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The morphological evaluation of the homograft wall in an electron microscopic study

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Cases of massive purulent infection of vascular prosthesis are demonstrated in this study. Infected prosthesis was substituted by arterial homograft, harvested during multi-organ procurement, and stored by the cold ischaemia method. In the follow-up period, the patients were divided into two groups, those treated with immunosuppression (n = 16) and those treated without immunosuppressive drugs (n = 13). The patients underwent resurgery, during which a fragment of arterial wall was taken for electron microscopic examination.

In the group with immunosuppression, the presence of the following structures was observed: endothelial cells, the intima, with a great number of elastic and collagen fibrils with fibrinogen inclusions, and active phagocyting myoblasts and myofibroblasts. In the group without immunosuppression electron microscopic examination showed the total destruction of the wall of the ruptured arterial homograft — absence of endothelium and sparse, damaged fibroblasts of the media or their degraded fragments, making a picture of cellular death.

Morphological analysis of the arterial wall and the clinical state of the patient suggest the necessity of immunosuppressive treatment after fresh arterial homograft transplantation.

key words: homograft, infection of vascular prosthesis, immunosuppression

INTRODUCTION

The common application of vascular prostheses has caused an increase in the number of patients operated on and thus an increase in the number of infections associated with the use of artificial materials, as well as complications following infection [1, 8, 10].

The unsatisfactory results of the treatment when the classical approach to infection control is employed have given rise to clinical trials aimed at tissue graft application [1, 2, 4, 6, 8, 9]. For vessel reconstruction both a patient's arteries and veins are used, as well as homological material — arteries or veins collected from a graft donor [1, 2, 4, 6–9]. The application of tissue material in place of synthetic substance makes the process of inflammatory focus healing easier [1, 2, 4, 6–9]. The problem still remains, however, of the viability of the graft in terms of its antigenicity, as well as the use of immunosuppression associated with this [3, 7, 8].

MATERIAL AND METHODS

In the years 2000–2002 29 patients with infection of the whole arterial prosthesis in the aortic-iliac-femoral segment underwent surgery. Fresh ar-

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terial allografts taken from graft donors were used. Homografts were stored by the cold ischaemia method at +4°C, in preserving liquid UW (University of Wisconsin) with antibiotics (lincomycin, vancomycin). Diagnosis was confirmed by clinical examination, laboratory investigations, bacteriological culture and imaging studies (USG Duplex-Doppler, computed tomography, scintigraphy with Technetium 99m labelled leukocytes). These studies were also applied in postoperative examination. During qualification of the patients for transplantation, blood grouping was conducted, and in the case of fresh arterial grafts, cross-matching tests were performed between the recipient serum and full suspension of lymphocytes and B cells from the recipient. Additionally, antigens of HLA histocompatibility system class I and II were determined, both of the recipient and the donor. 29 patients were divided into two groups. In 16 patients (group I) cyclosporin A was administered after tissue graft implantation at doses 1–2 mg/kg/24 hours, paralleled with the drug level measurements in serum. In 13 patients (group II) immunosuppression was not applied. In the last phase of the follow-up, both the patients with and without immunosuppression underwent surgery. Thus, during reoperation, material was obtained for electron microscopic studies. The follow-up examinations were complemented by studies of the vessel wall in a patient who had stopped taking immunosuppressive drugs.

RESULTS AND DISCUSSION

The patient profiles and the outcome of the operative treatment are collected in Table 1. Both laboratory and imaging studies confirmed regression of infection in all the patients. In both groups of patients operated on, specimens from transplanted arterial allografts were taken during resurgery — 9 months after transplantation without immunosuppression, and 12 months in the case of cyclosporin application. In a patient without immunosuppression a specimen was taken from the removed, ruptured iliac-femoral homograft. A patient treated with immunosuppression was operated on due to arterial embolism. During the surgery an edge of the incised branch of the homograft was taken. Electron microscopic examination showed total destruction of the wall of the fractured arterial homograft —absence of endothelium, sparse, damaged cells (fibroblasts) of the media, or their degraded fragments, forming a picture of cellular death. In the artery collected from a patient to whom immunosuppression had been applied and who was operated on due to acute limb ischaemia caused by arterial embolism, a different picture was observed made up of the following features: the presence of cells of mechanically-detached endothelium, thickened elastic lamina of the intima, the intima with a great number of elastic and collagen fibrils with fibrinogen inclusions, and active phagocyting myoblasts and myofibroblasts (Fig. 1). The activity of myofibroblasts of the transplanted vessel wall was evident in the electron microscopic pictures in the production of a high quantity of collagen fibrils. Fibrinogen inclusions are caused by its penetration from the arterial graft lumen. This patient stopped taking immunosuppressive drugs and 12 months later underwent surgery due to ischaemia of the left lower limb during thrombosis of the left branch of a bifurcated arterial homograft. A specimen from the homograft wall was taken intraoperatively. An examination with an electron microscope showed the absence of vascular

Table 1. The comparison of patients after arterial transplant

Characteristic	Group I	Group II	Statistic
Number	16	13	NS
Age	56 (42–68)	61 (50–71)	NS
Men	15	11	NS
Women	1	2	NS
Homograft Y	11 (69%)	9 (69%)	NS
By-pass	5 (31%)	4 (31%)	NS
Vascular complications	2 (12,5%)	8 (61.5%)	p = 0.0057
Death after vascular complications	–	2 (15%)	NS
Death	–	4 (31%)	NS

Figure 1. Arterial wall with Cyclosporin. Endothelium.

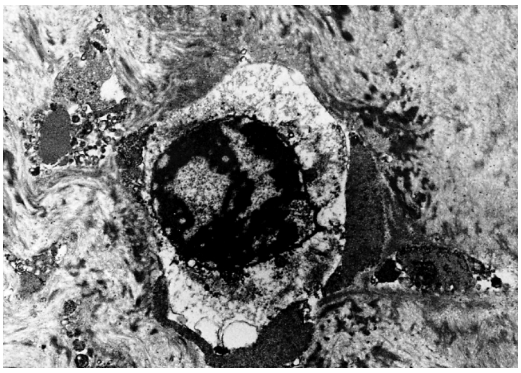

Figure 2. Arterial wall without immunosuppression. Apoptosis of cells.

endothelium cells and a picture of cellular apoptosis in the vessel wall (Fig. 2).

The main purpose of preparation of the arteries for transplantation was the maintenance of vascular endothelium [7–9]. This was possible, thanks to the storage of homograft in UW liquid at a temperature of +4 degrees Celsius and delicate preparation of the arteries from the surrounding tissues [1, 2, 7–9]. Electron microscopic study confirmed the presence of endothelium in an arterial homograft prepared for transplantation in such a way [7]. The choice of this type of homograft storage was caused by the reduced viability of the graft wall, as is documented in the literature, and retrograde changes to the epithelium on the application of deep freezing to the arterial homograft or allograft denaturation [5, 7, 8]. Destruction of the wall of such an arterial graft is especially evident on distant observation [2, 6–8]. However, when fresh arterial grafts with the preserved vascular endothelium are employed, different problems arise. These are associated with the use of immunosuppression in spite of infection, as well as the necessity for donors and recipients to be fully matched with respect to the compatibility of the main blood groups in the ABO and HLA systems [2, 7, 8]. Compatibility in the ABO system and negative cross-matching tests allow for the exchange of infected prosthesis into the allograft [2, 7–9]. Immunosuppression enables the process of degradation of the arterial graft wall to be stopped and its function sustained, which was possible due to the preservation of the arterial endothelium [2, 7, 8]. This observation was confirmed in the studies examining the wall of arterial graft with an electron microscope [7]. The absence of endothelium in homografts stored in a deep-frozen state accounts for the complications associated with the degradation of the graft wall [3–9].

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