Evaluation of Plasmatic Leucocyte Elastase Levels in Coronary Artery Disease

Ph. Bernadet, M.D., J. Bonnet, M.D., Th. Couffinhal, M.D., V. Touroulu, M.D., D. Benchimol, M.D., F. Drouillet, M.D., R. Crockett, M.D., and H. Bricaud, M.D.

SUMMARY

Leucocyte elastase may be involved in the structural modification observed in the atherosclerotic process. Therefore, we tested the usefulness of leucocyte elastase plasma level determination as a marker for atherosclerosis.

Plasma levels of elastase were determined by ELISA in 100 consecutive patients (mean age 56 ± 9.8 years) admitted to hospital for coronary angiographic investigation of chest pain. Eighty-seven patients had evidence of atherosclerosis, and 13 patients had normal coronary vessels. No significant difference in leucocyte elastase was found between the 2 groups, nor was there any relationship between elastase levels and the severity of atherosclerosis. However, relationships between plasma leucocyte elastase levels and various lipid fractions (Apo AI, LDL) and daily tobacco consumption were found. Leucocyte elastase may thus play a role not only by direct modification of the vessel wall, but also indirectly via risk factors such as dyslipoproteinemia and leucocyte toxicity.

Key Words:
Atherosclerosis Coronary artery disease Leucocyte elastase

ELASTASES are found in many tissues, including the pancreas, granulocytes, platelets, macrophages and monocytes, and smooth muscle arterial cells, and are thought to be involved in the physiopathology of numerous conditions ranging from infectious shock to pulmonary emphysema. They also appear to play an important role in the atherosclerotic process, by an action on the arterial wall through a balance between extracellular matrix neosynthesis induced by phenotypic change of smooth muscle cells and degradation processes. Indeed, elastases defined as enzymes which

From the Hôpital Cardiologique, Avenue de Magellan, 33604 Pessac Cedex and * Unité de Recherches INSERM U8, Avenue du Haut-Lévêque, 33600 Pessac, France.

Mailing address: Prof. Jacques Bonnet, INSERM U8, Avenue du Haut-Lévêque, 33600 Pessac, France.

Received for publication April 22, 1991.
Accepted September 17, 1991.
catalyze the solubilization of elastin through proteolytic cleavage, have proteolytic activity which is not restricted to elastin since these enzymes are as powerful as trypsin or chymotrypsin in cleaving soluble proteins like hemoglobin or denatured casein. They have a proteolytic action on various constituents of the arterial wall including elastin, proteoglycans and collagen. However, their role in pathological processes is not necessarily restricted to disturbance of the extracellular matrix. They may play an indirect role in the chronic inflammatory processes involved in atherosclerosis. Degradation peptides, especially those produced by elastase, have a chemotactic effect on circulating monocytes, favoring their accumulation in the arterial wall, and hence their participation in the atherosclerotic process.

Furthermore, after proteolysis certain macrophage-derived elastases appear to make alpha 1 proteinase inhibitor (alpha 1 Pi) a chemoattractant for polymorphonuclear cells, thereby stimulating the inflammatory response, and upsetting the balance between elastase and its main circulating inhibitor, alpha 1 Pi.

However, the exact role of elastases in the physiopathology of atherosclerosis is unclear. Some workers have suggested that they play a beneficial role since elastase levels have been found to be lower in the pancreas of patients with atherosclerosis, and administration of elastase can delay the development of atherosclerotic lesions in the rabbit fed a cholesterol-rich diet. Furthermore, cholesterol levels have been found to be reduced after oral administration of elastase to hypercholesterolemic rabbits.

Only a few studies have been carried out on elastases and their proteolytic activity in patients with atherosclerosis. High levels of elastase activity in aortic aneurysm fragments have been reported. Serum elastase activity has also been found to be altered in patients with atherosclerosis, and Baydanoff reported higher levels of elastin peptides in a population of atherosclerotic patients.

The source of the elastin-degrading activity in atherosclerosis could originate from different sources. During the first step of atherogenesis, where only functional alterations of endothelial cells are observed, the main source of elastase is surely dependent on monocyte infiltration and smooth muscle cell synthetic phenotype modulation, circulating pancreatic elastase being inhibited by plasmatic inhibitors. In complicated plaques, the rupture of endothelium allows the interaction between circulating cells, namely platelets and granulocytes within the subendothelial space. At this step one of the characteristic features of this disease is the fragmentation of elastin fibers within the media of the arterial walls. Even if smooth muscle cell and macrophage elastases participate in this process, platelet and granulocyte elastases
may be considered as main factors which aggravate the evolution of the atherosclerotic plaques.\textsuperscript{18,19}

In this study, we determined plasma levels of granulocyte elastase in patients with coronary atherosclerosis. This enzyme is the most prominent elastase and one of the most likely candidates for a role in complicated atherosclerosis.

**Patients and Methods**

**Patient population**

One hundred consecutive male patients (mean age 56.2±9.8 years) admitted for coronary angiographic investigation of chest pain or with a history of myocardial infarction (>2 months) were recruited. Different laboratory tests, plasma leucocyte elastase and elastase inhibitor levels were determined in all patients.

Eighty-seven patients had angiographically demonstrable atherosclerosis. Among these 87 patients (ATH group), 27 were stabilized after a 2-week period of unstable angina, 60 had stable angina, and 41 had had a myocardial infarction more than 2 months previously. None of the patients was in the acute phase of myocardial infarction or unstable angina.

Thirteen patients admitted for investigation of chest pain had no evidence of coronary atherosclerosis on coronary angiograms. Other arteries appeared free of atherosclerosis after clinical and Doppler analysis. These patients constituted the control group (CTR group). In all patients, the laboratory tests were carried out on the day before the hemodynamic investigations.

**Methods**

1) **Clinical evaluation**

Classical risk factors for coronary artery disease including smoking habits, years of tobacco consumption, and systemic arterial hypertension were recorded.

2) **Laboratory investigations**

These were divided into 3 main groups:

a) **Lipid profiles**: Cholesterol, triglycerides,\textsuperscript{20,21} HDL cholesterol, LDL cholesterol,\textsuperscript{22,23} apoproteins AI and B,\textsuperscript{24} ratios of total cholesterol to HDL cholesterol and Apo B to Apo AI.

b) **Hematologic data**: Leucocytes were counted in a Coulter counter (model B) (Coulter Electronics Inc.).

c) **Plasma factors**: Enzyme-linked immunoabsorbent assay (ELISA)
for elastase alpha 1 proteinase inhibitor complex (E-alpha 1 Pi) used the method described by Neumann et al\(^2\) with a two-site sandwich ELISA including antisera against granulocyte elastase and alpha 1 Pi (Merck, Paris, France). Plasma samples were rapidly incubated in plastic tubes coated with antibodies against elastase. After washing with buffer, the surface fixed E-alpha 1 Pi complex molecules reacted with alkaline phosphatase (AP) labelled antibodies directed against alpha 1 Pi. Under the conditions used, the activity of AP towards p-nitrophenylphosphate was proportional to the concentration of E-alpha 1 Pi in the sample.\(^2\) Concentrations (ng/ml) are given only for the amount of complexed elastase.

Alpha 2 macroglobulin (alpha 2 M) and alpha 1 proteinase inhibitor (alpha 1 Pi) were determined by a radial immunodiffusion technique with standardized immunodiffusion\(^8\) and specific antisera (Behring Werke AG). Fibrinogen level determined by Von Clauss' chronometric method\(^2\) was used as a marker of inflammation.

Fasting blood samples were taken before 10 a.m. Patients were requested not to smoke during the 12 hours prior to sampling. Blood was collected after clean puncture of the cubital vein. Lipids were determined immediately after sampling. Plasma samples for determination of E-alpha Pi were immediately frozen to \(-70^\circ\)C, and stored at that temperature until assay.

3) Coronary angiography and ventriculography were carried out on all patients using Jenkins' method after catheterization according to the Seldinger technique.\(^2\)

The severity of the coronary atherosclerosis was evaluated from two scores: Jenkins' score\(^2\) and a score referred to as the "mean atherosclerotic score" (MAS). Unlike Jenkins' score which excluded distal lesions, the MAS included the 15 segments of the AHA classification, the size of the segment involved and the severity and number of lesions in each segment. This mean atherosclerotic score was evaluated from a formula involving coefficients and points for each segment:

\[
\text{MAS} = \frac{(\text{points} \times \text{coefficient}) \times 100}{\text{coefficients}}
\]

This score was thought to define distal coronary atherosclerosis more accurately.\(^2\)

**Statistical analysis**

The parametric data were compared using Student's t-test and analysis of variance. Simple regression tests were used for the linear correlations.
The differences were considered to be significant if $p<0.05$.

**RESULTS**

The clinical findings and the incidence and severity of risk factors are listed in Table I. It can be seen that there was a significant age difference between the patients with and without evidence of atherosclerosis ($57.2\pm8.9$ vs. $49.5\pm12.3$; $p=0.007$). As expected, the atherosclerotic patients had higher levels of total cholesterol, LDL cholesterol and fibrinogen. Although there were more smokers in the atherosclerosis group (70% vs. 54%), the daily tobacco consumption was lower. This may have been due to the fact that the control group contained few but heavy smokers.

There were no significant differences between the 2 groups in the protease-antiprotease system: plasma leucocyte elastase, or rather E-alpha 1 Pi (Table II) and alpha 1 Pi and alpha 2 macroglobulin levels were comparable in the 2 groups. No significant relationship was found between E-alpha 1 Pi levels and Jenkins' score ($r=0.10$, NS) or MAS ($r=0.11$, NS). There thus appeared to be no relationship between granulocyte elastase levels and the severity of the atherosclerosis.

Regression analysis of E-alpha 1 Pi and various vascular risk factors showed a significant relationship between E-alpha 1 Pi and serum levels of LDL cholesterol ($r=0.24$, $p<0.05$), Apo AI ($r=-0.21$, $p<0.05$) and daily

| Table I. Clinical Findings and Risk Factors of Atherosclerotic Patients (ATH) and Control Patients (CTR) |
|---------------------------------------------------------------|-----------------|-----------------|-----------------|
|                                                                 | ATH ($n=87$)    | CTR ($n=13$)    | $p$             |
| Age (years)                                                   | $57.2\pm8.9$    | $49.5\pm12.3$   | 0.007           |
| Mean atherosclerotic score                                    | $207.4\pm117.4$ | $0$             | 7.9 E-8         |
| Total cholesterol (mmol/l)                                    | $6.5\pm1.05$    | $5.8\pm0.9$     | 0.016           |
| Triglycerides (mmol/l)                                        | $1.7\pm0.6$     | $1.8\pm0.9$     | NS              |
| HDL chol. (mmol/l)                                            | $1.2\pm0.3$     | $1.1\pm0.3$     | NS              |
| LDL chol. (mmol/l)                                            | $4.7\pm1.0$     | $3.9\pm0.9$     | 0.008           |
| APO AI (mg/100 ml)                                            | $128.9\pm25.1$  | $130.2\pm25.9$  | NS              |
| APO B (mg/100 ml)                                             | $145.7\pm25.7$  | $131.2\pm27.2$  | NS              |
| Smokers                                                       | $61 (70.1\%)$   | $7 (54\%)$      | NS              |
| Tobacco years                                                 | $24.4\pm15.3$   | $22.9\pm14.7$   | NS              |
| Tobacco daily consumption (cigarettes/day)                    | $3.7\pm7.5$     | $18.9\pm14.2$   | 2 E-5           |
| Fibrinogen level (g/l)                                       | $3.7\pm0.9$     | $3.1\pm0.6$     | 0.038           |
| Leucocyte count (giga/l)                                      | $6.9\pm1.9$     | $7.2\pm2.3$     | NS              |
| HTA (ASP $\geq$160 mmHg, ASD $\geq$95)                      | $31 (35.6\%)$   | $4 (30.8\%)$    | NS              |
Table II. Plasma Levels of the Protease-Antiprotease System in the Atherosclerotic (ATH) and Control (CTR) Groups

<table>
<thead>
<tr>
<th></th>
<th>ATH (n=87)</th>
<th>CTR (n=13)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocyte elastase (ng/ml)</td>
<td>125.7±127.1</td>
<td>142.2±174.6</td>
<td>NS</td>
</tr>
<tr>
<td>Alpha 1 Pi (mg/100 ml)</td>
<td>214.1±38.9</td>
<td>208.1±32.1</td>
<td>NS</td>
</tr>
<tr>
<td>Alpha 2 macroglobulin (mg/100 ml)</td>
<td>144.0±43.6</td>
<td>143.1±38.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table III. Regression Analysis between Leucocyte Elastase and Cardiovascular Risk Factors

<table>
<thead>
<tr>
<th></th>
<th>ATH (n=87)</th>
<th>CTR (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Age</td>
<td>-0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-0.05</td>
<td>NS</td>
</tr>
<tr>
<td>HDL chol.</td>
<td>-0.14</td>
<td>NS</td>
</tr>
<tr>
<td>LDL chol.</td>
<td>0.24</td>
<td>0.025</td>
</tr>
<tr>
<td>Apoprotein AI</td>
<td>-0.21</td>
<td>0.04</td>
</tr>
<tr>
<td>Apoprotein B</td>
<td>0.19</td>
<td>NS</td>
</tr>
<tr>
<td>Tobacco (years)</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Tobacco (daily consumption)</td>
<td>0.26</td>
<td>0.038</td>
</tr>
</tbody>
</table>

tobacco consumption (r=0.26, p<0.05) in the atherosclerosis group (Table III). No relationship was found between leucocyte elastase levels and the other lipids or other vascular risk factors, including age. No correlations were detected in the control patients.

**DISCUSSION**

Granulocyte elastase is an enzyme from the azurophilic granula of polymorphonuclear leukocytes. Like cathepsin G and collagenase, it is one of the neutral proteinases. Following different stimulations, the granulocyte may directly release proteinases. In plasma, granulocyte elastase is complexed with plasma proteinase inhibitors, mainly with alpha 1 proteinase inhibitor.31) A small fraction of the enzyme is bound to alpha 2 macroglobulin with a short half-life of the complex with alpha 2 macroglobulin.31) The dual antigenic specificities of the complex of human granulocyte elastase with alpha 1 proteinase inhibitor with the use of two specific polyclonal antibodies directed against the enzyme and its proteinaceous inhibitor, allow a specific immunochemical quantification of the complex of granu-
locyte elastase with alpha 1 proteinase inhibitor and thus a level of the deleterious release of granulocytic proteinase and namely elastase during atherosclerosis.\textsuperscript{25)}

Our results indicated that plasma levels of leucocyte elastase or protease inhibitors cannot be regarded as markers of either the presence or severity of coronary atherosclerosis in our patients. In agreement with other studies,\textsuperscript{32,33} we found no significant difference in elastase levels between patients with and without evidence of atherosclerosis, although Bihari-Varga\textsuperscript{33} found lower elastase-type activity in sera of male patients with atherosclerosis than in women with atherosclerosis, but also lower elastase-type activity than control patients. In our study, the method used for plasma elastase level determination was different. In a previous study, elastase levels were defined on the basis of the elastase-type enzyme activity of sera using a synthetic substrate according to the method of Bieth,\textsuperscript{34} and pancreatic elastase inhibitory capacity of the sera. Our data were based on the determination of human granulocyte elastase level in complex with alpha 1 proteinase inhibitor.

The lack of a relationship in our study might have been due to the method used for evaluation of coronary atherosclerosis. Visual analysis of coronary angiograms does not provide an accurate assessment in patients with mild stenoses of the coronary vessels (<40%) due to the compensatory dilatation of the artery.\textsuperscript{35} So, there is less of a relationship between visual analysis and true severity of atherosclerosis. However, the angiographic data are well correlated with the anatomical extent of the lesions.\textsuperscript{36} The absence of an elevation of leucocyte elastase levels in the atherosclerotic patients does not rule out possible local activity within the vessel wall.

The control group was significantly younger, but no linear correlation was observed between age and granulocyte elastase level. We did, however, find a correlation between circulating elastase levels and various risk factors for coronary artery disease. The least surprising concerned the smoking history. In agreement with Abboud\textsuperscript{37} we found no relationship between elastase levels and years of tobacco consumption, although there was a significant relationship between the daily tobacco consumption and elastase levels. Several authors\textsuperscript{37–39} have reported an elevation of circulating elastase after heavy smoking. This is probably due to an acute stimulation of elastase release from granulocytes and an increase in granulocyte number by demargination.\textsuperscript{37}

The positive relationship between leucocyte elastase and LDL cholesterol is noteworthy. Previous work had demonstrated that LDL is capable of promoting the release of elastase from granulocytes following in vitro in-
cubation and that this enzyme can cleave Apo B into fragments.\textsuperscript{40} Thus, it is possible that the proteolytic activity of elastase will be involved in the oxidative alterations of the supposedly atherogenic LDL\textsuperscript{41} which accumulates inside vessel wall macrophages by interaction with scavenger receptors.\textsuperscript{42}

The negative correlations between elastase and Apo AI may be indicative of proteolytic activity of the enzyme on this lipoprotein.\textsuperscript{43} It has been shown that polymorphonuclear elastase has proteolytic activity on the Apo AII of HDL\textsuperscript{3}.\textsuperscript{44} This elastase has also been found to have activity on the SAA apoprotein, a principal apoprotein of HDL\textsuperscript{3} in the inflammatory phase. The HDL\textsuperscript{3} particles appear to be modified with a loss of apoprotein AI at the expense of SAA apoprotein.\textsuperscript{45,46} Although our study did not evaluate HDL subfractions, our results suggest that elastases may be involved in the inflammatory modification of HDL particles and Apo AI in patients with atherosclerosis.

\textbf{Conclusion}

Our results show that leucocyte elastase levels are not a direct reflection of either the presence or the severity of coronary atherosclerosis. However, the elastase-antiprotease system may play an indirect role in the leional process via interactions with certain lipoproteins which have a well-established relationship with coronary artery disease and tobacco consumption.

\textbf{References}

7. Jacob MP, Brechemier D, Robert L, Hornebeck W: Variation of elastase type protease ac-
Vol. 33 No. 2

PLASMA LEUCOCYTE ELASTASE LEVELS

Vigil and elastin biosynthesis in rabbit aorta induced by cholesterol diet and immunization with elastin peptides. Artery 10: 310, 1982


