

Assessment of connective tissue fibres in walls of allogenic arterial grafts preserved by the method of cold ischemia - a preliminary report

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Abstract

The aim of the study was to evaluate microscopic changes in the wall structures of allogenic arterial grafts, preserved by the method of cold ischemia in relation to the storage period and to test the possibility of the storage period prolongation by allograft freezing at -70°C. The middle layer ultrastructure is well preserved till 30 days from allograft harvesting, however, allograft freezing results in total destruction of elastic and collagen fibres in the arterial wall. An application of allogenic arterial grafts, preserved by the method of cold ischemia till 30 days from their harvesting, seems an efficient therapeutic method in the treatment of patients with synthetic vascular graft infection. Further prolongation of the storage period at -70°C made the allograft useless for implantation.

Key words: arterial allograft, vascular graft infection, cold ischemia preservation, elastic and collagen fibres.

Introduction

Infection of synthetic vascular grafts is one of essential therapeutic problems in vascular surgery. Pharmacological treatment has, in these cases, an unfavourable prognosis, so, the standard procedure is to replace the infected graft [1, 2]. For this purpose, allogenic arterial grafts or silver-coated synthetic prostheses can be used [3, 4]. Both methods have been applied at the Department of Vascular Surgery and Transplantology of the

Medical University of Białystok since 2002. Due to better therapeutic effect, we prefer to use allogenic arterial grafts, preserved by the cold ischemia method, which, in comparison to commonly applied liquid nitrogen allograft freezing, does not require any special equipment and is characteristic of low costs [5]. The aim of the study was to evaluate microscopic changes in wall structures of allogenic arterial grafts, preserved by the method of cold ischemia, in relation to the storage period and to test the possibility of the storage period prolongation by allograft freezing at -70°C.

Material and methods

The allografts were prepared as follows. Sections of abdominal aorta with iliac and femoral arteries were harvested from organ donors, put in a solution, composed of cephalosoline (10 mg/100ml), metronidasol (7 mg/100ml) and heparin (5000u/100ml) and stored at 4°C. The allograft wall ultrastructure was assessed at 8 days and then between 20 and 30 days from allograft procurement. In order to test the possibility of the storage period prolongation, the allograft was frozen at -70°C after 30 days of cold ischemia. It was thawed in 0.9% NaCl ice solution after 14 days of storage at -70°C and its wall was once again examined. The evaluation of the allograft wall ultrastructure was performed by electron microscopy. At all the assessed storage periods, five specimens (about 1 mm³) of the allograft wall were taken from its different parts, fixed in 3.6% glutaraldehyde with 0.1mol/l cacodylate buffer (pH 7.4) for 3 hours, washed with the buffer and postfixed in 2% osmium tetroxide for 1 hour. The samples were then dehydrated in alcohol and propylene oxide and embedded in Epon 812. Ultra thin sections were cut from each specimen on a Reichert ultramicrotome, stained with lead citrate and uranyl acetate and studied under an Opton 900 PC transmission electron microscope.

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Figure 1. Considerable damage of the vessel internal layer with a total destruction of endothelial cells (arrow). Disintegrated smooth muscle cells (*) and well preserved elastic and collagen fibres in the allograft middle layer (Original magnification 4400 x).

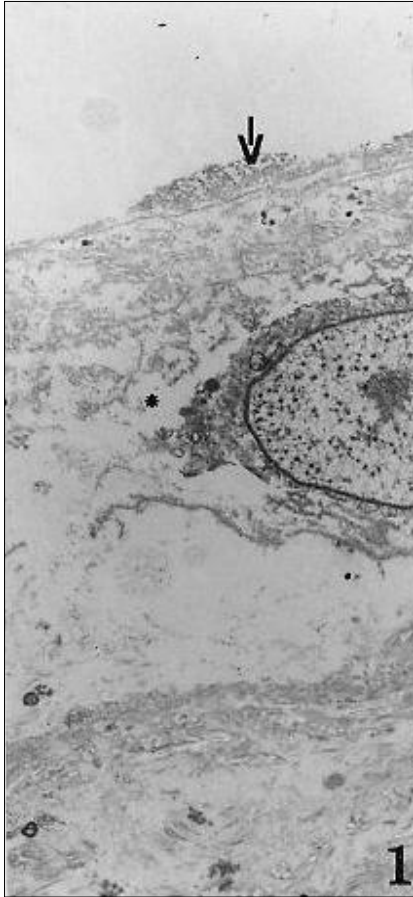


Figure 2. Well preserved collagen (C) and elastic fibres and fragments of disintegrated cells (*) in the allograft wall middle layer (Original magnification 4400 x).

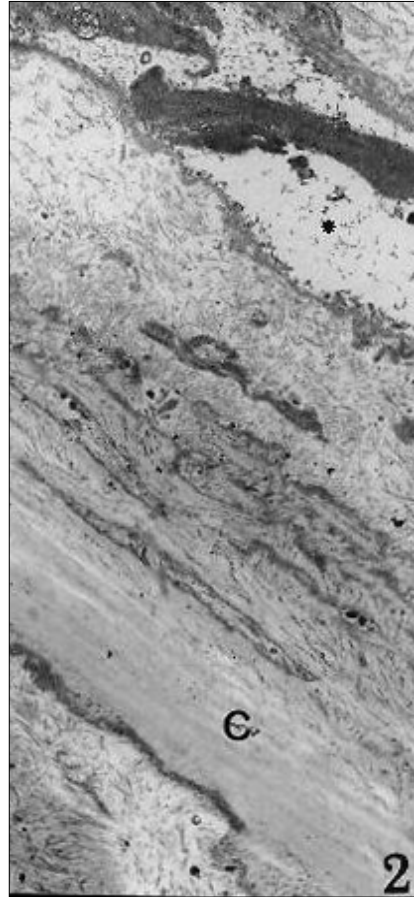
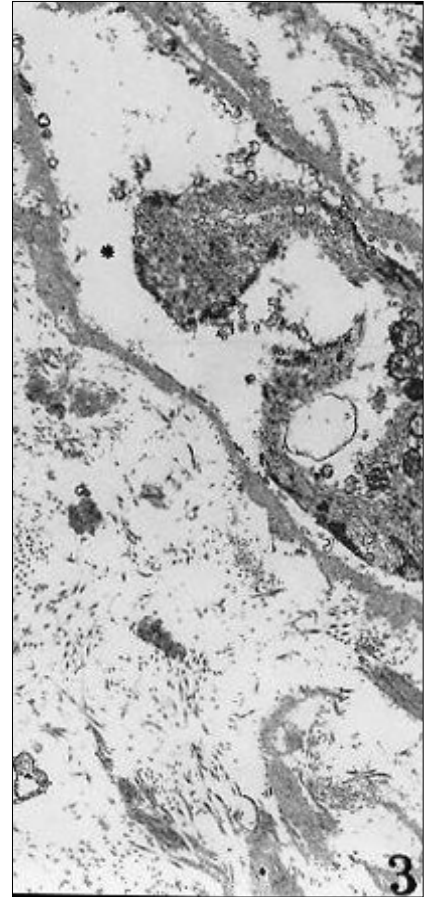


Figure 3. Total destruction of elastic and collagen fibres and disintegration of smooth muscle cells (*) in the allograft wall middle layer. (Original magnification 4400 x).



Results

After 8 days, a destruction of cells in all the layers of the allograft wall was observed, whereas the structures of elastic and collagen fibres in the middle layer were well preserved (Fig. 1). Their structure was quite well preserved till 30 days after allograft harvesting (Fig. 2), whereas prolongation of the allograft storage at -70°C caused a total destruction of elastic and collagen fibres in the allograft wall (Fig. 3).

Discussion

The method of cold ischemia provides elimination of viable cells with simultaneous good preservation of elastic and collagen fibres. Destruction of cells in the allograft wall and their content release into the preserving solution takes place during the first days of preservation. The lack of viable cells prevents the immunological response after allograft implantation, because native elastin and collagen of the same species do not show antigenic properties. It reduces the inflammatory reaction, protease activation and protects the structural proteins of implanted allograft against destruction [6]. Due to the lack of

viable cells, all the problems, associated with blood compatibility, as well as with histocompatibility, can be omitted. However, the short period, available for allograft application after its procurement is an essential limitation of the method of cold ischemia preservation. Prolongation of the storage period above 30 days at 4°C is not possible because of the damage of elastic and collagen fibres. Therefore, we tested the possibility of the storage period prolongation by freezing the allograft at -70°C . However the method of prolonged allograft preservation at -70°C occurred unsuccessful, due to the total destruction of connective tissue fibres, which are responsible for the mechanical resistance of the arterial wall. It could be supposed that water ice crystals, forming in the absence of dehydrating agents during the allograft freezing process, were the cause of ultrastructure damage. It seems that adding DMSO to preservation solution could avoid that. Nevertheless, a longer storage period of an allograft can be achieved by secondary liquid nitrogen freezing or lyophilization [7, 8].

Conclusion

Application of allogenic arterial grafts, preserved by the

method of cold ischemia till 30 days from their harvesting, is an easily accessible and simple method of treatment of patients with synthetic vascular graft infection. Prolongation of the allograft storage period by freezing at -70°C made the allograft useless for implantation because of damage and fragmentation of elastic and collagen fibres

References

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