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Morphological picture of the big saphenous vein following cryoobliteration of varices

Obraz morfologiczny żyły odpiszczelowej dużej po krioobliteracji żylaków

The last decade's novelty in surgical sclerotherapy of lower extremities is the use of low temperatures (5, 9). By using cryoprobe, freezing cryostripping procedures or sclerotherapy are performed safely, painlessly and quickly (3, 4, 6, 10).

It is generally known that cryoapplication is a king of treatment with an intentional, surgeon-controlled destruction of the tissue, therefore it seemed interesting to study the sphere of cryogen action in the histological aspect.

MATERIAL AND METHODS

For histological assessment, sections of a big saphenous vein (*vena saphena magna*) intrasurgically collected in the course of cryoobliteration of varices were used.

The procedure was performed using cryosurgical apparatus AK-1 produced by KRIO-METRUM in Warsaw, and nitrous oxide (N_2O) was the freezing agent. To the pathologically changed vessels there was introduced a probe of 1 mm in diameter, which froze the surrounding tissues to -80°C. Cryoapplication lasted for approx. 1 min. From the obtained tissue material there were made preparations which were assessed in the light microscope, transmission electron microscope and scanning microscope.

The paraffin sections were stained by the routine method with the use of hematoxylin and eosin (H+E), by the Masson's method and with resorcin-fuchsin, and were assessed by means of the light microscope. For ultrastructural examinations sections of venous vessel were fixed in 4% glutaralde-

hyde in cocodile buffer (pH 7.2 – 7.4). Next, the sections were dehydrated with ethyl alcohol of increasing concentration and embedded in Epon 812 (7). Halt–thin sections, 1 mm thick, stained with 1% methylene blue and Azur II were subject to verification, and a place for cutting ultrathin sections was chosen. The preparations were observed through the transmission electron microscope TESLA BS 500 and scanning electron microscope TESLA BS 301.

RESULTS AND DISCUSSION

Cryoobliteration of the big saphenous vein was followed by narrowing of the vessel, parietal thrombus was also observed (Fig. 1).

On the basis of histological examinations changes in the internal layer of the veins wall were found. The freezing zone was limited to the endothelium cells layer and distinctly affected the neighbouring cells of the subendothelial layer (Fig. 1).

Both the light microscope and electron microscope revealed cryonecrosis of the endothelium (Fig. 2). Endotheliocytes occurring in places were characterized by the untypical shape of the cellular nucleus proving its considerable shrinkage and accumulation of dense chromatin at the nuclear areola. Numerous vacuolar cytoplasmatic indentations led to discontinuation of the surrounding cells membrane (Fig. 3). In places, one could observe losses in the subendothelial layer and within the latter, losses in smooth muscle cells of the longitudinal or oblique system. These local erosions reached the media of the venous wall and were filled with blood morphotic elements (Fig. 4). In connection with cryonecrosis of the endothelium together with the basement membrane, the elements of the subendothelial layer were devoid of the tissular barrier. The morphological picture showed the distinct loosening of the intracellular structure of the endovein. The visible spaces isolating particular cells were filled with vacuolar formations.

The H+E staining revealed an intensive, homogeneous acidophilia of the cytoplasm in the myocytes of the subendothelial layer. This phenomenon, signallizing necrotic processes, was more strongly marked in peripheral cells located close to the cryoapplicator. The examinations carried out by means of electron microscope confirm this. In the myocytes, immediately under the endothelium, a considerable shrinkage of cellular nuclei was observed. This was manifested by deep meanders of the nuclear coat, which was accompanied by anomalies of intracellular systems of membranes. The endoplasmic reticulum canals were distinctly widened and mitochondria were swollen (Fig. 3, 5). Moreover, the examinations of the subendothelial layer ultrastructure revealed myocystes without traits of cryogenic destruction. These cells were usually located farther from the vascular cryoprobe

place of action. They were characterized by oval nuclei of smooth contours, distinct molecules and an increased number of polyribosomes in the cytoplasm (Fig. 6).

It is generally known that during the application of the liquid nitrogen there is formed an ice ball around the probe, and inside the ball, the so–called eutectic point, that is the area of permanent destruction of the frozen tissue, directly coming into contact with the cryoprobe (8). In this connection a total destruction and stratification of the internal membrane endothelium of the examined vein was observed. The carried out examinations suggest that the kind and intensification of changes in the cells of the studies structure depend on the location in relation to the source of the cryogen. It can be presumed that the proximity of the action area of the eutectic point was the reason why the adaptability of cells was broken. The destruction of plasmatic as well as intracellular membranes conditioning water barrier was observed in the cells. The fluid, connected with the hyaloplasm of the endoplasmatic reticulum interstice, after invading the cell caused its dilatation and also, it accumulated in mitochondria. Likewise, the increased acidophilia of the cellular cytoplasm, signallizing necrotic processes, was more strongly marked in peripheral myocytes immediately under the endothelium.

It seems that in the cells located at a proper distance from the cryoapplicator, the process of vitrification was not destructive, but on the contrary, a stimulating one. In the process of cryosclerotherapy it is a specially positive phenomenon because in consequence it leads to the production of colagen, may stimulate proliferation of connective tissue, which conditions cryoobliteration (1, 2). This allows to leave the "frozen" obliterated vessel without cryostripping (5).

It also seems that cryoapplication did not produce any changes in histological layers located far from the vascular probe. Therefore, the vitrification response in the middle and outer membrane cells of the vascular wall was not found. The microscopic picture of both layers was characteristic of the varicose vessels (1). Probably these structures were beyond the boundary of ice deposition of the cryoprobe.

It results from the observations made that the tissular reaction to freezing may be different – from small stimulation to total destruction due to a severe cryogenic trauma. Therefore, the endothelium and myocytes of the subendothelial layer located directly under the endothelium, were totally destroyed. The cells of the border–line area of action of ice–micromolecules showed metabolic stimulation. The structures beyond the scope of action of the applicator did not show any cryogenic changes.

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STRESZCZENIE

Badano metodami histologicznymi żyłę odpiszczelową dużą, która poddana była zabiegowi krioobliteracji żylaków. Stwierdzono, że strefa krionekrozy ograniczyła się do śródbłonka i wywierała wpływ na sąsiadujące komórki warstwy podśródbłonkowej. Procesem decydującym o destrukcji komórek poddanych zamrożeniu było uszkodzenie błon komórkowych, towarzyszyły mu zmiany struktury siateczki endoplazmatycznej i mitochondriów. Intensywność opisanych uszkodzeń zależała od odległości od punktu eutektycznego. Nie stwierdzono zmian kriodestrukcyjnych w błonie środkowej i zewnętrznej ściany badanej żyły.

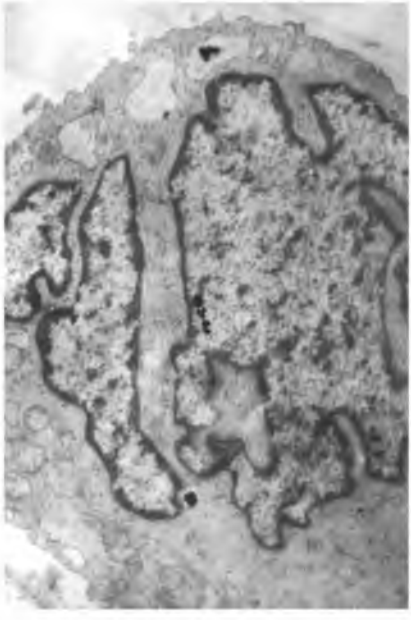

Fig. 5. TEM. *Vena saphena magna – stratum subendotheliale* cell after cryoapplication. Magn. 7200X

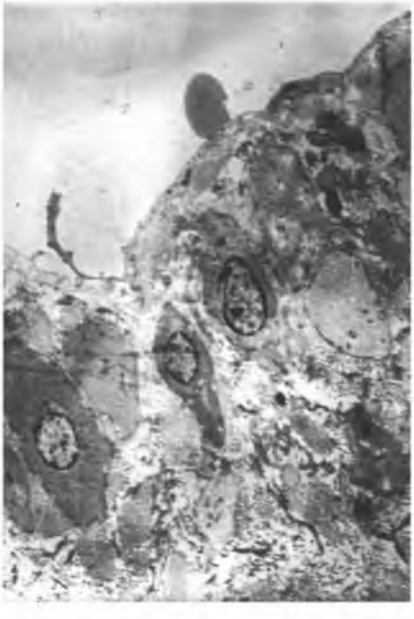

Fig. 6. TEM. *Vena saphena magna – stratum subendotheliale*. Magn. 1800X

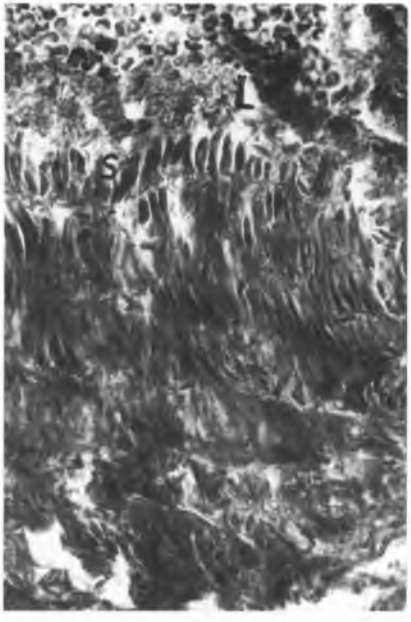

Fig. 1. *Vena saphena magna* – after cryoobliteration.
S – *stratum subendotheliale*. L – vessel's light. H+E staining. Magn. 48X

Fig. 2. SEM. *Vena saphena magna* – endothelium cryonecrosis.
Magn. 720X

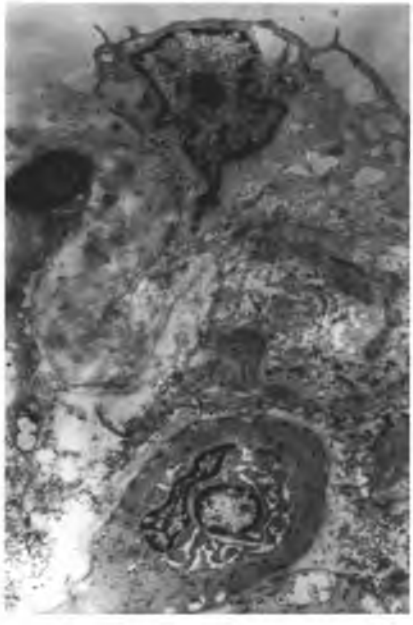

Fig. 3. TEM. *Vena saphena magna – tunica intima.*
Magn. 1200X

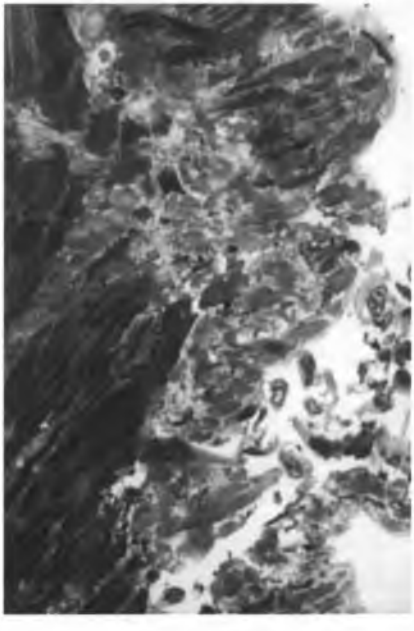

Fig. 4. *Vena saphena magna* - the damage caused by cryogen filled with blood morphotic elements. Staining after Masson. Magn. 48X