Blood Fibrinolytic Activity and Fibrinoid Necrosis of Small Arteries in the Rats Treated with Renal Cortical Extract

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SUMMARY

In order to determine the role of fibrinolytic system in the production of fibrinoid necrosis, blood fibrinolytic activities were measured in the rats nephrectomized and administered with the subcellular fractions (lysosomal or microsomal fraction) of the rat kidney cortical extract.

Tissue plasminogen activator was present in both fractions and blood fibrinolytic activity was increased in the rats so treated. But fibrinoid necrosis in pancreatic and mesenteric small arteries was observed only in those administered lysosomal fraction.

Direct relationship between the increase of blood fibrinolytic activity and the aggravation of the vascular degeneration could not be observed in this experiment.

Additional Indexing Words:
Tissue plasminogen activator Subcellular fraction

Fibrinoid necrosis was found in the small arteries of rats, nephrectomized and injected with the renal cortical extracts. Renin\(^1\) and/or vascular permeability factor\(^2\) was considered responsible for the production of fibrinoid necrosis in these experimental conditions. Jellinek\(^3\) proposed that the fibrinoid degeneration was caused by the damage of blood-arterial wall barrier and consisted with the diffusion of fibrin/fibrinogen and plasma protein together with degenerating cellular and fibrous elements, and pointed out the increase of fibrinolytic activity as one of the possible causes.

We previously observed that the lysosomal fraction in the subcellular fractions of the rat kidney cortical extracts had the highest renin activity\(^4\) and it produced severe fibrinoid necrosis in the small arteries of pancreas and mesenterium of bilaterally nephrectomized rats.\(^5\) In the same experiment, no vascular changes were produced by the microsomal fraction. Lewis

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and Ferguson\textsuperscript{6}) reported that fibrinolytic activity was present in the microsomal fraction of kidney extract.

This study was undertaken to examine the relationship between blood fibrinolytic activity and vascular changes in animals administered the kidney extracts in order to know whether the fibrinolytic system affects the production of fibrinoid necrosis.

**Materials and Methods**

Female rats of Wistar-King strain obtained from the Kyushu University Animal Center, were used in this study. Rat kidney cortex was separated by razor blades and homogenized with physiological saline. Subcellular fractionation of the cortical extract was done according to the method of Shibko and Tappel.\textsuperscript{7}) Renin concentration of the fractions was measured in bioassay by the method of Conradi et al.\textsuperscript{8}) Blood fibrinolytic activity was determined by euglobulin lysis time. The fibrinolytic activity of kidney extracts was examined on the standard fibrin plate made by plasminogen rich bovine fibrinogen and bovine thrombin. Standard histological staining procedures such as HE, PAS, PTAH, and AZAN were used for histological examination of the tissues fixed with 10\% formalin. Vascular permeability of serum proteins was observed by the immunohistological method, using FITC conjugated anti-rat serum rabbit gamma globulin and fluorescence microscope.\textsuperscript{9})

Two ml of kidney extracts (lysosomal or microsomal fraction) which contained 2 mg of protein, were intraperitoneally injected into rats in conscious state 6 hours after bilateral kidneys have been extirpated. The equal volume of physiological saline was used in the control group. In serial time intervals, such as 15, 30, 60, 120, 240 min, and 18 hours after the kidney extracts or saline injection, experimental animals were anesthetized by intraperitoneal injection of amobarbital sodium and sacrificed by bleeding from aorta to examine blood fibrinolytic activities and vascular lesions in mesenterium and pancreas.

**Results**

1. Renin concentration in the subcellular fraction.

In this work lysosomal and microsomal fraction were used, because the former had the highest renin activity and the high ability to induce vascular lesion and the latter neither had renin nor produced vascular lesions.\textsuperscript{5}) Renin concentration in the lysosomal fraction used in this study was 1.25 \( \mu \)g of synthetized angiotensin II/mg of protein. The microsomal fraction did not show the activity.

2. Tissue plasminogen activator activity in lysosomal and microsomal fraction.

In saline extractions, microsomal fraction had higher fibrinolytic activity than lysosomal fraction as shown in Table I. The same tendency was found
Table I. Tissue Plasminogen Activator Activity Examined by Standard Fibrin Plate Method

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Lysosomal fraction</th>
<th>Microsomal fraction</th>
</tr>
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<tbody>
<tr>
<td>Saline</td>
<td>34.8 ± 4.5</td>
<td>96.7 ± 15.8</td>
</tr>
<tr>
<td>2M KSCN</td>
<td>136.0 ± 31.7</td>
<td>176.8 ± 37.1</td>
</tr>
</tbody>
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Lytic area was expressed by mm². Mean ± SE.

![Graph](image)

Fig. 1. Blood fibrinolytic activities expressed as euglobulin lysis time (min) in rats injected with lysosomal fraction (L), microsomal fraction (M), or physiological saline (S). Mean ± SE.

in 2M KSCN extractions. No activity was present when heated fibrin plate was used.

In the following experiments, the fractions of saline extraction were used in order to avoid the toxic action of KSCN, although the materials extracted by 2M KSCN showed higher fibrinolytic activities than those by saline.

3. Changes in blood fibrinolytic activity after kidney extract administration.

Lysosomal or microsomal fraction or saline was injected into 6 hours-nephrectomized rats and after serial time intervals the rats were sacrificed to take blood from aorta for determination of fibrinolytic activity. The result was shown in Fig. 1. Euglobulin lysis time in control rats of saline injection was between 196 and 216 min and was fairly stable during the period of 15 to 240 min.

Significant shortening of the euglobulin lysis time was revealed at 60 min after the injection of lysosomal fraction and also at 120 min after microsomal fraction. It returned to the previous level or somewhat more prolonged than that in 2 to 3 hours.

Eighteen hours later, fibrinolytic activity could not be detected even in
Fig. 2. Fibrinoid necrosis in pancreatic arteries in rats injected with lysosomal fraction (L), microsomal fraction (M), or physiological saline (S). Immunohistological examination in 4 hours' experiment (left) and light microscopic examination in 18 hours' experiment (right).

Fig. 3. Fibrinoid necrosis in pancreatic small artery observed 18 hours after the injection of lysosomal fraction. HE ×260.

360 min incubation, the maximum duration of the measurement.

4. Vascular lesions (Fig. 2).

The typical arterial lesion (fibrinoid necrosis) was found by microscopic examination in pancreatic and mesenteric small arteries of the rat 18 hours after the injection of lysosomal fraction (Fig. 3).

Plasma protein deposition in the arterial wall in pancreas and mesenterium, which was considered the earlier changes of the fibrinoid necrosis, was observed in rats 4 hours and/or 18 hours after the administration of lysosomal fraction (Fig. 4).

Any vascular abnormalities were found neither in rats within 4 hours after injection of lysosomal fraction nor in all rats injected with microsomal fraction or saline.
DISCUSSION

Tissue plasminogen activator in the subcellular fractions of rat kidney cortical extract was found to be higher in microsomal fraction than in lysosomal fraction as was reported by Lewis and Ferguson in the dog tissues. The similar result was shown by Tignon and Palade in the subcellular fraction of rat lung. Ali and Lack, however, described that the lysosome-rich fraction of rabbit kidney had the high proportion of the tissue activator of plasminogen. The discrepancy may have been caused by the species difference of the animals.

Increase in blood fibrinolytic activity detected by the measurement of euglobulin lysis time was found earlier in rats injected with lysosomal fraction than in those with the microsomal fraction, even though the latter showed, in vitro, the higher tissue plasminogen activator activity. The protease activities, which were in lysosomal fraction and capable of attacking fibrinogen, should cause the shortening of fibrin clot lysis time.

Deposition of serum protein in pancreatic and mesenteric small arterial walls occurred predominantly in the animals given lysosomal fraction at the 4 hours experiment. The vascular change was thought to be an early manifestation of fibrinoid necrosis, observed by light microscope at the 18 hours experiment.

In the rats treated with the microsomal fraction the time of maximum increase in fibrinolytic activity was somewhat delayed, but no vascular changes could be demonstrated by fluorescent or light microscope. These data suggest that the increase in blood fibrinolytic activity elicited by tissue
plasminogen activator present in kidney extract is not a main factor of fibrinoid necrosis.

As to morphogenesis of plasmatic arterionecrosis, Ooneda et al.\(^{(1)}\) emphasized the importance of blood plasma infiltration into the arterial wall. And they considered the kidney as one of the factors which increase the vascular permeability. We previously reported that the renin rich fraction in the kidney extract had the highest pressor activity and caused a marked increase in vascular permeability. And we concluded that renin should be the most important factor to cause fibrinoid necrosis.\(^{(4)}\) Non-pressor factor(s) in the kidney had also been considered as a possible cause of the vascular injuries, such as permeability factor,\(^{(3)}\) necrotizing factor\(^{(13)}\) or non-pressor vascular lesion inducing factor.\(^{(14)}\) But there was no observation reported concerning the role of fibrinolytic activity in the development of the same kind of vascular lesions.

The present study may indicate that an increase in blood fibrinolytic activity, per se, is not a direct cause of fibrinoid necrosis. However the possibility could not completely be denied from the present experiment that it can somehow be related to the causative mechanism of fibrinoid necrosis.

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REFERENCES