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Developmental Changes of Vascular Lesions Produced by Renal Cortical Extract

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Periarteritis nodosa-like changes were reportedly caused by hypertension and immunological factor. The author experimentally produced necrotic change of arterioles and small arteries by administering a fraction of the rat renal cortex to the rat of unilateral nephrectomy and temporary contralateral urethral ligation, and carried out long-term observations of these vascular changes. As a result, it was demonstrated that these vascular changes developed to periarteritis nodosa-like change and fibrous thickening, independent from hypertension and immunological factor.

INTRODUCTION

Since Goldblatt’s renal artery constriction was introduced in 1934,1) various kinds of experimental hypertension have been produced. Many of the methods involve renal operation such as Goldblatt’s method, renal infarction, silk-perirenal operation and partial constriction on the aorta between the origin of the two renal arteries. Most of the animals that became hypertensive showed vascular diseases and these are generally called hypertensive vascular diseases.2) However, in these experiments the increase of blood pressure and vascular diseases were not necessarily in parallel with each other. As factors of renal origin other than the pressor factors such as renin–angiotensin, some vascular necrotizing
factor had been anticipated to exist. In the present experiment, a factor to show vascular permeability activity and vascular necrotizing activity without increasing blood pressure (vascular permeability and necrotizing factor: VPNF) was obtained from the rat renal cortex by ultracentrifugation. Since the time of observation of the rat with bilateral nephrectomy was restricted to only about 24 hours, longer survival of the rat was desired and unilateral nephrectomy and contralateral urethral ligation were performed which was followed by the removal of the urethral ligation 24 hours after the intravenous injection of VPNF. A long-term study of vascular diseases by this renal extract revealed changes from angionecrosis to periarteritis nodosa like change and cellulofibrous medial and/or intimal thickening.

**MATERIAL and METHODS**

Wistar strain female rats were used. Normal rats weighing 100-200 g were perfused from the abdominal aorta with physiological saline solution added with heparin (heparin 2500 units/physiological saline solution 1000 ml). After the removal of blood components, the kidney was resected and the renal cortex was collected. The renal cortex was crushed into pieces and 0.25 M sucrose was added to the renal cortex at the ratio of 3 ml to 1 g. The preparation was divided into four fractions by modified Hogeboom’s method. Dialysed Fraction 3 which consisted mostly of microsome was used in the present experiment. Beckman Model L-2 Rotor type 50 was used for ultracentrifugation. Experiments were performed at 0－5°C (Table 1).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The Fractionation of Rat Renal Cortex</th>
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<tbody>
<tr>
<td>Cortex of Rat Kidney</td>
<td>↓</td>
</tr>
<tr>
<td>Mince in 0.25M Sucrose</td>
<td>↓</td>
</tr>
<tr>
<td>Homogenize for 20sec. at 2000 r. p. m. in 0.25M Sucrose</td>
<td>↓</td>
</tr>
<tr>
<td>Centrifuge for 30 min. at 15000 r. p. m.</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Supernatant</td>
</tr>
<tr>
<td></td>
<td>↓</td>
</tr>
<tr>
<td>Ultracentrifuge for 60 min. at 40000 r. p. m.</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Supernatant (Fraction 4)</td>
</tr>
<tr>
<td></td>
<td>↓</td>
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<tr>
<td>Dialysis against 0.9% NaCl overnight</td>
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Normal rats weighing 100－150 g were subjected to unilateral nephrectomy and contralateral urethral ligation and the injection of dialyzed Fraction 3 at the dose of 6 mg of protein per 100 g of body weight from the caudal vein. Urethral ligation was removed 24 hours after the injection. The rats were autopsied upon collection of blood sample at the intervals of 24, 36, 48 hours, 3, 5 days, 1, 2 months after the injection, respectively.
A group with only surgical operation without injection and another group with injection without surgical operation were examined as the control groups (Table 2). The main objects of examination were the mesenterial and pancreatic vessels.

### Table 2

<table>
<thead>
<tr>
<th>Number of rats</th>
<th>130</th>
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<tbody>
<tr>
<td>1. To obtain extract</td>
<td></td>
</tr>
<tr>
<td>11. Control group</td>
<td></td>
</tr>
<tr>
<td>(1) Unilateral nephrectomy and contralateral urethral ligation without injection of extract</td>
<td>6</td>
</tr>
<tr>
<td>(2) Injection of extract without unilateral nephrectomy and contralateral urethral ligation</td>
<td>8</td>
</tr>
<tr>
<td>111. Experimental group</td>
<td></td>
</tr>
<tr>
<td>(1) Sacrificed at injection of extract</td>
<td>6</td>
</tr>
<tr>
<td>(2) Sacrificed 24 hours after injection of extract</td>
<td>15</td>
</tr>
<tr>
<td>(3) Sacrificed 36 hours after injection of extract</td>
<td>6</td>
</tr>
<tr>
<td>(4) Sacrificed 48 hours after injection of extract</td>
<td>8</td>
</tr>
<tr>
<td>(5) Sacrificed 3 days after injection of extract</td>
<td>10</td>
</tr>
<tr>
<td>(6) Sacrificed 5 days after injection of extract</td>
<td>10</td>
</tr>
<tr>
<td>(7) Sacrificed 1 week after injection of extract</td>
<td>15</td>
</tr>
<tr>
<td>(8) Sacrificed 2 weeks after injection of extract</td>
<td>8</td>
</tr>
<tr>
<td>(9) Sacrificed 1 month after injection of extract</td>
<td>6</td>
</tr>
<tr>
<td>(10) Sacrificed 2 months after injection of extract</td>
<td>6</td>
</tr>
</tbody>
</table>

The specimens were fixed with 10% formalin, dehydrated, embedded in paraffin and made into serial sections of 4μ in thickness. The sections were stained with Hematoxylin–Eosine stain, Azan–Mallory stain, elastic fiber stain by Weigert’s Resorcin–Fuchsin method, PAS stain and PTAH stain.

Blood pressure was determined with an automatic recording apparatus USM 105–T type and an automatic equilibrium recorder AS 13 type before operation, after unilateral nephrectomy and contralateral urethral ligation, at the time of injection of dialyzed Fraction 3, after the removal of urethral ligation, at the intervals of one week and before sacrificing the rats. From the blood sample collected at the time of sacrificing, serum was separated, and Blood Urea Nitrogen (BUN) level was determined and the production of antibody against renal cortical extract was examined by Ouchterlony method.

**RESULT**

1. **Macroscopic Finding**

Twenty-four hours after injection of renal cortical extract, the kidney on the side of urethral ligation showed mild hydronephrosis. Moderate hydrothorax and ascites were
also present in 7 of the 8 rats. Pancreatic edema was seen in 6 rats. Hemorrhagic spots were noted scatteringly in the mesentery of 3 rats. However, cerebral hemorrhage was not observed. Hydronephrosis which was slight two days after the injection of renal cortical extract, i.e., one day after the removal of urethral ligation, was not observed in any rat after the third day. After one week, a small amount of hydrothorax and ascites was present in 8 of the 15 rats and edema and hemorrhagic spots in the pancreas and the mesentery were noted in 12 rats. After two weeks, these findings were not present in any of the 8 rats but a small amount of hydrothorax and ascites still remained in a rat. In the rats after one month, no macroscopic change was present. In the control group, slight hydronephrosis as in the index group was present 24 hours after urethral ligation but it disappeared on the third day. Hydrothorax and ascites as well as hemorrhagic spots were not observed in any rat.

2. Microscopic Finding

Twenty-four hours after the injection of renal cortical extract, such findings mostly consisting of degeneration or necrosis of medial smooth muscle cells accompanied with fibrin-like material were noted in arterioles and small arteries. There was hardly any change in endothelial cells light microscopically nor was any change in the adventitia. The above changes were observed arterioles and small arteries. Hemorrhage form necrotized vessels were occasionally observed. The medial smooth muscle cells with degeneration and necrosis showed indistinct demarkation of cells (Photo. 1 and 2). Thirty-six hours after, angionecrosis covered the entire wall of the vessel. The number of degenerated and necrotized cells decreased. There was no infiltration of inflammatory cells (Photo. 3). Three days after injection, there were observed breakage of elastic fibers and hemorrhagic figure (Photo. 4).

Five days after injection, proliferation of cells being accompanied by fibrinoid degeneration was observed mostly in the media and adventitia. These proliferative cells consisted of spindle cells, lymphocytes, neutrophils and plasma cells (Photo. 5, 6 and 7). One week after, the proliferation of cells was present also in the intima. These changes were observed in many vessels and well resembled the lesions of vessels in periarteritis nodosa. The proliferation of cells was accompanied by fibrinoid degeneration in some vessels and not accompanied in some others (Photo. 8). In these vessels, the elastica lamina interna showed extension, dissolution and breakage (Photo. 9 and 10). At this time, the number of vessels with changes was reduced as compared with that 24 hours after injection.

At the lapse of 1–2 months after intravenous injection of renal cortical extract, localized increase of elastic fibers was observed at the thickened intima. Double layer structure was observed in some vessels (Photo. 11). Cellulosfibrous thickening of the media was also present in some other vessels (Photo. 12). Inflammatory cells such as neutrophils and lymphocytes were absent. Fibrinoid degeneration still remained in part of a few vessels. At this stage, the number of vessels with changes was further reduced as compared with that one week after the administration of renal cortical extract.
No histological change of vessels was observed in the control groups.

3. Blood Pressure

Blood pressure was low when urethral ligation was removed. However, 48–72 hours after, blood pressure returned to the value before treatment and retained normal value thereafter. There was no increase of blood pressure at the time of VPNF injection and during the entire course of experiment. In the control group, blood pressure at removal of urethral ligation was less reduced compared with the experimental group and the restoration of normal blood pressure was quicker (Fig. 1).

Fig. 1

4. Others

The production of antibody against rat serum VPNF was not observed by Ouchterlony method.

Serum BUN level increased in the group of unilateral nephrectomy and contralateral
urethral ligation, particularly in the group of renal extract injection, and gradually returned to normal level upon removal of urethral ligation (Fig. 2). There was no increase in the group of only renal extract injection without surgical procedure.

There was no significant difference in urine protein level between the group of unilateral nephrectomy and contralateral urethral ligation with renal extract injection, and the control groups (Fig. 3).

**DISCUSSION**

There have been a number of experiments to study vascular diseases by the use of extracts obtained from various organs.\(^6\)\(^7\)\(^8\)

Experiments to study vascular diseases using renal extract obtained by the centrifugal method or Sephadex method have been numerous such as those conducted by WINTER-NITZ,\(^9\) ASSCHER,\(^9\) KINOSHITA,\(^10\) YASUMURA,\(^11\) YAMAGUCHI,\(^12\) and others.\(^13\)\(^-\)\(^22\)

However, there is diversity in opinion among them as to whether the vascular necrotizing factor in the renal extract and the pressor factor consisting mostly of renin–angiotensin are identical or not. ASSCHER\(^9\) produced experimental hypertension and hypertensive vascular disease upon administration of endocrine kidney abstract obtained by ligation of the aorta. However, since administration of the same quantity of renin as that contained in this extract resulted in hypertension but not in vascular disease, he considered that the extract contained vascular necrotizing substance in addition to pressor substance. YASUMURA,\(^11\) YAMAGUCHI\(^12\) and others obtained from the renal cortex a factor to induce fibrinoid necrosis to the vessel and stated that this fibrinoid necrosis inducing factor had no pressor effect. SHIMOMURA\(^22\) dividing kidney of rat into subcellular fractions examined pressor effect and vascular necrotizing effect of those fractions and obtained a certain fraction with vascular necrotizing effect without pressor effect. By intravenous injection of that fraction, he observed an increase of vascular permeability and angioneurosis.
in arterioles and small arteries of the whole body. On the other hand, MASSON\textsuperscript{16,17} and CUSHBERT\textsuperscript{15} considered that renin–angiotensin was the cause of hypertensive vascular disease. ONOYAMA\textsuperscript{14} stated that, since vascular diseases including hydrothorax, pancreas edema and pulmonary edema were caused by lysosome fraction with high renin activity and pressor effect, and since microsome fraction had no pressor effect and required a large quantity to cause vascular lesions, the vascular necrotizing factor should be included in lysosome fraction.

The author obtained a subcellular fraction from the renal cortex by ultracentrifugation and observed sequentially early vascular changes caused by the intravenous injection of this fraction. Although bilateral nephrectomy occasionally resulted in slight degeneration in arterioles and small arteries after 24 hours, no vascular change was caused 24 hours after unilateral nephrectomy and contralateral urethral ligation nor was caused by the intravenous injection of the renal cortical extract into non-treated rats. In view of this, the stagnation of renal cortical extract in blood seemed required for the occurrence of vascular disease. For long survival of rats and long stagnation of the renal cortical extract, employed in the present study was the procedure to perform unilateral nephrectomy and contralateral urethral ligation and to remove the ligation 24 hours after intravenous injection of the extract. The intravenous injection of the renal cortical extract into thus treated rats resulted within 24 hours in increased vascular permeability and angionecrosis. Therefore, this renal cortical extract is called a vascular permeability and necrotizing factor (VPNF). The vascular changes 24 hours after VPNF injection in this procedure and those 24 hours after VPNF injection into rats of bilateral nephrectomy were almost the same in frequency and degree. In the group of unilateral nephrectomy and contralateral urethral ligation, 5–7 days after VPNF injection, there were infiltration and proliferation of inflammatory cells such as lymphocytes, neutrophils and plasma cells and of fibroblast–like cells into the entire layer of vessel being accompanied by fibrinoid degeneration. These changes quite resembled periarteritis nodosa in man.

These proliferative changes at the arterial wall heretofore reported are mostly related to hypertension such as those caused by experimental hypertension\textsuperscript{23–30} and those observed in spontaneous hypertensive rats\textsuperscript{31} (SHR). Proliferative changes were also observed in rats with systemic arteritis due to immunization with isologous renal extract.\textsuperscript{32} ALLISON\textsuperscript{23} by renal artery constriction and ZEEK\textsuperscript{25,26} and KUBOTA\textsuperscript{27} by the perirenal operation produced periarteritis nodosa-like change after continuous hypertension. SPIRO\textsuperscript{24} reported to have produced hyperplastic arteriolarsclerosis. AIKAWA\textsuperscript{29} produced hypertension by aorta constriction and observed arterial hypertrophy, arterial hyperplasia, arterial degeneration and associated fibrosis, and fibrinoid necrosis. No description of periarteritis nodosa–like change was made by SPIRO\textsuperscript{24} and AIKAWA\textsuperscript{29}.

It was feasible in the present study to produce experimentally periarteritis nodosa–like change without occurrence of hypertension. So far no antibody against this renal cortical extract has been demonstrated. This experiment is advantageous for observation.
of vascular diseases since the vascular change in a rat show almost the same histopathologic changes.

One month after the intravenous injection of fraction, the thickened intima showed generation of elastic fibers, and two months after, it was followed by additional change namely cellulofibrous thickening of the media. These changes after one month or more may well be said to show the figure of a type of fibrous thickening. However, the increase of elastic fibers may include the process of decomposition of elastic fibers. SEKIGUCHI and KOJIMAHARA who produced plasmatic arterionecrosis-like change by constriction of bilateral renal arteries observed cellulofibrous thickening of the intima which they called healing process of arterial lesion upon removal of constriction or administration of antihypertensive drug but no such thickening of the media.

In view of the fact that almost all vessels 24 hours after VPNF injection show some lesions but the frequency decreases during the course of long term observation, it seems that early vascular lesions are reversible and some of them may be repaired completely without leaving any trace.

The findings that there was no significant difference in BUN level and urine protein volume between the group of VPNF injection upon unilateral nephrectomy and contralateral urethral ligation and the control group suggest that these factors hardly participated in the occurrence of vascular lesions.

The induction of panarteritis and also cellulofibrous intima and/or medial thickening by early vascular lesion mainly consisting of medial smooth muscle cell necrosis independently from the blood pressure factors and immunological factors seems to occur as biological reaction against early vascular lesions including fibrinoid degeneration. Whereas periarteritis nodosa-like change and cellulofibrous intima and/or medial thickening have been considered as a histological feature of antigen-antibody reaction, it is demonstrated by the present experiment that these changes can occur irrespective of the production of antibody.

CONCLUSION

By ultracentrifugation of the normal rat renal cortex, a fraction to show increase of vascular permeability and vascular necrotizing effect without pressor effect (vascular permeability and necrotizing factor: VPNF) was obtained. Since the time of experiment using bilaterally nephrectomized rats is limited to about 24 hours, the present study employed the procedure to perform unilateral nephrectomy and contralateral urethral ligation and to remove the ligation 24 hours after the intravenous injection of VPNF so as to provide long survival of rats. From 24 hours after the intravenous injection of VPNF, early vascular changes mainly consisting of degeneration and necrosis of medial smooth muscle cells of the arterioles and small arteries were examined at designated intervals. As the result, the following were elucidated.
1. In comparison between the cases with VPNF injection upon unilateral nephrectomy and contralateral urethral ligation and those with VPNF injection upon bilateral nephrectomy, the vascular lesions 24 hours after the VPNF injection were almost the same in type, degree and frequency.

2. The kidney on the side of urethral ligation showed mild hydronephrosis, which returned to normal in 1–2 days after the removal of ligation.

3. Panarteritis was observed 5–7 days after the VPNF injection and it was sometimes accompanied by fibrinoid degeneration.

4. Proliferation of elastic fibers of the intima and cellulofibrous thickening of the media were observed in some vessels 1–2 months after the VPNF injection.

5. These changes occurred independently from hypertension, immunological factors and abnormality of BUN and urine protein levels.

6. Since the number of vessels showing these changes decreased with the lapse of time, it is considered that some of these early vascular changes is reversible.

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Photo. 1  24 hours after VPNF injection. The medial smooth muscle cells of small artery show degeneration and necrosis of cell level. Hematoxylin and eosin stain.

Photo. 2  24 hours after VPNF injection. Demarkation of the medial smooth muscle cells with degeneration and necrosis is indistinct. Hematoxylin and eosin stain.

Photo. 3  36 hours after VPNF injection. The change in Photo. 2 is spread to the entire circumference of the artery. PAS stain.

Photo. 4  3rd day. Dissociation of elastic lamina and hemorrhage are seen. Weigert stain.
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Photo 5  5th day. Cell proliferation with fibrinoid degeneration is seen in the intima, media and adventitia. Hematoxylin and eosin stain.

Photo 6  5th day. Similar findings to those in Photo 5. Hematoxylin and eosin stain.

Photo 7  5th day. Weigert staining of Photo 6.

Photo 8  7th day. Cell proliferation with fibrinoid degeneration is seen. This change can be called panarteritis. Hematoxylin and eosin stain.
Photo. 9 7th day. Weigert staining of Photo. 8.

Photo. 10 7th day. Cell proliferation is seen in the intima, media and adventitia but there is no fibrinoid degeneration. The internal elastic lamina is extended and broken. Weigert stain.

Photo. 11 1 month. Increase of elastic fibers is seen. Weigert stain.

Photo. 12 2 months. Cellulofibrous thickening of media is seen. Hematoxylin and eosin stain.