AN EXPERIMENTAL STUDY ON THE VASCULAR LESIONS CAUSED BY DISTURBANCE OF MICROCIRCULATION IN THE AORTIC WALL

INFLUENCE OF OBSTRUCTION OF THE LYMPHATICS IN THE AORTA AND PERIAORTIC TISSUES

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ABSTRACT

Distribution of the lymphatics and vascular changes caused by obstruction of the lymphatics in the aortic wall and periaortic tissues of dogs were investigated. By injecting India ink into the external iliac lymph nodes, the lymphatics in the aortic wall were observed in the superficial layer of the adventitia.

Obstruction of the lymphatics in the aortic wall was produced by injecting a solution of gelatin-hydrochloric acid into the external iliac lymph nodes.

The dogs were divided into three groups.

a) Ligated group
   Obstruction of the lymphatics and ligation of the inferior vena cava below the renal veins were performed.

b) Not-ligated group
   Only the lymphatic obstruction was undertaken.

c) Control group
   In general, accumulation of the interstitial fluids in the media, thickening of the intima and degeneration of the smooth muscle cells in the aortic wall were observed.

At early phase, the ligated group showed more rapid and severe vascular changes than the not-ligated group.

At late phase, no difference of changes was seen between the two group.

Vascular changes were severe and lasted long in the region where granulation tissues increased markedly around the obstructed lymphatics.

The above-mentioned facts suggest significance of the lymphatics related to vascular lesions.

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INTRODUCTION

There are many opinions about the etiology of diseases of the aorta such as atherosclerosis or medionecrosis and a multitude of theories have arisen to explain the pathogenic mechanism of diseases.

Recent studies showed that walls of blood vessels are functioning organs and have high metabolic activities. With the advancement in study of the nutrition of blood vessel walls, the importance of microcirculation in the aortic wall has been recognized.

Relationship between vascular lesions and the vasa vasorum has been investigated in detail.

Furthermore, microcirculation of the lymphatics is one of the important factors which influences the metabolic activity in the aortic wall. However, few observations of the lymphatic component in the aortic wall have been recorded and the significance of the lymphatics related to vascular lesions has not been assessed adequately.

The object of the present study is to investigate the anatomical structure of the lymphatics and to observe the vascular changes caused by obstruction of the lymphatics in the aortic wall and periaortic tissues of dogs.

MATERIALS AND METHODS

Fifty-three young mongrel dogs of both sexes with body weights of 15 to 25 kg were used.

1) Distribution of the lymphatics (11 dogs)

Distribution of the lymphatics in the aortic wall was studied by injecting 1.0 ml of dilute India ink or 50 per cent micropaque into the external iliac lymph node, bilaterally. The former specimen was made transparent and examined microscopically; the latter was x-rayed by Softex. (Hitachi Softex Co. Ltd.)

2) Obstruction of the lymphatics

In the preliminary experiments, various solutions (2.0 per cent phenol, 0.5 or 1.0 normal hydrochloric acid) were used for obstruction of the lymphatics. It was found that the lymphatics were obstructed most effectively with a solution of 0.5 N HCL, so a mixture of 0.5 N HCL with 10 per cent gelatin and 5 per cent India ink was used in the following experiments. Dilute India ink was used as index in microscopic study.

Under thiobarbiturate anesthesia, the peritoneal cavity was opened by a midline incision. Through a 27-gauge needle, 1.0 ml of the mixed solution was injected into the external iliac lymph node, bilaterally (Fig. 1).

The dogs were divided into three groups.

a) Ligated group (17 dogs)

Ligation of the inferior vena cava below the renal veins was performed after injection of the mixed solution into the external iliac lymph nodes.
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Fig. 1. Method of injection of the solution into the lymph node and visualization of the lymphatics in the aortic wall.

b) Not-ligated group (19 dogs)
Only injection of the mixed solution was undertaken. The inferior vena cava was not ligated.

c) Control group (6 dogs)
Through the above-mentioned technique, 1.0 ml of physiological saline was injected into the external iliac lymph nodes.

After the dogs were injected with 50 mg of heparin intravenously, they were sacrificed by injection of the overdosage of sodium thiobarbiturate at periods from two days to six months after the operation.

The abdominal aorta and periaortic tissues were dissected out from the spine with as little injury to the adventitial layer as possible and 50 per cent micropaque was infused into the aortic lumen under physiological pressure.

After fixation in 10 per cent formalin for two weeks, microangiograms of the specimens were taken with the method described by Clarke.

The same specimens were stained by hematoxylin-eosin, elastica-Van Gieson’s method, Mallory-Azan, toluidin blue of pH 4.1 and 7.0, PAS and PTAH method and studied histologically in comparison with the microangiograms of the vasa vasorum.

RESULTS

1) Distribution of the lymphatics
Plexus of the lymphatics were observed in the superficial layer of the adventitia but not in the media (Plate 1). The adventitial lymphatic channels coursed along the longitudinal axis of the aorta and reached the major lymph duct or cisterna chyli.

2) Ligated group
a) Four to seven days after the operation, accumulation of the interstitial fluids was observed in the media, especially adjacent to the internal elastic lamina. Intimal thickening was not observed. The elastic fibers of the media appeared to be intact (early phase).

b) Two to four weeks after the operation, accumulation of the interstitial fluids was observed in the inner side of the media. The internal elastic lamina and elastic fibers were well preserved. Smooth muscle cells were scarce and atrophic in the media. The obstructed lymphatics with India ink were seen in the adventitia and periaortic tissues (Plate 2, 3), (late phase).

c) Three to six months after the operation, slight intimal thickening was
observed in the localized regions. Accumulation of the interstitial fluids was recognized in the intima and media. The internal elastic lamina was almost normal, but elastic fibers were slender and irregular. Smooth muscle cells were scarce and atrophic (Plate 4). In the adventitia, increased granulation tissues were observed (late phase).

In general, the obstructed lymphatics were organized completely. The cause of this obstruction seemed to be due to intimal damage by hydrochloric acid followed by embolus of the injected mixture (esp. gelatin) and lymphatic fluid.

The inner surface of the inferior vena cava was covered by mural thrombus, about a few centimeter distal to the ligated portion.

3) No-ligated group
   a) Two to seven days after obstruction of the lymphatics (Plate 5) accumulation of the interstitial fluids was observed in the media, especially adjacent to the internal elastic lamina. The degree of accumulation was milder than that of the ligated group. Intimal thickening was not observed (early phase).
   b) Three to eleven weeks after the operation, intimal thickening, accumulation of the interstitial fluids and atrophy of the smooth muscle cells were also observed in the special regions where granulation tissues increased around the obstructed lymphatics in the adventitia and periaortic tissues (late phase).

In general, histochemical findings were similar in both the ligated and not-ligated groups. The changed areas of the intima and media stained well by pH 7.0 but partially by 4.1 of toluidin blue (Plate 6). The same regions were stained by PAS method and blue by Mallory-Azan method, but not stained by PTAH method.

Infiltration of inflammatory cells in the aortic wall was slight in all stages of both groups.

Microangiograms of the vasa vasorum showed a normal pattern (Plate 7).

4) Control group
   No abnormal findings were observed.

DISCUSSION

In investigating the metabolism in the vascular wall, it is important to elucidate the mechanism of microcirculation in the vascular wall and the influence of its disturbance. The normal aorta of a dog receives nourishment from two vascular sources. The outer part of the aorta is supplied by the vasa vasorum, while the inner part is nourished by direct permeation from the lumen of the aorta. Rich plexus of the vasa vasorum in the outer two-thirds of the media and the adventitia are observed\(^9\)\(^{10}\).

Many investigators have reported that fine networks of the lymphatics were present in the adventitia, but did not penetrate the media\(^{11}\)\(^{14}\). In our experiments, distribution of the lymphatics also was observed in the superficial layer of the adventitia (Plate 1).
Some investigators reported that it is difficult to occlude only the lymphatics and to produce a complete blockage of return of the lymph to the blood stream because of accessory anatomic channels.

On the other hand, there are many reports on injury of the adventitia. Application of acid, thermocautery or freezing of the vascular surface and freeing the blood vessel from the surrounding tissues produced deterioration of the muscle cells accompanied by plasma retention in the media. However, these experimental methods destroy both the vasa vasorum and lymphatics in the vascular wall. In our experiments, the lymphatics in the aortic wall and periaortic tissues were damaged by a solution of chemical substance (hydrochloric acid), which was carried by the physiological lymphatic flow. The microangiogram of the arterial vasa in the aortic wall in which the lymphatics had been obstructed showed a normal pattern. If the venous side of the vasa vasorum was obstructed, the contrast medium injected from the arterial vasa could not enter the aortic wall. Therefore, in our experiments, it was considered that only the lymphatics were damaged by a solution of hydrochloric acid and the vasa vasorum, both arterial and venous, were functioning adequately.

The lymphatics have rich anastomosis with the vein in normal situation and lymphatico-venous communications become fully operable two weeks after obstruction of the lymphatics. These lymphatico-venous communications are probably enlargement of pathways normally present, but they do not function except under the stress of increased volume or pressure within the lymphatics. If not-damaged lymphatics remained partially in the aortic wall, the accumulated fluids might flow out through the lymphatico-venous shunts or lymphatico-lymphatic communications. However, it was considered that the increased venous pressure induced by the caval ligation might inhibit the above-mentioned collateral pathways and reduce the compensatory reabsorptive abilities of the venous vasa.

At the early phase after the obstructed procedure, the ligated group showed more rapid and severe accumulation of the interstitial fluids than the not-ligated one. Difference of this finding was probably due to the elevated venous pressure by the caval ligation.

Many reports concerned with the influence of venous pressure on lymph flow have been made. Rotter stated that the movement of tissue fluid was disturbed when the blood pressure of the venous side of the vasa vasorum increased due to edema of the adventitia. Pick, et al. observed in dogs that combined compromise of both the venous and lymph outflow led to more significant occurrence of myocardial infarctions than by lymphatic occlusion alone.

After ligation of the inferior vena cava for pulmonary embolism or adjacent malignant diseases, some degree of elevated venous pressure is the usual finding in human being. Morets, et al. reported that femoral venous pressure was elevated to 15 cm from 8 cm of water until 6 months after the caval ligation. On the other hand, both animal and clinical studies by Gurewick and Nasbeth and
their co-workers have demonstrated that considerable collateralization occurred within two weeks after the caval interruption. In the limb replantation experiments in dogs, regeneration of the lymphatics was physiologically adequate by the eighth day. It was considered that because of the occurrence of collateral pathway and regeneration of the lymphatics, no difference of findings between the ligated group and not-ligated one was seen at the late phase. Moreover, accumulation of the fluids in the media was severe and lasted long in the localized regions where granulation tissues increased around the obstructed lymphatics in the adventitia and periaortic tissue so that the disturbance of drainage of the interstitial fluids progressed (Plate 2). Veress, et al. also reported that a local accumulation of plasma materials took place parallel with the deceleration of lymph drainage. Sandritter described the same observation in coronary artery at autopsy; semilunar shaped intimal thickening was observed in the special region where remarkable fibrosis existed in the adventitia.

In the presence of impairment of regeneration or anastomosis of the lymphatic system, plasma and metabolites in the aortic wall may flow out through the vasa vasorum, because of an overlap in the reabsorptive abilities of blood capillaries and lymphatics. However, proteins, lipoproteins, cholesterol, carbohydrates and enzymes are only absorbed by the lymphatic system, because of their molecular weight, size, structure and perhaps electrical charge.

It has been thought that the causes of the medial and intimal lesions observed clinicopathologically or experimentally were hyperpermeability, stagnation of the tissue fluids, and continuous hypoxia. Nagy, et al. proved that permeability of the coronary artery in dogs was increased pathologically by mechanical lymph congestion. As regards the mechanism through which permeability was increased, they suggested that lymph congestion caused disturbance of transport in the coronary wall and pH change with shift of the ionic milieu, and led to physico-chemical changes of the ground substance, which was followed by disturbance of the permeability and intimal edema.

The muscle cells are the only enzymically-active constituents of the media, thus their atrophy together with collagenous replacement must inevitably reduce the metabolic activity of the media. The ischemic atrophy of the media could be due to either impaired oxygen uptake from the lumen and diffusion of metabolites or obstruction of blood-flow in the vasa vasorum. However, in our experiments, it should seem that retention of the interstitial fluids in the media greatly contributed to the aetiology of degeneration of smooth muscle cells, because the vasa vasorum were functioning adequately in the aortic wall (Plate 7).

The quality of the accumulated fluids may also change with time. Jellinek, et al. reported that the blue color of the Azan staining turned pink and the Mallory negative yellow color turned bluish black in the case of plasma imbibition of long duration. In our experiments, the changes in staining like these were not observed.
Metachromasia positive and PAS positive substance increased especially in the inner side of the media where degeneration of muscle cells was recognized (Plate 6). Many investigators indicated that metachromasia positive substance accumulated in the damaged elastic tissue$^{43,44}$, though the role of it remains to be determined.

In our previous reports, new technical methods to obstruct the arterial$^{45,46}$ or venous$^{20}$ vasa in the aortic wall respectively were presented. Obstruction of the venous vasa produced stagnation of the interstitial fluids in the media, degeneration of the smooth muscle cells and destruction of the elastic fibers in the aortic wall. These aortic lesions were milder than lesions by obstruction of the arterial vasa. In the aortic wall, both the venous vasa and lymphatics have common functions as out-flow tracts of the interstitial fluids and metabolites. So, it might be possible that the vascular changes caused by disturbance of out-flow tracts have many similar aspects in their pathogenesis.

Further studies are needed to correlate the anatomical and pathological relationship between the vasa vasorum and lymphatics.

On the basis of the above-mentioned experiments and considerations, it seems reasonable that disturbance of lymph drainage in the aortic wall contributes to the aggravation of vascular lesions.

**CONCLUSION**

Lymphatics were observed in the superficial layer of the adventitia of the aorta in dogs.

Obstruction of the lymphatics in the aorta and periaortic tissues was produced by injecting a gelatin-hydrochloric acid solution into the external iliac lymph nodes. Ligation of the inferior vena cava was performed simultaneously in 17 dogs. In general, thickening of the intima, accumulation of the interstitial fluids and degeneration of the smooth muscle cells in the media were observed.

At an early phase, the ligated group showed more rapid and severe vascular changes than the not-ligated.

At a late phase, no difference of changes was seen between the two groups. Vascular changes were severe and lasted long in the regions where granulation tissues increased markedly around the obstructed lymphatics.

The role of lymph flow in the aortic wall and periaortic tissues was discussed.

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REFERENCES


23) Rotter, W., Über die Bedeutung der Ernährungsstorung, insbesondere der Sauerstoffmangels für die Pathogenese der Gefäßwandveränderungen mit besonderer Berücksichtigung der...
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40) Rottino, A., Medial degeneration of the aorta, a study of two hundred and ten routine autopsy specimens by a serial block method, Arch. Path., 28, 377, 1939.


LEGENDS

Plate 1. Transparent specimen of the transverse section of the aorta. Plexes of the lymphatics seen in the superficial layer of the adventitia.  x 15

Plate 2. Two weeks after lymphatic obstruction and caval ligation. India ink seen in the obstructed lymphatics (arrow). Granulation tissue in the adventitia increased. Hematoxylin-eosin.  x 50

Plate 3. The same specimen as shown in Plate 2. Accumulated fluid seen in the media. Smooth muscle cells scarce and atrophic. F: accumulated fluid. Hematoxylin-eosin.  x 200

Plate 4. Three months after the lymphatic obstruction and caval ligation. Cellular intimal thickening seen. Accumulated fluid observed in the intima and media. Smooth muscle cells scarce and atrophic. Hematocytin-eosin.  x 200

Plate 5. Seven days after obstruction of the lymphatics. Accumulated fluid observed in the media. F: accumulated fluid. Hematoxylin-eosin.  x 200

Plate 6. The same specimen as shown in Plate 2. Metachromasia positive substance seen in the inner side of the media (upper portion). Toluidin blue of pH 7.0.  x 200

Plate 7. Microangiogram of the same region as show in Plate 5. The vasa vasorum in the aortic wall shows normal distribution. L: Lumen  x 15