References


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