An Experimental Study on the Vascular Lesions Caused by Obstruction of the Vasa Vasorum (II). Special Consideration on the Deposition of Fat into Vascular Wall*

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It has been reported that disturbance of the vasa vasorum changes permeability, flow of tissue fluid, and nutrition in the vascular wall and becomes a cause of fat deposition in these regions. In this experiment, relation between fat deposition and disturbance of the vasa vasorum was studied using new method obstructing the vasa vasorum alone.

DISTURBANCE of the vasa vasorum have been discussed as the cause of vascular lesions. We have also reported normal distribution of the vasa vasorum and various vascular lesions caused by disturbance of the vasa vasorum at the abdominal aorta of dog such as, obstruction of the vasa vasorum alone^{1,2}, reconstruction of obstructed ones^{1,2}, their changes in the vein transplanted to the arterial system^{3,4}, bacterial infection through them⁵, and influence of bradykinin on the vasa vasorum⁶.

This series of experiments were done in order to observe vascular lesions in young mongrel dogs administered with cholesterol after obstruction of the vasa vasorum without any mechanical injury to outer coats of the aorta.

MATERIALS AND METHODS

Sixteen young mongrel dogs weighing approximately 10kgs were used in this series.

The obstruction of the vasa vasorum of the

abdominal aorta was done following the new method reported previously (Fig. 1)^{1,2,5}. That is, the abdominal aorta was clamped at two

Experimental Method

Fig. 1. The new method of obstruction of the vasa vasorum alone.

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levels just below the renal arteries and just above its bifurcation, and all branch arteries arising from this abdominal segment except the minute arteries to the spine were clamped as well. A mixture of 2000 units of thrombin in 50 mls of 10 per cent gelatin-saline was injected into the lumen of this segment of the abdominal aorta. Five minutes after the injection, the fluid in the aortic lumen was flashed from an incision made at the left common iliac artery. Then, the left common iliac artery was ligated and all clamps were removed.

Sixteen dogs were divided to 3 groups. A-group (8 dogs); following the operation, five grams of cholesterol and 0.5 gram of thiouracil were administered daily. B-group (5 dogs); following the same operation, five grams of cholesterol only was administered daily. Control group (3 dogs); in 2 dogs, the vasa vasorum was not obstructed by using injection of saline instead of gelatin-thrombin solution and following the procedure 5 grams of cholesterol and 0.5 gram of thiouracil were administered daily. In 1 dog, following the same operation, 5 grams of cholesterol was administered daily.

Total serum cholesterol was measured once every 2 to 3 weeks following ZAK-HENLY's method⁷. Microangiogram of the abdominal aorta and histological study on the liver, the kidney and the arterial system from the ascending aorta to the common iliac artery were done simultaneously at intervals varying from 2 weeks to 6 months postoperatively. The specimens were stained with hematoxylin-eosin, Sudan III, and by elastica VAN-GIESON's method.

RESULTS

All dogs were healthy after the operation except one dog died at the first operative day. Decrease of body weight was slightly. Average of the decrease was 0.8 kg from 2 weeks to 6 months after the operation.

Serum cholesterol: Total serum cholesterol before the operation ranged from 93 to 238 mg/dl. Average was 165 mg/dl. Total serum cholesterol was raised till 4 to 6 weeks post-operatively, and then decreased gradually (Fig. 2). The value of serum cholesterol did not become extremely high and remained in a relatively

Fig. 2. The straight line represents the group administered cholesterol with thiouracil and the dotted line represents the other group administered with cholesterol only.

low level. In the degree of the raise, there was no difference between A and B group.

Fat deposition: At the second week following the operation, fat was found already in the inner layer of the abdominal aorta of which the vasa vasorum had been obstructed (Fig. 3), but

Fig. 3. Accumulation of lipids was seen in the thickened intima (upper portion) at the second post-operative week. Serum cholesterol level was 206 mg/dl. A-group. Sudan III stain. $\times 40$.

in the other arterial system there was no deposition of fat. Accumulation of fat was also recognized in the intima and the inner one-third of the media at the 4th week following the operation, but this deposition of the fat didn't advance markedly in its degree from 4 to 28 weeks (Fig. 4). There was deposition of fat in the thoracic aorta in each of 3 dogs of 4 postoperative weeks, 3 and 6 months, but the extent was localized in the thin intima only. No deposition of fat in the abdominal aorta was recognized in only 3 dogs of 2, 4 postoperative weeks and 3 months.

Histological findings: At the second week after the operation, thickening of the intima

Fig. 4. A-group. Six months after the operation. Deposition of fat was also observed in the inner coat (upper portion), but the degree didn't progress markedly. Serum cholesterol level was 174 mg/dl at autopsy. Sudan III stain. $\times 40$.

and splitting and disappearance of the internal elastic membrane were seen. In the media

Fig. 5. Thickening of the intima, destruction of the internal elastic lamina, and fraying, splitting and disappear of the elastic fibers of the media were seen at the second week after the operation. A-group. Elastica Van-Gieson stain. $\times 40$.

Fig. 6. The same section to fig. 5. Note thickening of the intima, splitting of the internal elastic membrane, and the degenerate media. Hematoxylin-eosin stain. $\times 400$.

there were fraying and splitting of the elastic fibers were observed (Fig. 5, 6). Obstructed vasa vasorum were recognized in the outer-coats. These lesions progressed till 4 to 6 weeks similarly to the change caused by obstruction of the vasa vasorum alone^{1,2}, but no inflammation was observed in any periods.

In the control group, no change was observed in the arterial system and other organs.

Thiouracil showed no influence to both vascular pathology and serum cholesterol level in this experiment.

Microangiogram: Normal abdominal aorta of dogs has rich network of the vasa vasorum as human beings (Fig. 7). Anastomosis of the vasa vasorum to the vessels of the surrounding tissues were already seen at the second post-operative week, but the network was poor. Reconstruction of the vasa vasorum had progressed week by week and they seemed to be

Fig. 7. This is a full distended specimen of the normal abdominal aorta of dog. Rich network of the vasa vasorum can be recognized. $\times 25$.

Fig. 8. B-group. Four weeks after the operation. Note poor network and abnormal running of the vasa vasorum in contrast with normal one. $\times 25$.

come like normal figures in 3 months after the operation. In general there was a tendency that reconstruction of the vasa vasorum was poor in the severely damaged region (Fig. 8).

DISCUSSION

The vascular system of dog has rich network of the vasa vasorum as that of human beings does⁸. So it has seemed to be pretty difficult that fat deposition was able to be seen experimentally in the abdominal aorta of dog⁸. Many investigators⁸⁻¹⁰ have reported, in order to observe fat experimentally, necessity of abnormally high value of serum cholesterol caused by administration of large amount of cholesterol, thiouracil and Vitamin D, or thyroidectomy, and it also needs long periods. That is, STEINER et al.⁹ reported that lipid deposition were observed in the abdominal aorta of the dog 12 to 16 months after the administration of cholesterol only or cholesterol with thiouracil and the average cholesterol level had to exceed 450 mg/dl for more than one year before arteriosclerotic lesions were evidents. HASS¹¹ asserted that significant lipid deposition in normal arterial intima did not occur even in prolonged experiments unless the average serum cholesterol levels exceeded 350 to 400 mg/dl in rats and monkeys. There are even reports that lesions of the aorta couldn't be observed experimentally in high serum lipids level in rats¹².

Accordingly for the purpose to observe fat deposition in relatively low cholesterol level as human beings had atheroma, various experiments have been done as followings. Since SSOLOWJEW¹³ reported in 1930 experimental study that lipid deposition was able to observe in the injured arterial wall with hypercholesterolemia within 9 days after cauterization in rabbit, SCHLICHTER et al.⁸ showed also in 1949 that the addition of cholesterol associated with moderate hypercholesterolemia lead to the development of atheroma within 3 to 5 months in the ascending aorta injured by cauterization in dogs.

Further, experimental works for the same purpose had been done using the method freezing the arterial wall in rabbits and monkeys^{14,15}. CONSTANTINIDES et al.¹⁶ observed that the atheromata were observed in overlying the

patches of necrotic media produced using epinephrine and thyroxin with moderate hypercholesterolemia for only 20 days in rabbits, and more WILLIAMS¹⁷ observed at the interval of only 3 weeks postoperatively that intimal thickenings after the procedure of freeing a segment of femoral artery from its surrounding connective tissues were associated with an accumulation of lipid-laden macrophages in rabbits. TAKEDA¹⁸ also observed atheroma in rabbits by freeing the carotid artery from the surrounding tissue and covering it with synthetic resin.

These experiments succeeded to shorten the periods of lipid deposition with moderate hypercholesterolemia and all reports confirmed that injure of the arterial wall was necessary pre-existing conditions for accumulation of lipids, and the accumulation of lipids was always observed in the thickened intima. DE FARIA¹⁹ also considered by observing autopsy that changes of the intima and the media preexisted and played one of the important role for deposition of fats.

Different points of this experiments from above mentioned ones were as follows: high cholesterol feeding lasting few months before arterial injury was not used^{14,15}, and it was avoided to use abnormal ways such as limitation to only a few millimeter in injuring vascular wall¹⁵, acute death of medial cell by freezing or cauterization^{8,13-15}, over dosage of epinephrine and thyroxin¹⁶, intraperitoneal administration of cholesterol⁸, and moderate high cholesterolemia exceeded over 460-700 mg/dl^{8,16,18}. In this experiments, only the vasa vasorum of the abdominal aorta were obstructed so that direct mechanical injure to the arterial wall didn't occur. So it seemed that this experimental effects were purely caused by disturbance of the vasa vasorum.

Many investigators considered that the reason of accumulation of lipids after arterial lesions seemed to be hyperpermeability^{13,16,17}, changed ability of absorption¹³, storage of interstitial fluids¹⁵, and disturbance of flow of tissue fluid and lymph caused by disturbance of the vasa vasorum^{8,15,17,18} or by degeneration of the media^{14,16}. DOERR²⁰ have taken a serious meaning on change of permeability and disturbance

of flow of fluids through small venal vasa vasorum and lymph vessels for the genesis of arteriosclerosis. It has been also assumed that the arterial wall exhibits a certain permeability to cholesterol that allows a balance between the influx and removal so that no lipid accumulation occurs²¹. Some investigators have reported that changes of the elastic fiber in the arterial wall facilitated the rate of development of atherosclerosis^{10, 22}. As the elastic fibers were severely damaged following the obstruction of the vasa vasorum in this experiments (Fig. 5), this factor also may be related to accumulation of lipids.

As no fat was observed in 6 months after the obstruction of the vasa vasorum alone as reported previously², the other combined factors with disturbance of the vasa vasorum may be necessary for accumulation of lipids: slightly high cholesterolemia or change of fat metabolism and so on. Farther experiments need on these factors.

Why didn't vascular lesions and deposition of fat advance in this experiment? The reason was thought that serum cholesterol level decreased gradually (Fig. 2) and reconstruction of the obstructed vasa vasorum finished by approximately 3 months as reported previously².

There are many opposite opinions concerned with influence of thiouracil to atherogenesis^{9, 10}. Though no influence of thiouracil to serum cholesterol level and vascular lesions was seen in this experiment, more longer experimental periods may need in order to discuss it.

Anyway, in this experiment, if the vasa vasorum had been obstructed, fat was able to be observed in rather short period, even in a relatively low level of serum cholesterol, and only the aortic wall of which the vasa vasorum were obstructed. From these facts, it seemed to be reasonable that disturbance of the vasa vasorum may play an important role as underlying conditions in deposition of fat into the vascular wall.

SUMMARY

1. Fat deposition was able to be observed in 10 of 13 dogs administered cholesterol with thiouracil, at intervals from 2 weeks to 6

months after the obstruction of the vasa vasorum of the abdominal aorta, though the value of serum cholesterol was in a relatively low level.

2. There was a tendency that reconstruction of the vasa vasorum was poor in the severely damaged regions.

3. Relation to accumulation of lipids and disturbance of the vasa vasorum was discussed.

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