JOURNAL OF SURGICAL RESEARCH 200 (2016) 374–386

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

A novel method for long-lasting preservation of arterial grafts

Magdalena Gewartowska, PhD,[a,b,*] *Waldemar L. Olszewski, MD,*[a,b,c] *Olga Buyanowskaya, MSc,*[a,b] *and Malgorzata Frontczak-Baniewicz, DSci*[d]

[a] *Department of Surgical Research and Transplantology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland*

[b] *Department of Human Epigenetics, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland*

[c] *Department of Gastrointestinal and Transplantation Surgery and Clinical Laboratory, Central Clinical Hospital, Ministry of Internal Affairs, Warsaw, Poland*

[d] *Electron Microscopy Platform, Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland*

ARTICLE INFO

Article history:
Received 30 March 2015
Received in revised form
30 July 2015
Accepted 31 July 2015
Available online 5 August 2015

Keywords:
Arteries
Sodium chloride
Preservation
Allogeneic

ABSTRACT

Background: Autologous venous grafts generally give best results for arterial bypass grafting in cases of arterial stenosis. When no suitable venous graft can be found, synthetic prosthetic graft may be an alternative. Prostheses are easily accessible but susceptible to infection. In these cases, the replacement of infected prosthesis by the human arterial allograft is the best treatment option. The question arises whether we could prepare a graft meeting mechanical conditions of an artery immunologically inert and resistant to bacterial infection.

Materials and methods: LEW and BN rat aortic segments were placed in dehydrated sodium chloride and stored for 1 to 12 mo. Then, they were transplanted orthotopically as aortic grafts for 3 to 15 mo in syngenic and allogenic combination. No immunosuppression was used. Patency, pulsation, and frequency of development of aneurysms were studied. The tensile strength and maximum intraluminal pressures were measured. Morphology of grafts was evaluated on histology and electron microscopy. The endothelial and infiltrating cells were identified.

Results: Transplanted allogeneic aortic grafts preserved in anhydrous sodium chloride up to 12 mo remained patent for 15 mo. Hypertrophy of intima with endothelial cells lining the inner surface and single muscle cells between elastic fibers were seen. Normal structure of collagen and elastic fibers was maintained. Only minor-host mononuclear infiltrates were seen around the preserved allografts.

Conclusions: Rat aortas preserved in anhydrous sodium chloride retain patency and function even 15 mo after transplantation. Such grafts retain their wall structure and evoke only minor recipient reaction. Our results confirm that anhydrous sodium chloride may be used for arterial grafts preservation. Low immunogenicity is additional advantage.

* *Corresponding author.* Electron Microscopy Platform, Mossakowski Medical Research Centre, Polish Academy of Sciences, 5 Pawinskiego Str., Warsaw 02-106, Poland. Tel.: +48 22 608 64 45; fax: +48 22 608 65 90.

E-mail address: magdalena.gewartowska@vp.pl (M. Gewartowska).

0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2015.07.046

1. Introduction

Stenosis is the most common complication of prosthetic arterial bypass. Obstruction occurs in 30% of cases within 5 y from implantation [1].

Although only 0.5% to 6% of implanted prosthetic vascular grafts in the lower extremities are complicated by infection yet they have poor clinical outcome [2–5]. Colonization of prosthesis and proliferation of bacteria at the implantation site is part of the "foreign (dead) body reaction". Only in a few cases infection can be successfully cured with antibiotics [6]. In most cases, infected prosthetic grafts should be replaced by new conduits. Optimally, grafts used for replacement of infected prosthesis should not attract microbes and recipient immune cells. The "old arterial bed" is the best location for the new graft placement from the hemodynamic point of view.

Cryopreserved arteries are used for this purpose [7,8]. Cryogenic temperatures maintain vascular integrity over prolonged periods of time. However, cryopreservation causes damage to the endothelium [9], increases fragility of the graft [10,11], and reduces content of elastin fibers [12,13]. Additionally, thawing causes microcracks in the middle and inner layers of the vessel wall [11,14].

The susceptibility to cracking is aggravated when vessels are stored at very low temperatures and then quickly thawed [8,15]. Leaks at the anastomotic sites are more common than in the noncryopreserved arteries [8]. Moreover, cryopreserved allogeneic arteries evoke strong host immune reaction [16]. Finally, cryopreservation requires deep-freezing devices unavailable in most hospitals, especially in developing countries.

Another method of storage which, in contrast to cryopreservation, reduces the antigenicity is fixation in glutaraldehyde [17]. However, fixation in glutaraldehyde leads only to stabilization of collagen but no other components of the extracellular matrix, particularly elastin [18], which results in a low mechanical strength of transplanted arteries [19].

We have been searching for a simple arteries preservation method that would allow long-term storage, spare arterial wall structure and decrease its antigenicity. In our previous research, we investigated the influence of anhydrous sodium chloride preservation on various types of tissues and cells. This idea arose while we were performing research on lower limbs infections in tropical countries, especially India, where the devices for freezing tissues collected for histopathology were not easily accessible. For that reason, we decided to apply the method of osmotic dehydration of tissues. We showed that the osmotic dehydration may be an alternative method for storage and maintains the structure of skin and lymph nodes [20]. We reported that skin preserved in anhydrous sodium chloride evokes only minor immunologic reaction at the site of implantation [21]. In another study, we demonstrated that hepatocytes preserved in anhydrous sodium chloride and then rehydrated show almost unchanged cell morphology [22].

All these findings prompted us to investigate whether arteries can be preserved in this way and retain their mechanical properties and lack of local recipient reaction on grafting.

In this study, we investigated the patency, morphologic structure, elastic properties, and host reaction to allogeneic arterial grafts preserved in pulverized dehydrated sodium chloride.

2. Material and methods

2.1. Experimental groups

Experiments were carried out on 48 male rats divided into six groups (Table 1). Female rats were not studied because their hormones (estrogen and progesteron) fluctuation during the menstrual cycle might influence the results. Allogeneic Brown Norway (BN, RT1An) to Lewis (LEW, RT1Al) and syngeneic LEW (RT1AI) to LEW (RT1AI) aortas were preserved in anhydrous sodium chloride and transplanted orthotopically. Rats of 250 to 300 g body weight and 8 to 9 wk old were maintained in standard conditions and received standard rodent laboratory chow and water *ad libitum*. They originated from the medical research center husbandry. All experiments were conducted according to the European Federation of Laboratory Animal Science Associations guidelines and were approved by the IV Local Ethics Committee in Warsaw (resolution no. 23/2009).

2.2. Preservation and orthotopic transplantation procedure

Three-centimeter long segments of rat aorta were harvested under sterile conditions, dried on sterile gauze, placed in a 10-mm thick layer of pulverized, dehydrated (240°C for 1h) sodium chloride and stored in aluminum foil for a period of 1 to 15 mo at 4°C. Before transplantation, the specimens were rehydrated by immersion in 10 mL of 0.9% NaCl with 5% of BSA (bovine serum albumin) at room temperature. Aortas stored for 1–3 mo were rehydrated for 3 h, those stored for 6 and 12–15 mo for 24 and 48 h, respectively. Laparotomy was performed and upper and lower end-to-side graft anastomoses to abdominal aorta with ligature of host aorta between the anastomotic sites were performed.

Table 1 – Experimental groups.

Group	Type of transplant	Preservation time (mo)	Transplant follow-up (mo)
1a, 1b, 1c, 1d (control)	Nontransplanted ($n = 3$)	0, 3, 6, and 10	0
2a	Syngeneic ($n = 5$)	Nonpreserved	6
2b	Allogeneic ($n = 7$)		
2c	Allogeneic ($n = 2$)	Nonpreserved	12
3a	Syngeneic ($n = 4$)	1	12
3b	Allogeneic ($n = 5$)		
4a	Syngeneic ($n = 3$)	6	6
4b	Allogeneic ($n = 4$)		
5a	Syngeneic ($n = 3$)	12	3
5b	Allogeneic ($n = 3$)		
6a	Syngeneic ($n = 4$)	12	15
6b	Allogeneic ($n = 8$)		