Elastase-Induced Experimental Aneurysms in Rats

Samy Anidjar, Jean-Loup Salzmann, Danièle Gentric, Pierre Lagneau,
Jean-Pierre Camilleri, and Jean-Baptiste Michel

An experimental in vivo model of aortic aneurysm was established by perfusing an isolated segment of rat abdominal aorta with pancreatic elastase. Ten rats were used in each protocol. Saline-perfused aortas developed no aneurysmal dilations. Elastase-perfused aortas contained aneurysms in the perfused area and a total loss of elastic tissue. Control aortas contained no elastic tissue lesions. There was a quantitative relation between the amount of elastase perfused and aneurysm formation: 1–2 units induced neither macroscopic nor microscopic lesions; 3–6 units induced microscopic elastic tissue damage without macroscopic aneurysm; and more than 6 units produced aneurysmal dilation in all cases. In situ elastase secretion by macrophages was induced by perfusing rat aortas with thioglycollate-activated macrophages or with thioglycollate alone. There was aortitis without true aneurysm and a total loss of elastic tissue in the vicinity of activated macrophages within the aortic media. Perfusion of infra-aneurysmal amount of elastase (1 or 2 units) or thioglycollate plus plasmin (2 units) always induced a large aneurysm, whereas plasmin alone induced neither macroscopic nor microscopic lesions. These morphological results were supported by the significantly elevated elastolytic activity within the aortic wall of animals perfused with thioglycollate plus plasmin 9 days after perfusion (207.6±54.8 µg elastin-rhodamine lysed/18 hr; control rats, 25.43±11.13). The results suggest that the presence of elastase within the aortic media leads to aneurysm formation. Activated macrophages within the aortic media may be responsible for elastase secretion and elastic tissue destruction. Plasmin may enhance elastase activity and aggravate the aneurysmal lesion. 

(Circulation 1990;82:973–981)

Although the pathophysiological evolution of atheroma generally leads to stenosis and its ischemic consequences, atheromatous disease may, under certain conditions, also lead to aneurysms and their attendant life-threatening consequences including rupture and embolism. Most studies on the first pathophysiological pathway (stenosis) have used experimental models that involve lipid overload, endothelium injury, and, more recently, the role of inflammatory cells. This pathophysiological pathway of stenosis predominantly involves the intimal layer of the arterial wall.

In contrast, there are few experimental models of aneurysm. Aneurysm formation, in contrast with stenosis formation, predominantly involves medial layer of the arterial wall. Experimental models of aortic aneurysm are either spontaneous or induced by surgical or toxic manipulations. The “Blotchy mouse” is a typical model of genetically determined aneurysmal formation. Surgical or toxic (acetrizoate) destruction of the media can lead to aneurysm and so can perturbations of smooth muscle cell biosynthetic activities during development by theophylline or β-aminopropionitrile. However, Gertz et al. recently reported that calcium deposits within the media can cause fragmentation of the elastin network and inflammatory granuloma infiltration, which can induce arterial dilation.

Recent clinical investigations also indicate that elastase may be involved in the genesis of aneurysm in humans. Sumner et al., Dobrin et al., Busuttil et al., Campa et al., Cohen et al., and Powell and Greenhalgh have shown that there is a loss of elastic tissue and increased elastolytic activity in the media of human aneurysm. These changes suggest that the initial stages in the pathophysiology of aortic aneurysm involve elastolysis of the aortic media and that elastase plays a major role in the destruction of elastic tissue within the aortic wall.

This study describes an experimental in vivo model of aortic aneurysm that has been used to clarify the role of elastase activity in the genesis of aneurysm.
The results indicate that the presence of elastase within the aortic media is essential and that activated macrophages within the media can induce elastase secretion and, hence, elastic tissue damage. The results also show that plasmin enhancement of elastase activity can aggravate the aneurysmal lesion.

**Methods**

**Experimental Procedure**

A 1-cm segment of the abdominal aorta of male Wistar rats (300–320 g, Iffa Credo, Saint Germain sur l’Arbresle, France) was isolated and perfused. The animals were anesthetized with 6% sodium pentobarbital (0.1 ml/100 g body wt, Clin Midy, France) and a PE10 polyethylene catheter (Clay Adams, Parsippany, N.J.) was inserted into the femoral artery under a binocular surgical microscope (Zeiss, F.R.G.) until the tip reached the infrarenal abdominal aorta. The vena cava was dissected free from the aorta by a laparotomy; all collateral arteries were ligated, and the position of the catheter tip was verified.

The abdominal aorta was clamped at the level of the left renal vein and ligated around the catheter 1 cm downstream. This isolated 1-cm segment of abdominal aorta was then perfused with 2 ml of the appropriate test solution (rate, 1 ml/hr) from the lumen to the adventitia through the media. At the end of perfusion, the aorta was unclamped, the ligature and the catheter removed, the femoral artery ligated, and the aortic permeability verified. The wounds were closed, and the rats were returned to their cages. Animals were killed 3 weeks later.

**Morphology**

Rats were anesthetized as above, and a laparotomy was performed 3 weeks after surgical preparation of the model. The macroscopic appearance of the abdominal aorta was noted, and a PE20 polyethylene catheter (Clay Adams) was introduced into the aorta through the aortic bifurcation. The aorta was clamped above the left renal vein. Dubosq Brazil fixative solution was then perfused at a pressure of 120 mm Hg, and the vena cava was incised to prevent overpressure.

The whole abdominal aorta was then removed and embedded in paraffin for classic histological examination. Longitudinal 5-μm sections were stained with orcein for elastic tissue and with Masson trichrome for collagen fibers, fibrin, and nuclei.

The length of elastic disruption and the developed length of the aneurysmal dilation were measured on each section under an optical microscope by a morphometric method (Nachet Vision) (Figure 1).

Macroscopic aneurysms were defined by a length of elastic disruption greater than 1,500 μm and by the inability to obtain accurate measurement under microscopy. Dystrophic aortitis was defined by a length of elastic disruption smaller than 1,000 μm and by the ability to obtain accurate measurement under microscopy.

The elastin density in the normal aortic wall, in the intermediate wall (aneurysmal border area) and in the aneurysmal wall was quantified morphometrically as previously described14,15 (Figure 1). Elastin density was measured in aortas perfused with elastase, with macrophages, and with thioglycollate plus plasmin. Elastic tissue disappeared abruptly from the other perfused groups so that intermediate stages between the normal and aneurysmal wall could not be quantified. The number of lamellar units was determined on each section.

**Aortography**

Profile and lateral angiograms were obtained for some of the animals in each group to confirm the accuracy of macroscopic data. Contrast medium was injected into the aorta of anesthetized rats by a cannula inserted into an exposed carotid artery.

**Experimental Protocols**

**Perfusion with pancreatic elastase and physiological saline.** Ten rats were perfused with 15 units of hog pancreatic elastase (Type I; 1 unit = 1 mg elastin hydrolyzed for 20 minutes at pH 8.8, 37°C, Sigma Chemical Co., St. Louis, Mo.) in 2 ml normal saline; 10 other control rats were perfused with 2 ml saline alone. The 20 rats were killed at the end of the third week, and their abdominal aortas were removed for histological examination.

**Perfusion with other proteases.** Four other proteases were tested. Five groups of 10 rats were perfused with the same quantity of collagenase (Clostridium Type VII, Sigma Chemical Co.), papain (papaya latex, Sigma Chemical Co.), trypsin (bovine pancreas, Sigma Chemical Co.), chymotrypsin (bovine pancreas, Sigma Chemical Co.), or trypsin plus chymo-
trypsin. The perfused aortas were examined for the presence and size of aneurysm as described above.

Relation between elastase perfusion and aneurysm formation. Groups of three rats were perfused with different doses of hog pancreatic elastase (1, 3, 6, 10, and 15 units/animal) (Type I, Sigma Chemical Co.) and killed 3 weeks later. The presence of aneurysms and the histological extent of elastolysis were recorded.

Macrophage activation within the aortic media. Two protocols were tested: perfusion of the aorta with thioglycollate-activated macrophages (10 rats) and perfusion of the aorta with thioglycollate alone to induce in situ macrophage activation (10 rats).

Rat peritoneal macrophages were obtained by peritoneal lavage with phosphate-buffered saline containing 100 units heparin/ml; lavage was performed 4 days after intraperitoneal injections of 15 ml of 3% thioglycollate medium (Institut Pasteur, Paris France).16 Macrophages were isolated by centrifugation after hypotonic lysis of erythrocytes and resuspended in physiological saline solution (2 ml).

The time course of the elastolysis induced by macrophage activation was studied in 30 rats that had been perfused with thioglycollate alone and killed (n=3) 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 days after perfusion. The perfused aortas were then examined for elastolysis.

Plasmin. Ten rats were perfused with 1 (five rats) or 2 (five rats) units plasmin (from porcine blood, 3–5 units/mg, Sigma Chemical Co.) and were killed 3 weeks later.

Ten rats were perfused with an infra-aneurysmal amount of hog pancreatic elastase (1 or 2 units) plus plasmin (2 units) and killed 3 weeks later. Ten rats were perfused with thioglycollate plus plasmin (2 units) and killed 3 weeks later.

Quantification of Elastase Activity in Aortas Perfused With Thioglycollate Plus Plasmin and in Control Aortas Perfused With Saline

Ten aortas were perfused with thioglycollate plus plasmin. The aortas were removed 9 days later, and the elastase activity was determined.

Ten control aortas were perfused with saline.

Preparation of aortic homogenates. The perfused abdominal aorta was disrupted in 1.5 ml of 0.2 M Tris buffer, pH 8 with a polytron, and the homogenates were centrifuged at 3,000g for 20 minutes at 4°C.

Assay of aortic elastase activity. The substrate was elastin covalently labeled with rhodamine-β-isothiocyanate17 (from bovine ligamentum nuchae, Elastin Products Co., Owensville, Mo.). Reaction mixtures contained 1 ml of supernatant (aortic homogenates), 2 mg elastin-rhodamin, and 0.02% sodium azide.

Positive controls contained hog pancreatic elastase (chromatographically purified, 100 μl of 2.10^-3 M stock solution stored at 4°C, Byosis, France). The blank was obtained with Tris buffer alone. Tubes were incubated for 18 hours at 37°C. The reaction was stopped with 50 μl of 3 M acetic acid buffer at pH 5, and the tubes were centrifuged at 5,000g for 15 minutes.

The fluorescent intensities of the supernatants were determined in 12-mm cuvettes in a fluorometer with 546- (exitation) and 590-nm (emission) filters. The positive control supernatant was generally
diluted fivefold to obtain fluorescent readings within the range of the fluorometer.

Statistical Methods
Results are expressed as mean±SD. Comparison between groups was made by a one-way analysis of variance followed by Sheffe’s F test.

Results
Perfusion With Pancreatic Elastase and Physiological Saline
All the elastase-perfused (15 units/animal) aortas developed macroscopic aneurysms (>5 mm) (Figure 2); aneurysms occurred only in the perfused area. Saline-perfused control aortas developed no aneurysmal dilations (Figure 3).

All aortas were permeable 3 weeks after induction. The angiograms confirmed the macroscopic data (Figure 2); only the elastase-perfused aorta had aneurysmal fusiform dilations in the perfused area.

There were no elastic tissue lesions at the perfusion site in control aortas. The five lamellar units were always present, and smooth muscle cells appeared normal in control segments and in normal, nonaneurysmal segments of elastase-perfused aortas (Figure 4). There was a total loss of elastic tissue in the area of the aneurysmal dilation in elastase-perfused aortas. The aneurysmal vascular wall contained only collagen, and fibrin deposits were present in the aneurysmal lumen. Elastic tissue was fragmented in the undilated segments (intermediate wall) of the perfused aortic wall; its quantity was low and so was the number of lamellar units (Table 1). This probably represents early lesions preceding aneurysmal dilation.

Perfusion With Other Proteases
Perfusion with papain, trypsin, chymotrypsin, and collagenase produced elastic tissue disruption. The perfused areas lacked elastic tissue, and there was slight aneurysmal dilation with fibrin deposits in the lumen. Although the papain, collagenase, and trypsin groups all showed small multiple, microscopic aneurysms, there were always major elastic tissue lesions. Chymotrypsin induced significantly less (p<0.01) elastic disruption and microscopic aneurysm dilatation (Table 2).

Relation Between the Amount of Elastase Perfused and Aneurysm Formation
All aortas perfused with more than 6 units elastase developed macroscopic aneurysms similar to those obtained with 15 units. The amount of elastic tissue in aortas perfused with 3–6 units was considerably reduced, but it was still present at a few sites around live smooth muscle cells. Macroscopically, the aortas were more dystrophic than aneurysmal. Three units of pancreatic elastase induced slight elastic disrup-

TABLE 1. Elastin Density and Lamellar Units of Aortic Wall

<table>
<thead>
<tr>
<th></th>
<th>Elastin density (%)</th>
<th>Lamellar units (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal arterial wall</td>
<td>Intermediate wall</td>
</tr>
<tr>
<td>Elastase (15 units)</td>
<td>29.24±7.75</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Macrophage activation</td>
<td>31.51±8.45</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Thioiglycollate+plasmin</td>
<td>24.03±8.58</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Values are mean±SD.
tion similar to that produced by chymotrypsin. Six units of pancreatic elastase induced significantly \( F = 6.88, p < 0.05 \) more elastic disruption and larger aneurysmal dilatation than did 3 units.

### Macrophage Activation Within the Aortic Media

The passive transfer of thioglycollate-activated macrophages (10 rats) and perfusion with thioglycolate (10 rats) had the same results. The aortas were more dystrophic than aneurysmal in the area of perfusion. There were, microscopically, areas of total elastic tissue loss in the vicinity of macrophage granuloma from the adventitia to the media (Figure 5). The length of elastic tissue disruption was similar to that produced by 3 units pancreatic elastase or chymotrypsin.

The time course of elastolysis in thioglycollate-perfused aortas was studied in rats killed at regular intervals. The aortic wall was not significantly different from that of control aortas up to day 5. The structure of the aortic media was unchanged on day 7, but many macrophages were present in the adventitia. The media was completely invaded by many activated macrophages on day 9. Almost all the elastic tissue in this area was lysed in the vicinity of inflammatory granuloma (Figure 6).

### Role of Plasmin

The elastic tissue of the media was intact after perfusion with 1 or 2 units of plasmin, and the 5 lamellar units were conserved. The macroscopic and microscopic appearance of the plasmin-perfused aorta was similar to that of control aortas.

All the aortas perfused with small amounts of pancreatic elastase (1 unit) plus plasmin (2 units) developed macroscopic aneurysm in the perfused area (elastic disruption >1,500 \( \mu \)m). This aortic segment was enlarged and contained large thrombi. Three aortas had ruptured by the end of the second week. Microscopically, the area adjacent to the aneurysm was thin with fragmented elastic tissue, probably a result of diffusion.
All the aortas perfused with thioglycollate plus plasmin (2 units) developed macroscopic aneurysms (>1,500 μm) with microscopic and slight diffusion of elastic tissue lesions.

Quantification of Elastase Activity

All 10 aortas perfused with thioglycollate plus plasmin had significantly higher elastolytic activity (207.6±54.8 μg of lysed elastin-rhodamin/18 hr) than did the 10 saline-perfused control aortas (25.43±11.13 μg) (p<0.001) 9 days after perfusion.

Discussion

These experimental data with previous clinical results10–13 suggest that elastolytic activity plays a major role in aneurysm formation. Elastolytic activity has also been shown to be involved in the pathogenesis of pulmonary emphysema.18

Janoff and Scherer19 proposed that connective tissue can be damaged by leukoproteinase. The suggestion that elastase activity from neutrophils promotes lung emphysema stimulated considerable research on elastase and its pathophysiological role in connective tissue disease and its inhibition by α1-protease inhibitor. This was supported by animal experiments indicating that only elastolytic enzymes were able to produce experimental emphysema.20

Even though pathogenesis of emphysema is not superficially closely related to the pathogenesis of aortic aneurysm, emphysema remains a good model for studying the relation between the extracellular matrix (particularly elastic tissue) and the occurrence of elastolytic activity and can shed new light on the pathophysiology of aneurysm.18

The present experimental models of aortic aneurysm have all shown a failure of the medial elastin...
network. The mutation on the X chromosome of the Blotchy mouse induces a defect in intestinal copper absorption. Systemic copper deficiency leads to a dysfunction of lysyl oxidase, an enzymatic activity that affects the cross-linking of elastin and collagen fibrils. As described above, Blotchy mice develop aneurysmal dilation of arterial wall and pulmonary emphysema. The incidence of aneurysm in Blotchy mice increases with age, reaching 100% by 6 months. The anatomic variation in the sites of aneurysm formation and their basic histological structure have been determined. The progression to a markedly degenerated wall with aneurysm formation is gradual, with increasing infiltration of inflammatory cells and a progressive loss of elastic tissue.

Other models of spontaneous dissecting aneurysm are more difficult to interpret in terms of their pathophysiology. The turkey has provided another frequently studied model. Certain strains, such as the Broad-Breasted-Bronze and the Broad-white, have high blood pressure and early atheroma formation, which are lesions that occur before aneurysmal ruptures of the aorta. Most aneurysms in poultry occur in the abdominal aorta and are associated with cystic medial degeneration. It was suggested that these lesions result from defective nutrition of the aortic wall, which lacks a vasa vasoorum. The stallion has also been used as a model of spontaneous aortic aneurysm. There is medial degeneration at the level of the aortic ring, leading to aortic rupture. These models, which are more the result of dissection than true aneurysm, nevertheless show defects in the structure of the extracellular matrix within the media of the aortic wall, whatever the initial mechanism.

Economou et al first produced provoked experimental aortic aneurysms in the dog by intramedial injections of toxic acetyrlazate. The same investigators developed another model by surgical resection of 70% of the aortic media. Gertz et al produced an experimental model of arterial dilation of the rabbit common carotid artery by periarterial application of calcium chloride in vivo. There was progressive focal arterial dilation, limited to the site of calcium application. The calcium-elastic tissue complex was the focus of an inflammatory infiltrate including neutrophils, lymphocytes, monocytes, and multinucleated giant cells. This recent study suggested that the interaction of calcium with elastic fibers is an important pathogenic factor in initiating an inflammatory response and developing arterial dilation.

Yokoyama et al developed an experimental model of aortic aneurysm by administering theophylline or caffeine to embryonic chicks. Microscopic examinations revealed thinning of the media due to a marked decrease in the number of medial cells, with a widened intercellular space and fragmented, dispersed elastic and collagen fibers. This model suggests that aneurysm formation is due to hypoplasia of the arterial wall resulting from inhibition of smooth muscle cell proliferation and of the secretory function of extracellular matrix components.

Most of these spontaneous and provoked aneurysms showed a deficiency in the elastin network structure, but they were models very different from the possible pathophysiology of acquired aneurysm in humans. Clinical investigations suggest that there is an increase in elastase activity. The present model demonstrates, as do others, that the destruction of elastin network within the medial layer is a necessary step in the genesis of aneurysmal dilation. The perfusion of an isolated 1-cm segment of aorta probably caused injury of the aortic wall by hypertension and stretch. But rupture of the aortic wall never occurred with this model. The clinical restriction of aneurysm in patients to arterial area exposed to repeated external or internal stress (terminal abdominal aorta and femoral and popliteal artery) suggests that injury of the arterial wall contributes to aneurysmal dilation. This is supported by the experimental data of Bomberger et al, showing the effect of medial injury and of a hyperlipidemic diet on the genesis of aneurysm in rabbits. Nevertheless, despite the wall stress that occurred in our model, perfusion of saline or molecules without direct elastolytic activity, such as plasmin, did not induce aneurysmal dilation. Therefore, injury of the arterial wall without elastase activity is not sufficient to induce elastolysis and aneurysmal dilation.

In our model, hog pancreatic elastase destroyed the elastin network within the media and, thus, induced macroscopic aneurysm. We have attempted to induce aneurysmal dilation by incubating the external part of the aorta (after dissection and clamping) with elastase (data not shown). This manipulation induced neither elastolysis in the media nor aneurysmal dilation. Hence, elastase activity and the media layer of the aortic wall in the elastolytic process must be involved in the induction of an aneurysmal dilation.

Elastolysis is not specific to pancreatic elastase. Other proteolytic enzymes, such as papain, trypsin, chymotrypsin, and collagenase, which are not elastin specific but also attack insoluble elastin, can also induce elastic disruption and small aneurysmal dilatation.

Clinical investigations have shown that the size of and prognosis for aneurysms are proportional to the elastase activity within the aortic wall. In our experimental model, the extent of elastin destruction and the appearance of microscopic and macroscopic lesions were related to the amount of perfused elastase. Although the elastase activity in the pathophysiology of acquired aneurysmal dilation cannot be compared with a simple model of pancreatic elastase perfusion, this pharmacological manipulation confirms that elastase activity, whatever its origin, can cause destruction of the elastin network and, hence, aneurysmal dilation.

Macrophage activation was induced with thioglycollate to provoke in situ elastase secretion as in the probable pathophysiology of acquired aneurysm. White cells can secrete elastase activity as has been shown in studies on the pathophysiology of emphysema. There are two enzymatically distinct
types of elastin breakdown by inflammatory cells: classic neutrophil-derived soluble elastase that is sensitive to protease inhibitors and the macrophage-mediated breakdown that is largely cell associated and relatively resistant to inhibitors. Human neutrophils produce a serine proteinase that is active at neutral pH and has marked elastolytic capability. The enzyme has been purified and characterized. Neutrophil elastolytic activity depends on the secretion of soluble enzymes that are more readily inhibited by soluble protease inhibitors. This dependence on soluble elastase for elastin degradation can explain the absence of direct contact between elastin and neutrophils.

Murin macrophages contain three elastinolytic proteinases: a macrophage-synthesized metalloprotease, a serine protease that is probably neutrophil elastase internalized by the macrophage, and a cell-surface thiol-protease. The metalloenzyme is synthesized in small amounts but it secreted into the medium and is resistant to inhibition by the serum. By internalizing neutrophil elastase, the macrophage protects this enzyme from α-protease inhibitor and carries it to sites of inflammation. Resident macrophages adhere to the extracellular matrix. As shown by in vitro experiments, elastase inhibition by surrounding inhibitors is impaired when the cells are in close contact with the substrate. The macrophage surface-associated thiol-protease is a very efficient elastase when the cells contact the substrate because the elastinolytic reaction is not inhibited by serum elastase inhibitors. Chapman et al. observed that elastin degradation by cells in contact with the substrate was 10–30-fold higher than degradation by cells in the same culture that were physically separated from elastin.

Our model confirms, in vivo and in the arterial wall, that passive transfer of activated macrophages or direct activation of macrophages within the arterial wall by thioglycollate can induce “in situ” elastolytic activity. The elastolytic activity of macrophages seems to be limited to the vicinity of inflammatory cells as in “in vitro” studies. Nevertheless, induction of macrophage activation within the arterial wall can induce limited elastolysis areas and some aspects of aortitis without true aneurysm. This experimental result can be explained by insufficient elastolytic activity associated with the activated macrophages.

In vitro studies have demonstrated that plasmin can cooperate with elastolytic activity. Latent collagenase and plasminogen activator are also secreted by macrophages. These proteinases may act in cooperation to cleave elastin fibers in vitro. Werb et al. and Jones and Werb examined the degradation of metabolically labeled extracellular matrix rich in elastin, glycoprotein, and collagen by intact thioglycollate-induced activation of macrophages cultured on the matrix. They observed that plasmin accelerated elastin degradation by macrophages only when the matrix contained glycoproteins. They concluded that the role of plasmin was to eliminate matrix protein that restricted the interaction of macrophage elastase with the matrix elastin. Chapman et al. also observed a similar phenomenon. Human alveolar macrophages cultured on matrix containing a mixture of fibrin and elastin only degraded the elastin in the presence of plasmin. Banda and Werb reported that macrophages secrete up to 80% of their elastase in a latent form that can be activated by sodium dodecyl sulphate, whereas Chapman et al. suggested that plasmin can also activate latent macrophage elastase activity in the presence of elastin. Plasmin can cooperate with elastase activity either by attacking matrix glycoproteins or by activating a pro-elastase.

All the above “in vitro” studies indicate that elastin degradation is a cooperative process involving multiple proteinases. Plasmin and elastase are especially effective in degrading insoluble elastin. Plasmin alone has no measurable effect on purified insoluble elastin. Serum and serum-derived tissue proteinase inhibitors can localize elastase activity in the vicinity of the cell surface.

Our model provides in vivo confirmation of the cooperation between elastase and plasmin in the degradation of the elastin network. A low concentration of elastase, which did not induce aneurysmal formation alone, induced aneurysm in the presence of plasmin. These results demonstrate that plasmin can facilitate the interaction between elastase and its insoluble substrate in vivo, whereas plasmin has no direct elastolytic activity. Plasmin also cooperated with elastase activity from in situ activated macrophages in our model. Thioglycollate-activated macrophages only induced elastolysis area in the elastin network and produced an appearance of aortitis, without true aneurysm. Similar manipulations in the presence of plasmin, which had no direct elastolytic activity, induced an aneurysm in all cases.

This point is very important in the pathophysiological evolution of a human aneurysm toward rupture because fibrinoid thrombus is always present in aneurysms. But neither in vitro data nor the present in vivo results define the role of fibrinoid thrombus in the evolution of aneurysm. Fibrin induces fibrinolytic activity, which can cooperate with elastolytic activity.

Our morphological results have been confirmed by measurements of elastolytic activity within the aortic wall of rats perfused with thioglycollate plus plasmin. A proteinase can be considered an elastase if it can solubilize mature cross-linked elastin. Several proteinases having different catalytic sites can do this. The elastolytic property of a given proteinase, therefore, cannot be predicted from its ability to cleave a synthetic substrate or even a soluble elastin derivative. Its action must be verified experimentally on insoluble elastin. Hence, insoluble elastin must be used to quantify the “elastase activity” of cell or tissue extracts. Of course, once a proteinase has been shown to possess elastinolytic activity, any convenient artificial substrate may be used to study the enzyme. Elastin covalently labeled with rhodamine-β-
isothiocyanate was used in this study because it is a very sensitive substrate and because its soluble breakdown products are readily quantified.17 We confirmed that activated macrophages plus plasmin can induce a very significant attack on insoluble elastin.

In conclusion, the present experimental model of in vivo aortic aneurysm in the rat has provided evidence indicating that the presence of elastolytic activity within the aortic media can induce aneurysm development. Other proteases can also attack the elastin network and induce aneurysmal dilation. There is a quantitative relation between perfused elastase and aneurysm formation. Activated macrophages within the aortic media may be responsible for the secretion of elastase and for elastolysis, and plasmin enhances macrophage elastolytic activity in vivo.

Acknowledgments

We thank Drs. J.G. Bieth and C. Boudier, INSERM U237, Strasbourg, France, for their assistance.

References


Key words • macrophages • plasmin • arterial wall • elastic tissue
Elastase-induced experimental aneurysms in rats.
S Anidjar, J L Salzmann, D Gentic, P Lagneau, J P Camilleri and J B Michel

_Circulation_. 1990;82:973-981
doi: 10.1161/01.CIR.82.3.973
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/82/3/973