METHODS

Modeling of Phlebothrombosis

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A new model of phlebothrombosis has been developed. Thrombotic mass is prepared *in vitro* by mixing dog's blood with thrombin. Retracted clot is injected transcutaneously in ligated segment of the jugular vein. The veins are examined visually and microscopically 1, 3, 5, 7, 10, and 14 days after injection. It is found that the thrombus is loosely bound to the vascular wall within 8-10 days. Endothelial desquamation starts on the 3rd-5th day.

Key Words: *thrombosis; modeling; external jugular vein*

High incidence and inadequate results of conventional therapy of deep venous thrombosis pose a number of problems that can be solved experimentally. Therefore, a model of thrombosis reflecting with maximum accuracy the course of thrombosis in humans is necessary.

Thrombosis modeling can be arbitrarily divided into two groups:

1. Injection of compounds that stimulate thrombus formation (thrombin or thromboplastin) into a temporally ligated denuded blood vessel [1,3-5].
2. Injection of thrombosis-stimulating antigen into sensibilized and stenosed vein [4].

However, these models to a greater extent correspond to thrombophlebitis, since they involve damage to the vascular wall and surrounding tissue as a result of local inflammation. In addition, it is impossible to create a model of obturating thrombosis due to intravasal retraction of blood clot.

In this study we attempted to develop a simple and reliably reproducible model of phlebothrombosis with maximum correspondence to clinical manifestations of the disease in humans.

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MATERIALS AND METHODS

Twenty-two adult mongrel dogs (body weight 17-35 kg) of both sexes were used. They were operated under aseptic conditions and thiopental (25 mg/kg) anesthesia with fentanyl premedication (0.1 mg/kg). The external jugular vein (EJV) was chosen for thrombosis modeling, which allowed us to control the development of thrombus by palpation. In order to prevent intravasal retraction and simulate obturating thrombosis, thrombotic mass was prepared *in vitro*. For this purpose 20-40 ml of blood collected from a superficial vein on the leg was incubated in a sterile vial with 3-4 ml of warmed thrombin (250 U/kg) solution for 40-60 min at room temperature. Then the mass was squeezed through several layers of medical gauze to separate retracted clot. The clot was transferred into a 20-ml syringe. Dog's neck was thoroughly shaved and treated with iodine, after which the EJV was clearly seen. The distal segment of the vein was transcutaneously ligated by the method of Klapp, which is employed in the surgical treatment of varicose veins. The proximal segment of the vein at the angle of mandible jaw was punctured with an injection needle (6F according to the French scale) and catheterized by the method of Seldinger. The thrombotic mass was injected into the vein through a

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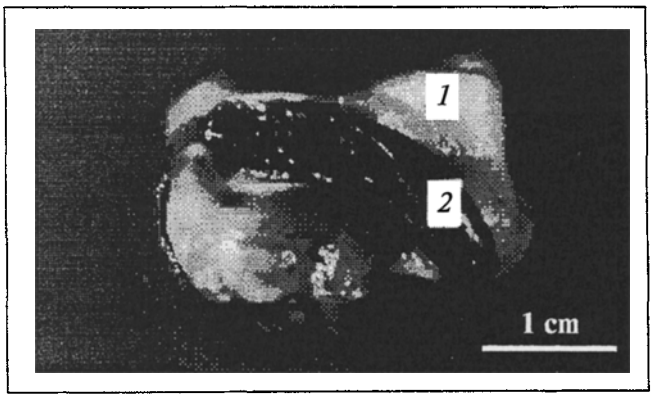

Fig. 1. Thrombus (*2*) formed in the external jugular vein (*1*) on day 8 after injection of thrombotic mass.

catheter to fill the entire vein, which was controlled visually and by palpation. The catheter was then removed, and the proximal segment of EJV was ligated downwards the site of punction analogously to the distal segment. The opposite EJV, in which thrombosis was reproduced by the another method [3], served as a control.

The vein were dissected on days 1, 3, 5, 7, 10, and 14 after the operation and examined visually.

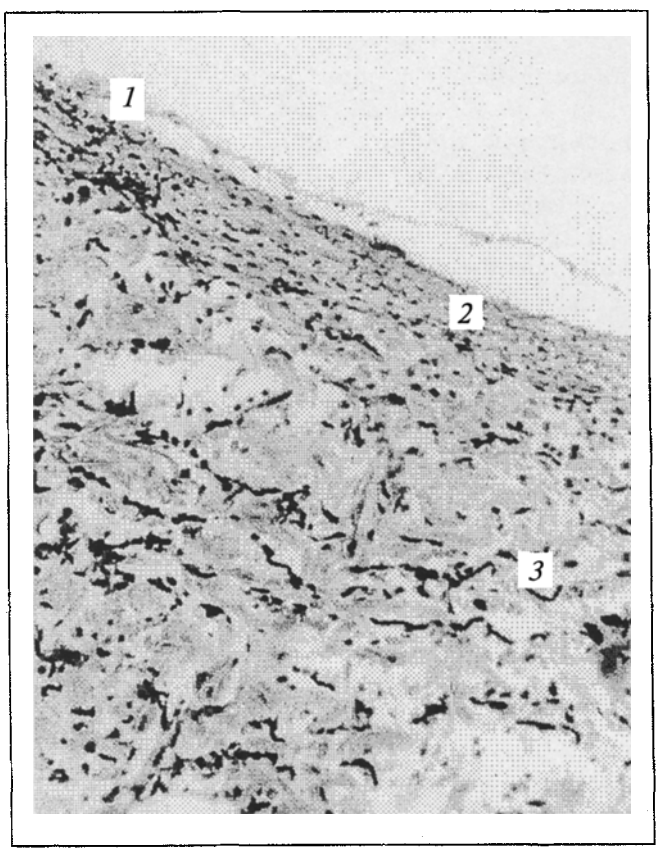

Fig. 2. Light microscopy of vein wall on day 5 after infection of thrombotic mass. Exfoliation of the intima (*1*), leukocytic infiltration of the media (2) and adventitia (*3*). Staining with hematoxylin and eosin, ×90.

Segments (1-cm long) were fixed in 10% neutral formalin, embedded in paraffin, stained with hematoxylin and eosin or picrofuchsin by the M.S.B. method, and studied by light microscopy.

RESULTS

By palpation, thrombus did not formed in two dogs, presumably due to a low activity of thrombin. In other dogs, thrombus was palpated throughout the entire observation period (1-14 days). Obturating thrombus was not attached to the vascular wall for 7-8 days (Fig. 1).

According to the light microscopy data, the vein wall remained practically unchanged within the first 24 h after injection of thrombotic mass. Endothelial structure was preserved, and there was no inflammatory reaction in the deep layer of the vein wall.

Slight focal desquamation of endothelium was observed on days 3-5 (Fig. 2). Moderate diffuse leukocytic infiltration occurred in the media, and leukocytes were seen in the surrounding tissues. Stasis was observed in *vasa vasorum*.

Total endothelial desquamation started on day 7. Fibroblast and small individual capillaries entered the thrombus from the intima. The empty spaces in the thrombus were covered with endothelial cells. There was no pronounced inflammatory reaction in the close proximity to the vein. Moderate thickening of the vein wall could be attributed to impaired blood outflow via *vasa vasorum*.

On day 10, fibroblasts and capillaries ingrowing into the thrombus gave rise to collagen fibers. Vascular wall was thickened, and perivascular tissue was sclerotized. Although the degree of the thrombus organization increased considerably, the thrombus was not attached to the vascular wall.

On day 14 a well-organized thrombus was firmly attached to the vein wall.

Organization of the thrombus produced in EJV by the method [3] started on the first day, since the inflammatory reaction from the surrounding tissues also involved the vein wall. As a result, the thrombus was attached to the vascular wall on days 3-5.

Thus, production of the thrombus using a minimal invasive method without denudation of blood vessels and subsequent development of periphlebitis provides a model adequately reflecting clinical course of the disease (in this case phlebothrombosis). Preparation of thrombotic mass *in vitro* allows one to avoid intravasal retraction of the clot and provides a model of obturating thrombosis. Thrombus organization starts on days 3-5 after injection of thrombotic mass, and the thrombus is not firmly attached to the vein wall for 8-10 days.

This model of phlebothrombosis can be used in experimental studies of structural and ultrastructural changes with subsequent choice of optimal terms of thrombectomy.

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Immunological Aspects of Alisat in Patients with Diabetes Mellitus

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The effect of long-term (12 months) therapy with the garlic-containing preparation Alisat on some parameters of immunity is studied in 52 patients with noninsulin-dependent diabetes mellitus (type II). It was found that Alisat increases natural resistance in the beginning of therapy and induces no remote immunopathological responses.

Key Words: *Alisat; diabetes mellitus; immune system*

Garlic-containing preparations produce various effects in numerous pathological states [1-3]. The information regarding the use of these preparations in patients with diabetes mellitus (DM) is scarce [4]. We failed to find any data on the effect of garlic preparations on the immune system of DM patients.

In the present study we investigated the time course of some immunity parameters in patients with type II DM (noninsulin-dependent) treated with Alisat, a garlic-containing preparation of prolonged action.

MATERIALS AND METHODS

The study enrolled 52 patients with type II DM (18 men and 34 women) aged 42-66 years (mean age 52±1.5 years). The duration of the disease varied from 1 to 20 years. Ten patients had mild DM, 41 patients had moderate DM, and 1 patient had severe DM. In 8 patients diet was used as sugar-reducing monotherapy; other 44 patients received oral hypoglycemic preparations. In all patients, carbohydrate metabolism was compensated or subcompensated. Alisat was prescribed as 1 tablet (300 mg garlic powder) 2 times daily at a 12-h interval. The patients received no special diet or other medication. The immune status was assessed by the factors of nonspecific resistance and specific humoral immunity. The activity of phagocytosis was expressed as the phagocytic index. The intensity of phagocytosis was represented as phagocytic number in experiment with nitro blue tetrazolium (NBT) with evaluation of spontaneous and stimulated phagocytosis. The contents of M, G, and A immunoglobulins were determined by Mancini immunodiffusion technique. The concentration of circulating immune complexes was measured by precipitation in the presence of 4% polyethylene glycol-600. The patients were examined

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