Cryopreserved and fresh valved aortic homograft conduits in a chronic sheep model: Haemodynamic, angiographic and histological comparisons

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Introduction

In 1949, Gross (1) demonstrated that simple freezing to — 72 °C of the canine aortic homograft, followed by subsequent implantation, resulted in a high rate of failure due to aneurysm and rupture. Since that time there have been major advances in preservation methods. We thought that it was important to repeat the large animal experimental model studies performed by Gross in order to compare storage of aortic homografts using current techniques of cryopreservation relative to simple storage in a nutrient medium at 4 °C.

Materials and Methods

15 aortic homografts were harvested immediately after death of donor sheep with a mean weight of 23 kg at approximately 4 months of age. Eight homografts were immersed for 48 h at 4 °C in an antibiotic solution composed of cefoxitin 240 mg/ml, lincomycin, 120 mg/ml, polymyxin B, 100 mg/ml, vancomycin, 50 mg/ml, and amphotericin, 25 mg/ml, in Eagle's cell culture medium. The homografts were then stored for 1—4 days in plain Eagle's culture medium at 4 °C prior to implantation (Group A). Seven homografts were placed in ice-cold normal saline in a sterile container and were shipped to the CryoLife procurement plant. Following immersion for 48 h at 4 °C in the same antibiotic solution as Group A, these homografts were frozen in a controlled rate liquid nitrogen freezer (— 196 °C) with 10 % DMSO as cryoprotectant (Group B).

The homografts were inserted into recipient sheep of similar size and weight as the donor sheep, between the right ventricle and pulmonary artery bifurcation without the use of cardiopulmonary bypass. The main pulmonary artery was divided and oversewn. Right heart catheterisation studies were performed at 6 weeks, 4 months, and 9 months. A pullback gradient was obtained between the pulmonary artery and right ventricle. Cardiac output was calculated using thermodilution and right ventricular and pulmonary artery angiograms were performed. 50 % of the animals were sacrificed at 4 months and the remainder at 9 months. Histological analyses were performed in New Zealand.

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Results

There were three early deaths. Two of these animals were from the fresh group. One animal had an unrecognized right haemothorax and one remained in persistent congestive failure following prolonged intraoperative resuscitation. One animal from the cryopreserved group died suddenly 11 weeks after surgery. At post mortem examination, the homograft was found to have ruptured. Histologically, there was evidence of infection.

The weight gain of the animals (51 %) was similar between the two groups. There was an increase (73 %) in transconduit gradient with time, starting with a gradient of between 20—30 mm at 6 weeks after surgery, increasing to approximately 40—50 mm at 4 months with a further slightly increase by 9 months. There was no significant difference ($p = 0.67$) between the gradients in the cryopreserved group and the fresh group.

Figure 1 illustrates the typical angiographic appearance of the homografts. There was frequently a gradient at the point of take-off of the large single first branch trunk, arising from the aorta. Anastomotic gradients were also found, as in the proximal anastomotic stenosis shown in Fig. 2. Fig. 3 is the angiogram taken 6 weeks after surgery in the animal from the cryopreserved group that died 11 weeks after surgery. There is a kink at the midpoint of the homograft with a filling defect which may be an infected thrombus. There is also a proximal bulge in the posterior wall of the homograft where the rupture subsequently occurred. All homograft conduits were found to be heavily calcified by 4 months from the time of surgery. This was verified both by X-ray and histologically.

The homograft conduits were examined histologically from two points of view, namely, as a conduit looking at the aortic wall and as a valve looking at the valve

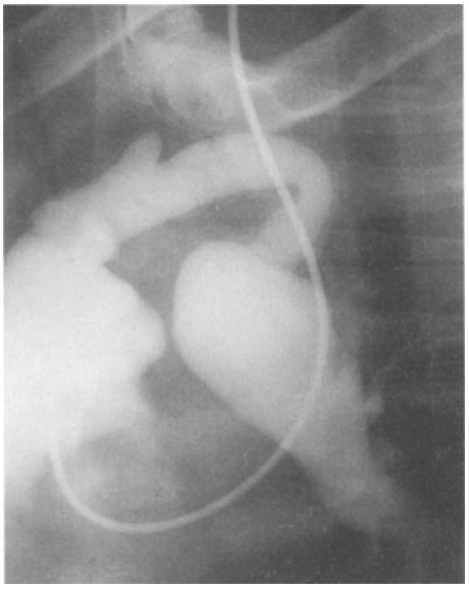

Fig. 1. Typical angiographic appearance of sheep homograft placed between the right ventricle and pulmonary artery. Prominent calcification of the homograft wall is visible.

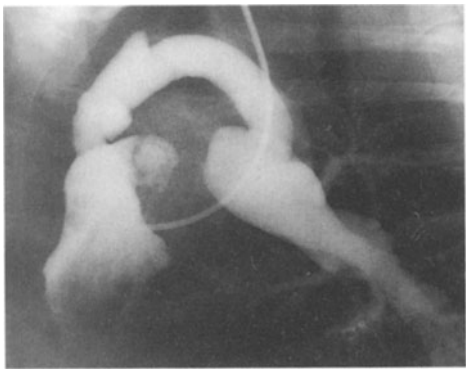

Fig. 2. Proximal anastomotic stenosis between the right ventricle and aortic homograft.

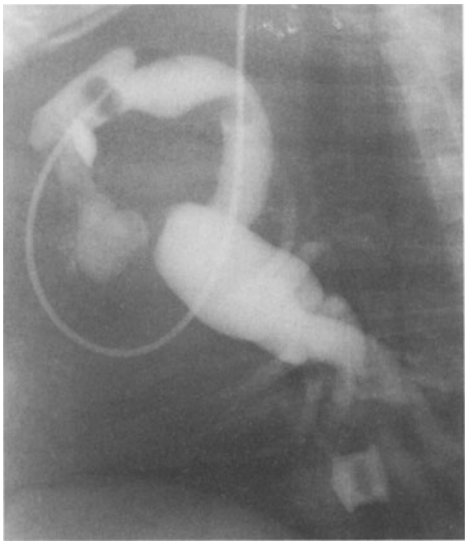

Fig. 3. Pulmonary angiogram 6 weeks following surgery in an animal which received a cryopreserved homograft. A filling defect distal to the midpoint kink is noted, together with a bulge in the posterior wall proximally, which was the site of subsequent rupture.

leaflets. The normal sheep aortic wall has particularly prominent smooth muscle within the media. Islands of smooth muscle are surrounded by elastic fibres. After 9 months of implantation, the most dramatic finding in the aortic wall was the disappearance of smooth muscle, often with persistence of spaces which had been occupied by these muscle islands. There was frequently some fibrosis within the adventitia and often red blood cell infiltrates within the adventitia. Calcification was always present. Often there were areas of separation between calcified plates and the remainder of the aortic wall. Some conduits showed relatively heavy lymphocytic infiltration in the adventitia. This may represent an immune phenomenon. Careful comparison of the fresh and cryopreserved groups failed to reveal any significant difference from the point of view of conduit calcification, loss of muscle cells, or intimal proliferation. There was a suggestion that lymphocytic infiltration

within the adventitia was more prominent in the cryopreserved group than in the fresh group.
There was a variable loss of cellularity from valve leaflets following either method of donor treatment. However, intracuspal thrombus of unknown significance appeared more prominent in the cryopreserved group relative to the fresh group. There was a surprising persistence of endothelium on the valve cusps with both methods of preservation. However, factor VIII immunofluorescence was not used to positively identify the cells which were morphologically indistinguishable from endothelial cells.

Conclusions

This study demonstrates that in the sheep model, cryopreservation with current techniques does not significantly alter the functional integrity of aortic homograft conduits placed between the right ventricle and pulmonary artery relative to antibiotic treated aortic homografts stored in nutrient medium for up to 4 days. There are some minor differences in histological appearance with a suggestion of greater immune reactivity of the cryopreserved homografts. The significance of intracuspal thrombus, seen within the valve leaflets of the cryopreserved homografts but not in antibiotic treated homografts stored at 4 °C, is not known.

References

1. Gross RE, Bill AH, Peirce EC (1949) Methods for preservation and transplantation of arterial grafts. Surg Gyn Obstet 88: 689—701

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