

LYMPHATIC ARTERIOPATHY: DAMAGE TO THE WALL OF THE CANINE FEMORAL ARTERY AFTER LYMPHATIC BLOCKADE

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ABSTRACT

The effect of lymph stasis on the histological, biochemical, and elastic properties of the femoral artery were studied after regional lymphatic blockade in 36 dogs. Dogs were sacrificed 4-21 days after operation. Histologic changes of the femoral arterial wall (interstitial edema, degeneration in the muscle layer or media, thickened adventitia with dilated lymph vessels, and fibrosis) developed after regional lymphatic blockade. Characteristic metabolic alterations of the arterial wall (anerobic catabolism of carbohydrate, increased lactate and glycosamine content) accompanied the morphological changes. Distensibility of the femoral artery decreased and greater elastic stiffness developed after regional lymphatic blockade. These results in conjunction with other experimental and clinical data support the concept that insufficient lymphatic transport within the blood vessel wall may contribute to the genesis and progression of arteriopathies.

Transmural plasma flow (i.e., influx of plasma constituents and nutrients into the intima and across the media) and interstitial drainage within the adventitia maintain homeostasis within the blood vessel wall. Blood vascular wall drainage depends in part on intramural lymphatics located within the adventitia. Proteins and other macromolecules that "leak" from the plasma into the blood vessel wall can

only be removed by adventitial lymphatics. Insufficiency of lymphatic transport promotes accumulation of plasma proteins and lipoproteins in the blood vascular wall accompanied by proliferative and degenerative intramural damage (1,2). These observations have incriminated disturbances in lymphatic clearance as a factor in the pathogenesis of some arteriopathies.

In this study, we investigated the effect of experimental regional lymphatic blockade on the histomorphological, biochemical, and biomechanical properties of the canine femoral artery; in other words, can lymph stasis induce arteriopathy?

MATERIALS AND METHODS

Experiments were carried out on 36 mongrel dogs of both sexes, weighing 12-20kg under sodium pentobarbital narcosis (30mg/kg). Regional lymphatic blockade of the femoral vascular area was produced by ligating the regional collecting lymphatic trunks. After administering Evans blue dye subcutaneously into the hindpaw, the lymphatics and lymph nodes of the hindlimb were readily visualized. In the crural canal, the lymphatic collectors accompanying the femoral artery and vein were ligated (~3-4 lymph trunks in each dog). On the contralateral hindlimb, only a sham operation was performed. Dogs were sacrificed 4-21 days following lymphatic ligation.

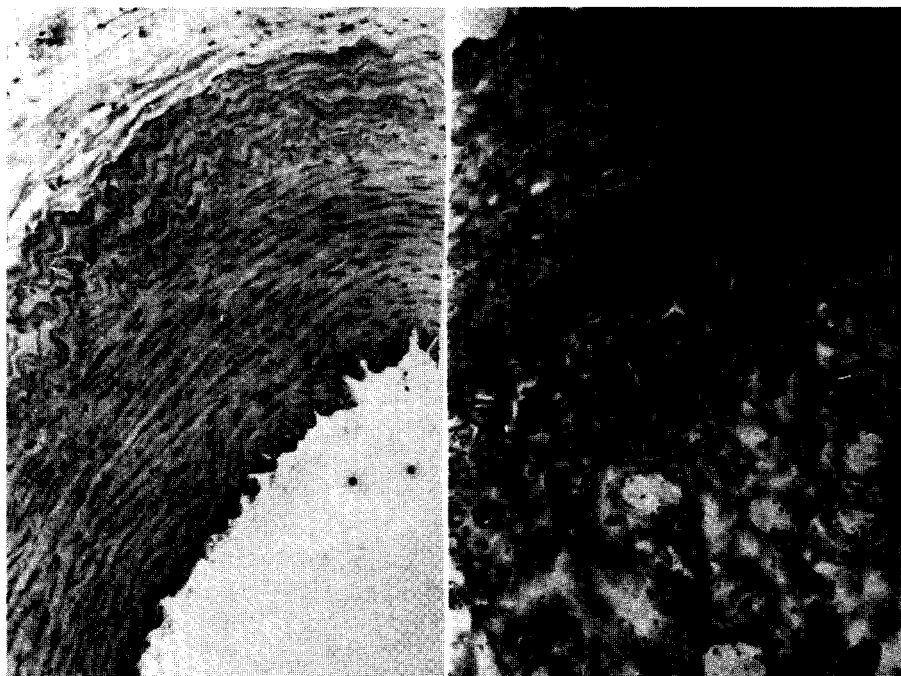


Fig. 1. Characteristic histologic changes in the femoral artery following regional lymphatic blockade (lymph stasis). On the left (sham operation), light microscopy shows on cross-section that the media consists largely of smooth muscle and some collagen and elastic fibers. The adventitia is formed mainly by collagen and elastic fibers. On the right, 10 days after lymphatic blockade (lymph stasis), light microscopy shows edema in the subendothelial layer, in the media, and also swelling of smooth muscle cells. The tunica adventitia is thickened and contains interstitial edema, dilated lymph vessels and infiltration by mononuclear cells. Hematoxylin and eosin stain. (x400)

Histomorphological study

Specimens from both femoral arteries were taken immediately after killing the dogs. Both light and electron microscopy were used for histologic studies. For light microscopy, the specimens were fixed in 10% formalin embedded in paraffin and stained with hematoxylin-eosin, Van Gieson, Azan, and toluidine blue. For electron microscopy, the femoral arterial segments were fixed promptly in cold 4.5% glutaraldehyde in 0.1M cacodylate buffer at pH 7.4. Durcupan ACM embedded ultrathin sections were subjected to transmission electron microscopy.

Biochemical study

About 10mm cylinder segments were cut from the femoral arteries. The

arterial segment was freed from the loose periadventitial tissue and 1.0g was used for biochemical investigations. Cytochrome C oxidase, succinyl dehydrogenase and uridine diphosphate dehydrogenase (UDP) enzyme activities were determined by enzymatic Farb test. O₂ consumption and CO₂ production was measured by manometry. The lactate content of the arterial wall was determined by LDH UV-monotests, and hexosamine content by colorimetry. For measuring the proteoglycan content CPC and gel filtration technique (3) was used, and for collagen content a colorimetric method (4) was applied.

Biomechanical study

Seven-thirty mm segments from both femoral arteries were transected and were

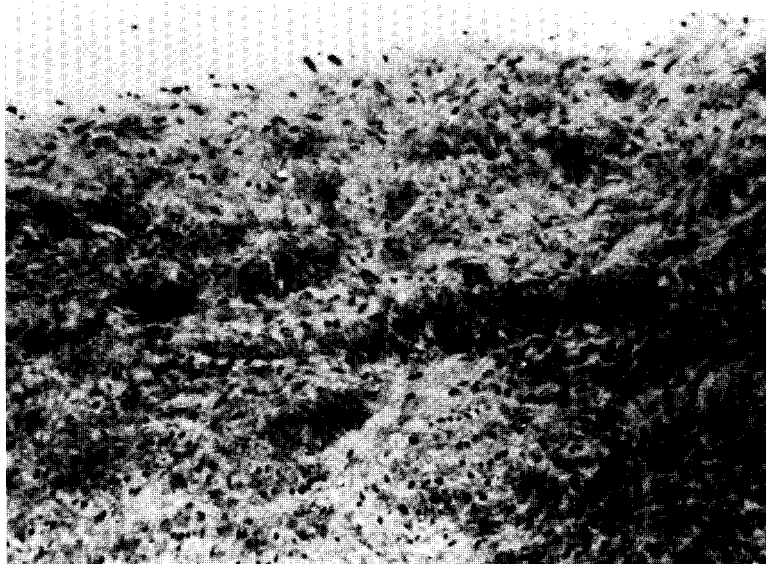


Fig. 2. Pathologic change of the connective tissue of the canine femoral arterial wall 14 days after regional lymphatic blockade (lymph stasis). Light micrography (toluidine-blue staining) shows bulby vacuolated material within the media and adventitia between connective fibers. This material resembles acid glycosaminoglycan. (x700)



Fig. 3. Muscle cell destruction in the femoral artery of the dog 12 days after regional lymphatic blockade (lymph stasis). Electron micrography (x20,000) shows interstitial edema and slight muscle swelling with vacuoles (arrows).

placed into Krebs-Ringer solution. The intraluminal pressure was altered slowly in the range of 5-250mmHg. External

diameter of the arterial segment was measured by a cantilever strain-gauge transducer and was recorded as a func-

Table 1
Biochemical Properties (mean \pm SEM) of the Canine Femoral Wall with and Without Regional Lymphatic Blockade

	Control (sham)	Experimental (lymphostasis)
Lactic acid content (mM/g protein)	36.5 \pm 1.00	50.50 \pm 72.00
Sialic acid content (mg/g protein)	2.64 \pm 0.52	5.02 \pm 0.62**
Hexosamine content (mg/g protein)	348.60 \pm 42.10	562.2 \pm 49.20**
Collagen content (mg/g protein)	43.40 \pm 8.20	56.80 \pm 0.80*

*p<0.05
**p=0.01

tion of the intraluminal pressure following total relaxation of the vascular smooth muscle (5). Subsequently, norepinephrine (3×10^{-5} mol l⁻¹) was added to the tissue bath and the recording was repeated ("active" curves).

For statistical analyses of the experimental data, the Student's t test was applied.

RESULTS

Edema of the hindlimb developed in 22 dogs on the side of the lymphatic blockade. Histopathologic changes developed in the femoral artery in each dog with lymph stasis. Subendothelial edema in the intima and interstitial edema and swelling of the smooth muscle cells in the media were detected. Electron microscopy revealed patchy necrosis and swelling of the mitochondria with ruptured cristae in the muscle layer. The adventitia was enlarged and contained dilated lymph vessels partly filled with granular material, interstitial edema, and infiltration by macrophages. Fibrosis in the arterial wall regularly developed after 10 days following lymphatic blockade. Histological changes were similar after shorter or longer lymph stasis (i.e., dogs killed between 4-11 or 12-21 days following lymphatic blockade). Intramural fibrosis,

however, was more regularly seen in the latter group. The thickness of the media (on frozen section) was $225 \pm 14 \mu\text{m}$ in controls compared with $256 \pm 16 \mu\text{m}$ in lymphatically blocked femoral arterial segments ($p < 0.01$). Characteristic histologic changes are shown in *Figs. 1-3*.

Biochemical properties of the femoral arterial wall also changed with insufficient lymphatic drainage. Metabolism became more anerobic. Thus, the activity of enzymes involved in oxidative metabolism (succinyl dehydrogenase, cytochrome oxidase) decreased, while that of uridine diphosphate dehydrogenase (involved in anerobic catabolism of carbohydrate) increased. Oxygen consumption of the femoral arterial wall significantly decreased (by 45%), while CO₂ production markedly increased (by 30%). The lactic acid content of the femoral arterial wall increased by 38%, the sialic acid content by 90%, the collagen content by 31%, and the hexosamine content by 61% (*Table 1*).

Changes in femoral arterial mechanics also developed with impaired lymphatic drainage. The distensibility of the passive wall component decreased by 27 and 59% respectively, while that of the isobaric active tangential strain decreased by 54%. Moreover, wall thickness increased, the inner radius decreased and the tan-

Table 2
Geometric, Elastic and Contractile Properties (mean \pm SEM) of Canine Femoral Artery with and Without Lymphatic Blockade

	Control (sham)	Experimental (lymphostasis)
Outer radius (mm)	1.94 \pm 0.11	1.77 \pm 0.11**
Inner radius (mm)	1.69 \pm 0.11	1.42 \pm 0.12**
Cross section of lumen (mm ²)	9.36 \pm 1.16	6.85 \pm 1.11**
Tangential force (Newton/m)	22.6 \pm 1.5	19.0 \pm 1.7**
Wall thickness (μ m)	243 \pm 18	343 \pm 35*

*p<0.05
**p=0.01

gential force diminished (*Table 2*). No histopathologic or biochemical changes were detected in the control (sham operated) contralateral femoral artery.

DISCUSSION

Experimental data have suggested that lymph stasis induces blood vessel wall damage. Thus, blockage of lymphatic transport from the blood vascular wall promotes histopathologic changes in the coronary arteries (1,2,6,7) and in the aorta (8,9). Moreover, the vascular biomechanics of the arterial wall are also deranged with impaired lymphatic drainage (10,11). The present results demonstrate that insufficient lymphatic transport similarly produces damage to a major peripheral artery.

Experimental or "artificial" lymphatic arteriopathy is characterized first by the development of histologic changes in the arterial wall, namely, interstitial and intracellular edema, degenerative changes in the smooth muscle layer, lymph stasis in the adventitia, and intramural fibrosis. Metabolic changes accompany the histomorphologic derangements. Thus, the arterial wall shifts toward anerobic metabolism and the collagen and aminoglycan content of the femoral artery itself are increased.

Changes in the biomechanical properties of the femoral artery also develop with insufficient lymphatic drainage. The distensibility of the arterial wall decreases both in the relaxed and activated state. Taken together, these data suggest that insufficient transport of liquid and protein within the blood vascular wall contributes to the pathogenesis of some arteriopathies.

In this regard, it is noteworthy that impairment to the arterial wall occurs with experimental chronic lymphedema (2,12). Clinical observations, too, suggest that inadequate lymph drainage contributes to the genesis and progression of some blood vasculopathies. For example, after lymphatic blockade, propagation of atherosclerosis is promoted (13-15). Similarly, destruction and obliteration of regional lymphatics by irradiation induces coronary arterial disease (16,17). In the long run, however, further experimental and clinical studies are needed to determine more precisely the pathomechanism of impaired lymphatic drainage induced arteriopathies particularly over many months and years of ongoing lymph stasis.

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