Experimental Aortitis
Aortic Lesions Induced by a Serine Protease
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SUMMARY
The etiology of aortitis syndrome (Takayasu's arteritis) is unknown. This study was designed to show whether aortic and pulmonary artery lesions might be induced by a small dose of protease in the circulating blood of rabbits.

Serum trypsin activity was increased transiently but significantly by an intravenous infusion of the enzyme at the rate of 1000 IU/min for 100 min. The aortic wall was significantly thickened. Marked edema and focal tears were seen in the intima and media and a mild inflammatory reaction in the adventitia of rabbits sacrificed 2 weeks following trypsin infusion. The lesions were also present in rabbits sacrificed at 6 weeks. They were observed in 7 of 8 rabbits given the same dose of chymotrypsin-A4, but not in control rabbits infused with saline alone.

The lesions were observed frequently in the ascending aorta and arch, but were rare in the descending aorta. The main pulmonary artery showed similar lesions in about half of the studied rabbits.

These results show evidence that a diffuse lesion of the aorta and pulmonary artery may be induced by a small dose of serine protease in the circulating blood.

Additional Indexing Words:
Aortitis syndrome Takayasu's arteritis Fibronectin Pulmonary artery lesion

AORTITIS syndrome (Takayasu's arteritis) is a chronic inflammatory disease of the aorta, main branch arteries and pulmonary artery, but the etiology remains unknown. An experimental model would be useful for studying its etiology, but unfortunately none exists at present.

Fibronectin, a glycoprotein, connects smooth muscle cells to fibrous
elements in vascular walls,\textsuperscript{31-5} but is dissolved by serine protease.\textsuperscript{6} A part of this protease activity is present transiently in the circulating blood in spite of inhibition by serum proteins.\textsuperscript{7} Therefore, it is probable that vascular wall tissue might be damaged by a small amount protease in the circulating blood. Because of its elastic architecture and hemodynamic exposure, the aorta may be injured more severely than other vessels.

This study was designed to show whether a diffuse lesion of the aorta and pulmonary artery could be induced in rabbits by an intravenous infusion of a small dose of serine protease.

**Materials and Methods**

Twenty-four rabbits (2.5–3.0 Kg in weight) were used. They were anesthetized with 25 mg/Kg of pentobarbital sodium (iv). Anesthesia was maintained by an additional 25 mg added at 20 min intervals during the protease infusion.

A protease solution was infused intravenously through an ear vein at a rate of 1 ml/min (1000 IU/min) for 100 min. The rabbits were sacrificed at 2 or 6 weeks after the infusion. At that time the aorta and pulmonary artery were perfused from the heart for 30 min with a 6% solution of glutaraldehyde in saline. After being fixed by the infusion, the intact aorta and main pulmonary artery were taken out with the heart and again fixed for 1 week. The ascending aorta, aortic arch at the level of the subclavian artery, descending thoracic aorta at the level of the diaphragm and main pulmonary artery were examined by routine histological methods and HE stain. The diameter and wall thickness of the ascending aorta were measured with a microscope.

The protease solution was prepared as follows: Bovine crystalline trypsin (Mochida) or bovine crystalline chymotrypsin-A4 (Sigma) was used. Each enzyme was dissolved to a concentration of 1000 international units/ml in saline at the beginning of the infusion. Trypsin was infused to 12 rabbits. Eight of them were sacrificed at 2 weeks after the infusion and 4 at 6 weeks. Saline was infused by the same methods to 4 rabbits that were sacrificed at 2 weeks and that served as a control group.

Serum trypsin activity was determined in 6 of the rabbits following trypsin infusion by Haverback's\textsuperscript{8} and Hayakawa's\textsuperscript{9} method. Briefly, 0.1 ml of serum, 1.0 ml of buffer solution and 1.0 ml substrate solution were mixed and incubated for 60 min in a 37°C water bath with agitation. The reaction was then stopped by adding 2.0 ml of 30% acetic acid. The enzyme activity was determined by absorbance at 410 μm with a spectrophotometer (Hitachi
The blank was made as follows: The same serum and the substrate were incubated separately and then mixed simultaneously with 30% acetic acid. The serum was used immediately after sampling 1.0 ml of venous blood from the opposite ear. The substrate solution contained 1.0 mg/ml of \( \alpha \)-N-benzyl-DL-arginine p-nitroanilide·HCl (Sigma) in distilled water. The buffer solution was Tris buffer (pH 8.0, 0.1 M) with 0.04 M CaCl\(_2\). The standard curve was made using solution of a known trypsin activity. Student’s t test was used for statistical analysis.

**RESULTS**

1. Serum trypsin activity in 6 rabbits following trypsin infusion

   Serum trypsin activity was 8.5±2.5 units/ml before the infusion, and 33±11 at 15 min (p<0.001 vs before), 44±10 at 30 min (p<0.001), 52±15 at 60 min (p<0.001) and 52±14 at 100 min (p<0.001) during the infusion. After stopping the infusion, the activity decreased gradually to 48±17 units (p<0.001) at 15 min, 30±19 at 60 min (p<0.005) and 6.5±1.9 at 24 hours (NS). These results showed that serum trypsin activity was elevated transiently and significantly by the infusion.

2. Histology of the ascending aorta

   Trypsin infusion: The intima and media of the ascending aorta of rabbits sacrificed at 2 weeks after the infusion was markedly edematous. The smooth muscle cells were atrophic and focal tears (interruption of a few muscle layers) in the media were observed. The adventitia showed a mild inflammatory reaction involving an increase in capillary vessels and collagen fibers with a few inflammatory cells (Figs. 1 and 2). The wall thickness of

![Fig. 1. Ascending aorta of a control rabbit that was infused with saline, showing no lesions. I=intima; M=media; A=adventitia. (×300)](image)
Fig. 2. Ascending aorta 2 weeks after trypsin infusion. The wall was thick, compared to the control. Magnification is the same as Fig. 1. (×300) The intima (I) and media (M) were markedly edematous, and the adventitia (A) showed a mild increase in capillary vessels and collagen fibers with a few inflammatory cells.

the ascending aorta was 0.50±0.07 mm in the 8 treated rabbits and 0.27±0.04 mm in 4 control rabbits (p<0.001). The ratio of wall thickness/transverse outer diameter was 0.16±0.03 in 8 and 0.09±0.01 in 4 control rabbits (p<0.05) (Table I). Similar lesions were observed in 4 rabbits sacrificed at 6 weeks after the infusion (Fig. 3).

Chymotrypsin-A4 infusion: The ascending aorta of rabbits sacrificed

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Follow up period (weeks)</th>
<th>No. of rabbit</th>
<th>Histology (No. of rabbit)</th>
<th>Main pulmonary artery (edema, tear)</th>
<th>Size of ascending aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Edema</td>
<td>Focal tear</td>
<td>Mild inflammation</td>
</tr>
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<td>Saline (Control)</td>
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<td>4</td>
<td>0/4</td>
<td>0/4</td>
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<td>Trypsin</td>
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</tr>
<tr>
<td>Chymotrypsin-A4</td>
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<td>4</td>
<td>3/4</td>
<td>2/4</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>8</td>
<td>6/8</td>
<td>7/8</td>
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</tbody>
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The table shows number of rabbits with each lesion/total studied. * p<0.001, +p<0.01, ** p<0.05 vs control.
Fig. 3. Ascending aorta 6 weeks after trypsin infusion. A focal tear and related fibrous changes were observed in the intimal side of the media. I=intima; M=media; A=adventitia. (×150)

Fig. 4. Ascending aorta 2 weeks after chymotrypsin-A4 infusion. A small focal tear (arrow) in the intimal side of the media, mild edema of the intima (I) and media (M), and mild inflammatory reactions of the adventitia (A) were observed. (×150)

Fig. 5. A large focal tear of the ascending aorta seen 2 weeks after chymotrypsin-A4 infusion. I=intima; M=media; A=adventitia. (×300)
at 2 weeks after the infusion was edematous but less so than those of rabbits infused with trypsin. Focal tears were observed frequently and the adventitia also showed a mild inflammatory reaction (Figs. 4 and 5). The wall thickness was $0.39 \pm 0.09$ mm (p<0.01 vs control) (Table I).

Saline infusion (Control): The 4 control rabbits showed minimal edema in a few layers of the intimal portion of the media. There was no evidence of marked edema, focal tears or inflammatory reaction (Fig. 1).

3. Distribution of the arterial lesions

Trypsin infusion: Marked edema of the ascending aorta or arch was observed in 11 of 12 rabbits sacrificed at 2 and 6 weeks, and focal tears were present in 4 of 12. Mild inflammatory reactions were observed in the adventitia of all 12. The main pulmonary artery showed edema in 6 of 12 and a focal tear was present in 1 of 12. The descending aorta showed edema in 1 of 12 but none had focal tears (Fig. 6).

Chymotrypsin-A4 infusion: Edema of the ascending aorta or arch was present in 6 of 8 rabbits, but focal tears were more frequent than in the trypsin infused rabbits, being observed in these areas in 7 of 8 rabbits. The pulmonary artery showed edema in 3 of 8 and a focal tear in 1. None of the 8 rabbits exhibited edema or focal tears of the descending aorta (Fig. 6).

The lesions were more common in the ascending aorta and arch. Edema

![Fig. 6. Distribution of arterial lesions. The black column is the number of rabbits with focal tear and the gray the number of rabbits with edema. These are out of a total of 8 rabbits infused with chymotrypsin-A4 and of 12 rabbits infused with trypsin and sacrificed at 2 or 6 weeks. Both focal tears and edema were frequently seen in the ascending aorta and arch, in the main pulmonary artery (pulmonary trunk) in about half, and rarely in the descending aorta at the level of diaphragm. The edema was more frequent following trypsin infusion and focal tears more frequent following chymotrypsin-A4 infusion.](image-url)
was more common following trypsin infusion and focal tears more frequent following chymotrypsin-A4 infusion.

**DISCUSSION**

Marked edema and focal tears of the aorta were observed frequently in rabbits infused intravenously with trypsin or chymotrypsin-A4. The wall of the ascending aorta was significantly more thickened in the enzyme infused rabbits than in the control. The main pulmonary artery lesions may be induced by an intravenous drip infusion of a small dose of protease.

Serum trypsin activity, measured simultaneously in 6 of the rabbits, was transiently but significantly elevated by the infusion. Trypsin and chymotrypsin combine immediately with serum trypsin inhibitor or macroglobulins in the circulating blood, but it was evident from in vitro studies that the enzyme has some activity even when complexed with serum globulins. Thus, the induced arterial lesions seem to be related to the enzymatic action on the vessel walls.

The complex of the enzyme and serum macroglobulin in the circulating blood permeated immediately through vessels and the serum concentration decreased by half in 6–8 min. The complex, however, was shown to remain in the connective tissue for 30 min in studies by Ohlsson et al. Serine protease dissolves fibronectin by which the tissue elements of vascular wall are connected. Therefore, when the active enzyme remains in the circulating blood or in the vascular wall, it seems to dissolve the fibronectin of the vascular wall and it may weaken the wall. Vasoactive agents such as histamine released by the proteases may have some effects on the induced lesions.

The architecture of the aorta and pulmonary artery consists of alternating layers of smooth muscle cells and elastic fibers. This architecture may be injured more easily by fibronectin dissolution than that of a muscular type artery. In addition, the aorta and main pulmonary artery may be affected more directly by hemodynamic forces than other peripheral vessels. Marked edema and focal tears were frequently observed in the ascending aorta, arch and main pulmonary artery in this study. This is evidence that the enzyme may readily induce lesions in large elastic arteries.

The rabbits did not show any changes during the infusion, although tachypnea and peripheral vascular dilatation occasionally appeared for short periods of time. Body weight did not change during the study. No other organs showed any macroscopic changes at autopsy regardless of time of sacrifice following infusion although they were not examined histologically.
The distribution of arterial lesions in this study was like that in aortitis syndrome, but the induced lesions were different in that they exhibited more edema and focal tears, and less inflammatory reactions than those of aortitis syndrome. One of the reasons for this may be that the lesions in this study were acute, compared to the chronic inflammation in aortitis syndrome. The lesions seen in rabbits sacrificed at 6 weeks were almost same histologically as in those sacrificed at 2 weeks. Six weeks follow-up might be too short to study the course of these lesions.

In conclusion, the results show evidence that a diffuse lesion of aorta and pulmonary artery may be induced by a serine protease.

References