

HISTOLOGICAL, IMMUNOHISTOCHEMICAL, TEM AND SEM INVESTIGATIONS IN THE VALVE-CUSP FREE BORDER OF GREAT VARICOSE SAPHENOUS VEIN

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The authors studied venous valve cusps from surgically removed great saphenous vein with essential lower limb varicosis from 20 patients without data about previous thrombophlebitis. Venous valves without varicose alterations were used as controls. The histological, immunohistochemical, TEM and SEM techniques were applied. In morphologically complete valve cusps from non-varicose great saphenous vein, a small marginal thickening was observed by routine histology. The cells visualised in these thickenings showed positive reaction against anti-vimentin but negative reaction against anti—smooth muscle actin antibody. In varicose vein valve cusps a marginal thickening with considerably larger diameter was histologically observed. In the fibrin-like material which was disposed around these thickenings, proliferation of fibroblasts as well as collagen fibres' depositions were seen. The SEM study showed partial “rollings” of the free cusp border. In the marginal thickening, the cells showed negative reaction not only to anti—smooth muscle actin but they also lost the reaction to anti-vimentin monoclonal antibody. The process mentioned above advanced, it occupied a new part at the valve cusp and so the cusp shortened. According to our hypothesis, this was one of the ways of initiating and advancing incompetence in primary varicosis.

Key-words: Venous valve insufficiency, valve cusp marginal thickening, vimentin, alpha-smooth muscle actin

As it is well-known, venous valvular incompetence plays an impor-

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tant role in the development of varicosis (7,13,15). In the last decades important structural changes that occur in saphenous vein valves during the varicose process were described (2,6,8-10,12). Recently, special attention was

paid to the so-called "marginal thickening" of the valvular cusp free border (4,10). However, knowledge about the formation of this kind of morphological changes of the valve-cusp free border as well as about its exact role for valvular incompetence development is scanty (10). As venous valvular alterations are considered more and more important for the venous varicosity (2,3,6,8,11,12,14), the objective of the present work is to study the morphological changes of the valve-cusp free border during the development of the varicose process.

MATERIAL AND METHODS

Material selection

Segments of great saphenous veins were removed from 10 cases without varicosity and 20 cases with primary varicosity of the lower limbs. The material of the first group was obtained from 4 cadavers, from 1 amputated lower limb, and from non-varicose great saphenous veins taken from 5 patients to whom this vein was used as autologous arterial bypass graft. The material of the second group consisted of surgically removed varicosely dilated great saphenous veins from 20 patients without anamnestic data about previous thrombophlebitis of the lower limbs.

Macroscopic observations and specimen harvesting

The veins were carefully opened longitudinally so as to expose the valves which were removed with adjacent

parts of the venous wall, gently rinsed in saline and fixed as described later. All valves were examined by stereomicroscope. The valves of the first group without any alterations were selected for the present study only. The degree of valve incompetence (atrophy) of the valves of the second group was carefully described. For pathological analysis, representative samples were taken and were divided in parts for light, transmission electron (TEM) and scanning electron microscopic (SEM) examinations. Immunohistochemical stainings were used, too.

Light microscopy

The specimens were fixed in neutral-buffered formalin and, after routine processing, embedded in paraffin wax. Five- μ m sections were cut and one series of them stained after standard histological techniques such as haematoxylin-eosin, orcein, AZAN and by the methods of Mallory and Van-Gieson for light microscopy investigation. The histological sections were taken longitudinally across the valve to show the cusp, the valve agger, and the portions of the vein wall proximal and distal to the valve.

Immunohistochemistry

Immunohistochemical staining for vimentin and alpha-smooth muscle actin was carried out by using monoclonal antibodies purchased from Sigma and by a standard avidin-biotin-peroxidase method. The sites of antibody depositions were visualized by

substrate that contained hydrogen peroxide (H_2O_2) and a chromogen. Two different chromogens were applied in parallel, 3-amino-9-ethyl carbazole (AEC) and 3,3' diaminobenzidine tetrahydrochloride (DAB). Control sections in which the first antibody was omitted and which were treated with biotinylated negative control (product of Sigma) showed that the level of non specific staining, in all cases, was very low.

Transmission electron microscopy

Tissue specimens were fixed in 4% glutaraldehyde in phosphate buffer, pH 7.4, postfixed for 2 h in 1 % OsO_4 , dehydrated in graded series of ethanols and embedded in Durcupan. Ultrathin sections were viewed in a JEM 7A transmission electron microscope.

Scanning electron microscopy

These specimens were fixed in 4 % glutaraldehyde in phosphate buffer, pH 7.4, postfixed in 1 % OsO_4 , rinsed twice in distilled water, dehydrated through a graded ethanol series, immersed in hexamethyldisilazane for 5 min, and then air-dried. They were gold palladium-coated and examined in a Jeol JSM-35CF scanning electron microscope (Soquelec LTD., Montreal, Quebec, Canada) at a 15-25 kV accelerating voltage.

RESULTS AND DISCUSSION

Histologically, in morphologically complete valve cusps from non-

varicose great saphenous veins a small marginal thickening is observed. On higher magnification, in sections of the cusps oriented parallelly to the longitudinal axis of the vein the shape of this thickening appears elongated and ellipsoid. The cells visualized in the stroma of normal valvular cusps such as sub-endothelial cells and "stroma cells" are sparse. In normal veins of our material they contain intermediate filaments. The vimentin nature of these filaments is expressed by positive reaction against anti-vimentin monoclonal antibody. The immunohistochemical investigation of -smooth muscle actin shows positive cells only in the valve agger and as exception in the proximal part of the valvular cusps but not in their terminal part. In the valve cusps of varicose great saphenous vein there is a irregular marginal thickening of various shape and considerably larger diameter. Both the light microscopy and SEM analysis demonstrate that these thickenings consist of a "rolled" terminal part of the valve cusp around which a fibrin-like material has been deposited (Fig. 1 and Fig. 2). Proliferation of connective tissue cells in these fibrinoid deposits can easily be seen. After immunostaining the cells in these thickenings demonstrate completely negative reaction against anti-smooth muscle actin antibody. In addition, most cells in the thickenings of valve-cusp free border in varicose vein lose the positive reaction for vimentin.



Fig. 1. A morphologically incomplete valve of a varicose saphenous vein and a marginal thickening of larger diameter. Haematoxylin-eosin stain. Microphoto. Magn 10x10

In the varicose great saphenous vein valvular cusps a marginal thickening with various shape and considerably larger diameter (10) is revealed around which a fibrin-like material is deposited. Proliferation of connective tissue cells in these fibrinoid deposits are visible. According to our opinion, the marginal thickenings mentioned above arise based on a “rolling” of the free cusp border. As this process advances, it occupies a new part of the valve cusp and the cusp becomes shorter. One of the consequences of this structural alteration is that the course of the valvular cusp changes so as to control the di-



Fig. 2. A morphologically incomplete valve of a varicose saphenous vein and an irregular marginal thickening. SEM. Magn. as shown

rection of the blood flow. On the other hand, in the present study immunocytochemistry by means of antibodies against two intracellular proteins documents and compares the nature of the cellular population in the valve-cusp free border of non-varicose and varicose great saphenous veins (1,5,9). The most significant immunohistochemical finding in varicose saphenous veins is the fact that, in the marginal thickening, the cells show negative reaction not only to anti—smooth muscle actin, but most of them also lose the reaction to anti-vimentin monoclonal antibody (9). The vimentin is a distinct subclass of intermediate filaments typical of many mesenchymally derived cells and a variety of distinct non-epithelial cells of other tissues (1,5). In our opinion, the

change of the type or amount of intermediate filament expression in the cells of the valve-cusp free border is very important because it may phenotypically reflect certain pathologic disorders of the vascular system.

Finally, we believe that these alterations of the valve-cusp free border are very important when the decision for the reconstructive operations of the venous valves should be made (16).

CONCLUSION

The thickening of the free border and the shortening of the valve cusp in combination with the histochemical alterations may be one of the ways of arising and advancing incompetence of the valves of varicose saphenous veins.

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Хистологични, имунохистохимични, ТЕМ и СЕМ изследвания в свободния ръб на клапното платно при варикоза на голямата подкожна вена

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Резюме: Проведени са хистологични, имунохистохимични, ТЕМ- и СЕМ-изследвания върху материал от оперативно отстранена голяма подкожна вена от 20 пациенти с есенциална варикоза без данни за прескаран тромбофлебит. За контрола са изследвани клапи от голяма подкожна вена без варикозни промени. Използвани са хистологични, имунохистохимични, ТЕМ- и СЕМ-методи за изследване. При морфологично пълноценните клапни платна на неварикозната голяма подкожна вена посредством рутинното хистологично проучване се установява леко задебеляване на свободния им ръб. Клетките, наблюдавани в тези задебеления, показват положителна реакция към антивиментин, а отрицателна - към антиятлото на анти-алфа-гладкомускулния актин. При повечето клапи на варикозно разширената голяма подкожна вена хистологично се установява значително по-голямо задебеление на свободния ръб на клапните платна. В подобния на фибрин материал, разположен около тези задебеления, се наблюдава пролиферация на фибробласти и отлагане на колагенни влакна. СЕМ-проучването показва частично "завиване на руло" на свободния ръб на клапното платно. В областта на задебелението му клетките показват отрицателна реакция не само към анти-алфа-гладкомускулния актин, но загубват и реакцията си към моноклоналното анти-виментин антиятло. Процесът напредва и заема нови участъци на клапното платно, така че то се скъсява. Съгласно нашата хипотеза това е един от пътищата за започване и развитието на клапната некомпетентност при първичната варикоза.