Experimental Vascular Lesions Elicited by Collagenase in Rats

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Purified collagenase which attacks only native collagen and gelatin was injected intravenously into the normal and DCA hypertensive rats to determine whether damage in collagenous tissue of the vascular wall would result in diffuse necrotizing lesions of the vessels. In the normal rats subclinical edema, proteinuria, and mild degenerative lesions in the small arteries and arterioles were produced. In the DCA hypertensive rats generalized edema, hematuria, decrease in plasma total protein, diffuse necrotizing renal and vascular lesions similar to those of malignant hypertension were caused. The results suggest that the collagenase accelerates hypertensive vascular disease by increasing vascular permeability.

Necrotizing renal and vascular damage is the characteristic feature of the malignant phase of hypertension. Sustained hypertension and associated hemodynamic changes have been considered to play important roles in the pathogenesis of the lesions, but it is still uncertain that high blood pressure is a sole determining factor.

The participation of humoral, vasculotoxic substance in the development of the lesions has also been considered. Masson et al. elicited both hypertension and renal and vascular lesions in uninephrectomized rats by the chronic treatment with crude or semipurified renin of hog or rat.

The present studies were undertaken to determine whether damage in the connective tissue of the vascular walls would result in diffuse necrotizing lesions of the vessels. Collagenase which attacks only native collagen and gelatin was used to cause a selective damage in the collagenous tissue.

Materials and Methods

Thirty-four female Wistar rats, weighing 150–160 Gm. were uninephrectomized and divided into 4 groups. Group I (5 rats) served as control. Group II (10 rats) received intravenous injections of 0.5 mg./Kg. collagenase every other day for 14 days. Group III (7 rats) and IV (12 rats) were injected subcutaneously with...
1 mg./day of an aqueous DCA suspension. In Group IV intravenous injection of collagenase was started on the 22nd day of DCA treatment as Group II.

The rats were fed Oriental Laboratory chow. Animals of Group I and Group II were given tap water to drink ad libitum. Group III and IV were maintained on 1% NaCl solution. Body weight was measured regularly twice a week and blood pressure was determined at least once a week by the microphonic tail method\textsuperscript{10,11} after warming at 45°C for 5 min. without anesthesia. Several rats were individually placed in metabolism cages and daily excretion of urinary proteins was measured by Tsuchiya's method. Plasma total protein and blood urea nitrogen were measured by refractometer (Hitachi) and U-Ni-GRAPH (Warner-Chilcott) respectively. Animals in Group I and III were exsanguinated by inserting a cannula into the carotid artery at the end of the 5th week. Group II and IV were also killed at the end of each treatment. All animals were autopsied and examined grossly. Kidney, heart, liver, brain and other main organs were removed, dissected free of extraneous tissues, weighed fresh and then fixed in neutral 8% formalin. Tissues were stained with hematoxyline and eosin, Mallory's azan, periodic acid fuchsin (PAS), Weigert's elastic tissue stain and silver impregation by Bielschawsky-Pap's method. Renal and vascular lesions were graded from 0 to 3+ according to the severity index described in the previous report.\textsuperscript{12}

Clostridium histolyticum collagenase purchased from Sigma Chemical Company was purified by zone electrophoresis on potato starch and lyophilized.\textsuperscript{9} The preparation hydrolyzed 0.76 μ moles of glycylproline per min. per mg. The activity of collagenase increased to 10 times by the purification while caseinolytic activity reduced to 1/10. The rate of increase in free α-amino group which was determined by ninhydrin colorimetric method was used as the index of collagenolytic activity.\textsuperscript{9}

**RESULTS**

*Body Weight (Fig. 1–2)*

Five of 10 rats in Group II increased their body weights 15 to 25% for 2 weeks of collagenase administration while the control group gained only 5 to 14%. Growth was somewhat inhibited by the DCA treatment in Group III. In the DCA-collagenase-treated group (Group IV) 4 of 12 rats increased their body weights 13 to 47% as a result of fluid retention. In contrast 3 rats emaciated with cachexia, 2 of which died. One rat which gained 84 Gm. within 24 hours became comatose and manifested oliguria, hematuria and generalized edema.

*Blood Pressure (Fig. 3)*

Blood pressure ranged from 82 to 117 mm.Hg with an average of 89.0 mm.Hg in Group I. Administration of collagenase had no remarkable effect on blood pressure. Hypertension developed in all animals in Group III and IV which received DCA and 1% NaCl solution. Blood pressure became slightly lower in Group IV after 2 weeks of collagenase treatment.

*Excretion of Urinary Proteins, Blood Urea Nitrogen, Plasma Total Protein (Table I)*
Urinary proteins increased in Group II and IV. There was no increase in blood urea nitrogen in Group II, III and IV compared with the control, except one case in Group IV which was 49.0 mg./100 ml. Plasma total protein decreased in Group IV. Two rats with fluid retention showed 4.0 and 4.1 Gm./100 ml. respectively.

**Organ Weight** (Table II)

The weight of the kidney, brain, lung and liver increased in Group II. Cardiac and renal enlargement was prominent in hypertensive groups. The weights of heart, lung and liver in Group IV exceeded those in Group III, but no appreciable difference was noted in the kidney and brain weight.
Fig. 3. Effect of collagenase treatment on the blood pressure of the normal and DCA hypertensive rats. The cross bars represent standard deviations.

Table I. Changes of Blood Urea Nitrogen, Plasma Total Protein, Excretion of Urinary Proteins and Severity of Lesions Produced by Repeated Injection of Collagenase

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Rats</th>
<th>Blood Urea Nitrogen (mg./100 ml.)</th>
<th>Plasma Total Protein (Gm./100 ml.)</th>
<th>Excretion of Urinary Proteins (mg./day)</th>
<th>Incidence of Lesions</th>
<th>Severity of Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>25.2* ±0.5</td>
<td>6.3 ±0.1</td>
<td>3.8 ±0.9</td>
<td>0/5**</td>
<td>0 0</td>
</tr>
<tr>
<td>Collagenase-treated</td>
<td>10</td>
<td>22.0 ±0.4</td>
<td>6.2 ±0.1</td>
<td>6.3 ±0.7</td>
<td>0/10</td>
<td>0.3 0.2</td>
</tr>
<tr>
<td>DCA-treated</td>
<td>7</td>
<td>24.9 ±3.1</td>
<td>6.2 ±0.2</td>
<td>35.6 ±11.5</td>
<td>3/7</td>
<td>1.4 1.3</td>
</tr>
<tr>
<td>DCA-collagenase-treated</td>
<td>11</td>
<td>22.9 ±3.1</td>
<td>5.2 ±0.3</td>
<td>121.2 ±29.5</td>
<td>7/9</td>
<td>2.1 1.8</td>
</tr>
</tbody>
</table>

* Results are expressed as mean values with standard errors.
** Number of rats which revealed lesions over 2+/number of rats examined.

Macroscopic Findings

The organs of rats given collagenase alone were grossly normal. Specific lesions were present only in the DCA-treated and DCA-collagenase-treated series. These were grayish-white, pathy lesions on the surface of the right ventricle and kidney, small brownish-white nodules along the mesenteric arteries, submucosal hemorrhage of the gastrointestinal tract and petechial cerebral bleeding. Accumulation of peritoneal, pleural and pericardial fluid was also observed in the DCA-collagenase-treated group. Subcutaneous
**Microscopic Findings**

**Collagenase-treated**: There was early and focal involvement in the glomeruli of the kidney. The basement membrane was thickened and the capillaries were occluded focally with amorphous material (Fig. 5). In the small arteries and arterioles of the kidney, heart and gastrointestinal tract, the media of the vessels was weak in staining with vacuolar changes of cytoplasm.

**DCA-treated**: Lesions were obvious in 3 of 7 animals in this group. The most striking and distinctive feature was wide-spread vascular damage in the small arteries and arterioles of the various organs which was characterized by necrosis of the media with PAS positive fibrinoid materials. The endothelium tissues were edematous and swollen to a thickness of 1 cm. (Fig. 4).

**Table II. Comparison of Organ Weight among 4 Groups**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of Rats</th>
<th>Kidney (Gm./100 Gm. of Body Weight*)</th>
<th>Heart (Gm./100 Gm. of Body Weight)</th>
<th>Brain (Gm./100 Gm. of Body Weight)</th>
<th>Lung (Gm./100 Gm. of Body Weight)</th>
<th>Liver (Gm./100 Gm. of Body Weight)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0.36**</td>
<td>0.51</td>
<td>0.90</td>
<td>0.62</td>
<td>4.10</td>
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<tr>
<td></td>
<td></td>
<td>0.04</td>
<td>±0.03</td>
<td>±0.04</td>
<td>±0.05</td>
<td>±0.22</td>
</tr>
<tr>
<td>Collagenase-treated</td>
<td>10</td>
<td>0.39</td>
<td>0.55</td>
<td>1.04</td>
<td>0.74</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.02</td>
<td>±0.01</td>
<td>±0.03</td>
<td>±0.03</td>
<td>±0.08</td>
</tr>
<tr>
<td>DCA-treated</td>
<td>7</td>
<td>0.43</td>
<td>0.78</td>
<td>0.98</td>
<td>0.67</td>
<td>4.69</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.03</td>
<td>±0.06</td>
<td>±0.05</td>
<td>±0.07</td>
<td>±0.20</td>
</tr>
<tr>
<td>DCA plus Collagenase-treated</td>
<td>11</td>
<td>0.54</td>
<td>0.84</td>
<td>0.94</td>
<td>0.86</td>
<td>5.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.02</td>
<td>±0.04</td>
<td>±0.02</td>
<td>±0.08</td>
<td>±0.18</td>
</tr>
</tbody>
</table>

* Body weight at the time of sacrifice was used. Previous weight was applied in the case which showed extreme loss or gain prior to death.

**Results are expressed as mean values with standard errors.**

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*Fig. 4.* Subcutaneous tissue of a DCA-collagenase-treated rat, showing predominant edema (left). That of a DCA-treated (right).

*Fig. 5.* Kidney of a collagenase-treated rat, showing slightly increased mesangium and thicken basement membrane of the glomeruli (PAS stain).
was enlarged and swollen, and elastic lamina was segmentally or completely lost in the involved area. Glomerular capillary loops were partially obliterated with diffuse or granular deposits and capsular spaces were often occupied by this material. Tubules were dilated and tubular epithelium was atrophic and regenerative. Interstitial cells and fibers were proliferated in the involved vessels to various degree (Fig. 6).

In the heart there were numerous islets of hyalinization of the myocardial fibers and eventual fibrosis mainly situated within the wall of the right ventricle. Small vessels showed focal or diffuse fibrinoid necrosis with extravasation of amorphous substance in the perivascular tissue. Inflammatory perivascular

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**Fig. 6.** Malignant nephrosclerotic lesion of a DCA-treated rat. Damaged glomeruli are swollen and degenerative. Tubules are dilated with hyaline casts. (Mallory's azan stain)

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**Fig. 7.** Damaged arterioles in the right ventricular wall of a DCA-collagenase-treated rat which reveal fibrinoid necrosis of the media and intima. Homogenous, PAS positive material is exudated in the adjoining interstitial tissue. Vascular lumen is occupied by fresh thrombus. (Mallory's azan stain)

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**Fig. 8.** Glomerulus and afferent arteriole of a DCA-collagenase-treated rat, showing degeneration of tufts, hyaline cast in Bowman's capsular space and proliferation of interstitial cells and fibers. (PAS stain)

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**Fig. 9.** Damaged arterioles of the kidney in a DCA-collagenase-treated rat. Collagen fibers laminate surrounding the vessels, vascular lumen being reduced in size. (Mallory's azan stain)
proliferation similar to poliarteritis nodosa was observed in small vessels of the pancreas, gastrointestinal tract and mesentery.

DCA-collagenase-treated: The severity and extent of lesions became greater by superimposition of the effect of collagenase. Vascular lesions were more commonly found in this group (Fig. 7-8). Especially, prominent proliferative reaction was noteworthy. Collagen fibers formed laminated structures in the adventitia and perivascular tissues of the affected vessels with concentric hyperplastic thickening and fibrosis of all coats of the walls (Fig. 9). In the glomeruli synechiae were found between the tufts and capsular membranes and they often changed to fibrous masses. The tubules were either atrophic or fibrotic secondary to damage of the glomeruli. In some cases round cells infiltrated into the interstitial tissues.

The incidence of the diseased rats and the averaged severity of lesions in each group are summarized in Table I. No case showed damage over 2+ in Group I and II. Clearly defined lesions were observed in 3 of 7 rats in Group III and 7 of 9 rats in Group IV. The severity of lesions were also higher in Group IV.

DISCUSSION

Repeated injection of collagenase into the normal rats produced proteinuria, subclinical edema and mild degenerative lesions in the small vessels. These changes suggest increase in vascular permeability by collagenase. The enzyme caused generalized edema, marked proteinuria, decrease in plasma protein and necrotizing renal and vascular lesions similar to those of malignant hypertension in the DCA hypertensive rats. The changes were apparently identical to those by the DCA-treatment only, but more extensive. The effect of collagenase is small in the normal rats but may act synergically with high blood pressure and may accelerate hypertensive vascular disease.

Collagenase is a proteinase with high degree of substrate specificity and acts only on native collagen and gelatin. The in vitro effect of collagenase on collagen fibers has been investigated. Reticulin and collagen fibers in tissue segments exposed to collagenase were dissolved.13),14) Lamination of collagen fibers in the perivascular tissues of the affected vessels in Group IV may be implied as an evidence of repeated injury by collagenase and subsequent reaction of the tissues. Non-specific proteinases were tested whether they could produce similar vascular lesions. Trypsin or nagase injected intravenously into uninephrectomized rats failed to elicit vascular change (unpublished observation). Masson et al.15) showed no effect by the subcutaneous injection of trypsin into DCA hypertensive rats. Kellner and Robertson16) elicited focal
necrosis of the myocardial and skeletal muscle by intraarterial injection of papain or ficin into the rats but blood vessels were not involved. Rabbits given intraarterial injections of elastase exhibited focal edema and small hemorrhage due to local capillary damage.\textsuperscript{17} Swelling and fragmentation of elastic fibers of aorta with medionecrosis aortae cystica were recognized in hyaluronidase administered rats by Yamakawa et al.\textsuperscript{18} Since these enzymes produced no lesions similar to those caused by collagenase, the possibility that the changes reported in this study were merely non-specific injuries due to a substance exogenously administered could be excluded. It is generally accepted that high blood pressure and associated hemodynamic changes are important determinants in the development of vascular lesions. The observation of Byrom and Dodson\textsuperscript{2} that the sudden strain placed on the arterial walls by repeated injections of Ringer's solution into the aorta produced focal necrosis of the small arteries in the kidney will suggest the causal relation between high blood pressure and vascular damage. On the other hand, the possibility of participation of a vascular permeability factor in the pathogenesis of the lesions has been raised by Winternitz et al.\textsuperscript{5} and more recently by Asscher and Anson.\textsuperscript{4} They demonstrated that bilaterally nephrectomized rats and renal hypertensive rats developed generalized edema, leakage of plasma protein, visceral hemorrhage and arterial necrosis which resembled lesion of malignant hypertension by intraperitoneal administration of saline extract of autogenous or homologous kidney. They pointed out that altered vascular permeability might play a role, as was suggested by Schürmann and MacMahon,\textsuperscript{19} in the production of lesions.

Kaley\textsuperscript{20} reported that bacterial endotoxin of Escherichia coli had the property to induce fibrinoid degeneration of the glomerular basement membrane and occlusive lesions of capillaries with PAS positive substances in the DCA-treated rats. Maekawa and Hayashi\textsuperscript{21} noted acute renal and vascular lesions similar to those of malignant hypertension in the DCA-treated rats given kidney adenosine triphosphatase fraction. Therefore this type of renal and vascular lesions are a uniform pathological manifestation and a subsequent reaction of the vascular walls to various damaging substances which increase vascular permeability. This may play an important role as well as the elevation of blood pressure in the pathogenesis of hypertensive vascular disease.

**Summary**

Vasculotoxic effect of purified collagenase was investigated in the normal and DCA hypertensive rats.

1. Body weight increased with fluid retention in collagenase treated
and DCA-collagenase-treated rats.

(2) Blood pressure elevated in the rats given DCA and 1% saline for drink and it decreased slightly after 2 weeks of collagenase treatment.

(3) Proteinuria developed in the collagenase-treated and DCA-collagenase-treated rats with decrease in total plasma protein in the latter. No elevation of blood urea nitrogen was recognized.

(4) Mild degenerative lesions in the small arteries and arterioles were observed in the collagenase-treated rats. Necrotizing renal and vascular lesions, arteriolar fibrinoid necrosis, glomerular degeneration and perivascular proliferation of collagen fibers were observed in the DCA-collagenase-treated rats which were identical in nature with lesions caused by DCA treatment only but more extensive and severe.

(5) These observations suggest that collagenase increases vascular permeability and serves as augmenting or accelerating factor in the development of hypertensive vascular disease.

Acknowledgement

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References