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Cryopreserved aortic allograft replacement of infected prosthetic grafts in man: processing and clinical results

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Abstract Aortic allografts preserved at 4 °C have been used successfully for the replacement of infected prosthetic grafts, but have a limited storage duration and this does not allow for rigourous security of the allograft. Original cryopreservation protocol has been developed, characterized by the use of polyethylene glycol 20000 30 g/l associated with 12.5 % DMSO, high concentration of antibiotics (lincomycin 300 mg/l, vancomycin 125 mg/l), controlled freezing rate, and storage in the vapour phase of liquid nitrogen (– 150 °C). Cryopreserved arterial allografts were used for the replacement of infected prostheses in 22 patients. In 2 patients, allograft-related dysfunction was observed, 3 patients died in the early postoperative period from non-allograft-related causes, and, 17 patients were cured of infection without any dysfunction of the allograft. These results are comparable to replacement by fresh allograft, but cryopreservation allows for better microbiological security, long-term storage, and, thus, better management of the available allografts.

Key words Cryopreservation · Arterial allograft · Prosthetic infection · Graft infection

Introduction

Prosthetic graft infection remains a severe complication in reconstructive aortic surgery observed in 2 % of patients [6] after primary surgery, and in 3–4 % with redo surgery [5]. In situ replacement with "fresh" aortic allografts stored at 4 °C has been used with encouraging early and midterm results [2], and has been proved to be more resistant to bacterial infection than prosthetic grafts [3]. However, conservation at 4 °C has a limited duration of storage and does not allow for rigourous viral security of the allograft. Cryopreservation is the optimal method ensuring the long-term conservation and security of allografts required for tissue banking. To date, clinical experience with cryopreserved aortic allografts for the treatment of prosthetic graft infection is still limited [4, 7].

We present the technical protocol developed to ensure a high quality cryopreservation of aortic allografts, and the results obtained for the first 22 patients.

Materials and methods

Procurement and preparation of cryopreserved aortic allografts

Arterial allografts were harvested from cadavers as part of a program to retrieve multiorgan transplant tissue. Bacteriology and virology tests were performed for all donors as required by French legislation. In case of organ transplantation from the same donor, a complete serology of the organ recipient was performed 3 months after transplantation. After harvesting, arteries were transported in an extracellular Krebs-like solution (Na 130.3, K 5.36 Ca 1.82, Cl 11.6 mmol/l) containing macromolecules, polyethylene glycol (PEG) 20000 (30 g/l) or gelatine (30 g/l), and incubated for 48 h at 4 °C after supplementation with high concentration antibiotics, vancomycin 125 mg/l and lincomycin 300 mg/l, to ensure bacterial decontamination. The allografts were then placed in 100 ml extracellular Krebs-like solution containing 12.5 % DMSO and 30 g/l PEG 20000 in a cold resistant bag (Gambro). Incubation time was determined by proton NMR spectroscopy which allows a fast determination of the permeation kinetic of cryoprotective agents and to determine their effect on water structure. Two hours were necessary to ensure the equilibration of cryopro-

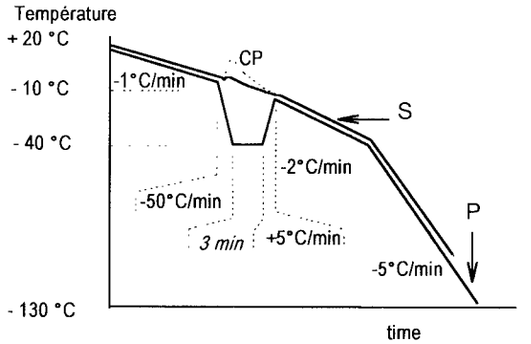

Fig. 1 Computer-controlled freezing rate during cryopreservation of aortic allografts. *P* Programmed temperature, *S* sample temperature, *CP* suppression of the crystallization peak

tective agents in the whole aortic wall [1]. DMSO was equilibrated with intra- and extracellular water, and PEG with extracellular water only. The allografts were cryopreserved with a computer-controlled cooling apparatus (Digit-Cool), which conducted the heat produced during freezing away from the cryoprotective solution (Fig. 1), and then stored in the vapour phase of a liquid nitrogen container (−150 °C). Prior to implantation, the allografts were thawed by immersion in a water bath thermostated at 37 °C for 2 min and then rinsed in successive baths of saline solution containing decreasing concentrations of DMSO (8, 4, and 2 %).

Patients

Between January 1994 and December 1996, the "Assistance Publique Hôpitaux de Paris" tissue bank has supplied cryopreserved arteries to seven vascular surgery centers for the replacement of infected prostheses. There were 22 male patients with a mean age of 63.4 ± 10.9 years (range 43–84). Arterial allografting was performed as an emergency in 3 patients and electively in 19 patients. The infected prostheses were 16 Dacron, 4 polytetrafluoroethylene, and 2 Goretex bypasses. The types of aortic reconstruction and the organisms grown from infected prosthetic grafts are described in Tables 1 and 2, respectively. Multiple organisms grew in 3 patients, whereas no organisms were cultured in 2 patients. All surviving patients underwent digital arteriography or conventional techniques before discharge. Routine late follow up included clinical and duplex scanning at 1 month and every 3 or 6 months according to the protocol followed in each department.

Results

In 2 of the 22 patients, allograft-related dysfunction was observed. One patient was a late death (4 months) due to cryopreserved allograft rupture (poorly documented), and the other was an early allograft thrombosis (36 h) in a patient with a combined bypass (PTFE + CPAA). Three patients died in the early postoperative period from non-allograft-related causes: one colic necrosis (day 2), one heart failure (day 4), and one colic ischemia and pneumopathy (day 30). Seventeen patients were cured of infection without any dysfunction of the cryopreserved aortic allograft. Among these patients, the mean follow-up period was 9 ± 7 months (range 2–24). Eight patients were monitored from 2 to 5 months, 7 patients from 6 to 12 months, and 2 for up to 24 months. Microfractures, without any consequences, were observed only in the first allograft implanted in 1 patient with a survival greater than 24 months.

Table 1 Types of aortic reconstruction

Reconstruction	Number of patients
Aortofemoral	9
Aortoaortic	4
Aortoiliac	3
Iliofemoral/popliteal	3
Axillofemoral	2
Femoropopliteal	1

Table 2 Organisms grown from infected prosthetic grafts (multiple organisms in three patients)

Organism	Number of patients
Staphylococcus aureus	8
Enterobacter cloacae	4
Escherichia coli	3
Staphylococcus epidermidis	2
Streptococcus	2
Pseudomonas aeruginosa	2
Salmonella typhimurium	1
Bacteroides fragilis	1
None	2

Discussion

The present data support the assumption that cryopreserved aortic allografts resist bacterial colonization in vivo. The mechanism of infection resistance of cryopreserved allografts remains unclear, however, antibiotics used for bacterial sterilization during the preparation of the allograft could play an important role, such as described for antibiotic-bonded prosthetic grafts. The particular cryopreservation protocol in which the arterial allograft is loaded with a high antibiotic concentration, gives clinical results comparable to fresh allograft preserved at 4 °C [2, 3]. But conservation at 4 °C is time limited and this makes these allografts unavailable to a significant number of patients. Organization of cryopreserved aortic allograft banking allows for better microbiological security of the graft, long-term storage, and, thus, better management of the available allografts even for urgent reconstruction.

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