Structural degeneration of pulmonary homografts used as aortic valve substitute underlines early graft failure

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Received 17 January 2002; received in revised form 25 June 2002; accepted 3 July 2002

Abstract

Objectives: The limited availability of donor valves and experimental evidence that pulmonary valves can withstand systemic pressure made us use cryopreserved pulmonary homografts as aortic valve substitutes. We observed a high incidence of early reoperation because of severe graft insufficiency due to cuspal tears. The mid-term results are evaluated in this study and histological analysis of explanted homografts is performed to investigate the cause of graft failure.

Methods: From December 1991 to April 1994, 16 patients (13 male; mean age 37.3 years, range 21–59 years) underwent aortic valve replacement with a cryopreserved pulmonary homograft. The indication was endocarditis (n = 4), bioprosthesis degeneration (n = 3) or congenital aortic valve disease (n = 9). All homografts were implanted freehand in the subcoronary position. All patients were contacted for follow-up and recent echo-Doppler studies were reviewed. Six explanted homografts were examined microscopically using routine histological techniques to analyze changes in cell population, collagen and elastic fiber structure.

Results: Follow-up was complete in all patients. Reoperation was required in ten patients because of severe graft incompetence (mean implantation time 5.9 years, range 2.8–8.0 years). In two patients, recurrent endocarditis was the cause of graft failure. In the other eight patients the leaflets looked pliable and thin with gross tearing in one or more cusps. The histopathologic changes observed were remarkably similar in all examined grafts: the cusp tissue was almost non-cellular and the collagen fiber structure had mostly disappeared. At the site of rupture, the tissue had become thin with strongly degenerated collagen and elastic fiber structure. In the six patients with a homograft remaining in situ, echo-Doppler showed trivial to mild insufficiency in five cases and moderate to severe in one case, whereas no significant gradients were observed.

Conclusions: We concluded that structural reduction of cell number and degenerative alterations in the molecular composition of the extracellular matrix in valve tissue is the main cause of early graft failure in this series. The use of cryopreserved pulmonary homografts in the systemic circulation is therefore not advised.

Keywords: Cryopreserved pulmonary homografts; Pathology; Pulmonary homograft; Pulmonary allograft

1. Introduction

Aortic valve replacement with an aortic valve homograft is a well-established procedure. The aortic valve homograft has a low gradient, a low risk of thromboembolic events, a high resistance to endocarditis and acceptable long-term results when compared with other bioprosthetic and mechanical valves [1,2]. Prompted by the limited availability of donor valves, our group started to use pulmonary valve homografts as aortic valve substitutes in the early 90s. An argument to support this policy was that in vitro biomechanical testing had shown that the pulmonary valve is able to withstand the higher pressure in the systemic circulation [3]. In addition, calcification of the pulmonary homograft wall had been reported to be less than that of the aortic homograft wall tissue [4]. Furthermore, excellent long-term results of the pulmonary autograft in the aortic position were published [5,6].

In 1994, the first cases of acute cusp rupture of pulmonary homografts in the aortic position were reported [7,8]. The procedure was halted in our center, in expectation of longer follow-up. In the following years, redo surgery was performed on several patients for the same reason. Meanwhile, others reported graft failures as well and disappointing results after 5 and 7 years of follow-up [9,10]. We report our experience with cryopreserved homografts in the aortic position (1991–1994), with an emphasis on histological analysis of six explants.
2. Patients and methods

2.1. Patients

Between December 1991 and April 1994, 16 patients (13 male, three female) underwent aortic valve replacement with cryopreserved pulmonary homograft. The mean age at the time of implantation was 37.3 years, ranging from 21 to 59 years. The indication for replacement surgery was mechanical prosthesis endocarditis in one, active bacterial endocarditis of the native aortic valve in two, aortic valve insufficiency as the result of rheumatic disease at a younger age in one, bioprosthesis degeneration in three and congenital aortic valve disease in nine patients. Three patients with congenital aortic valve disease had previous aortic valvulotomy. In cases of active bacterial endocarditis, a homograft was chosen as a valve substitute because of its known high resistance to bacterial endocarditis and in one case it facilitated reconstruction of the damaged aortic annulus. The other patients were all young with a well-considered choice for a homograft, in order to avoid anti-coagulant therapy. The use of aortic or pulmonary homograft valves was determined by first availability of a homograft with the required annulus size.

2.2. Homograft preparation

All homograft valves were acquired from the Heart Valve Bank Rotterdam and prepared and cryopreserved according to their Standard Preparation Protocol [11]. The mean donor age was 33.8 years (range 16.3–56.3 years). Two valves were obtained from ‘domino hearts’, 11 from heart beating donors and three from non-heart beating donors (warm ischemic times limited to 2.5 h). All valves were sterilized in a low dose antibiotic solution for 24 h and dimethylsulfoxide 10% was used as a cryoprotective agent [11].

2.3. Implantation technique

Preoperative transthoracic echocardiographic (TTE) measurements and intraoperative sizing were used to determine the homograft size. No mismatches occurred in this series. The left and right coronary sinuses were scalloped, while the non-coronary sinus remained intact. All valves were inserted freehand in the subcoronary position with continuous sutures, using 4/0 or 5/0 polyprolene. The non-coronary sinus was incorporated in the aortotomy suture.

2.4. Clinical follow-up

Before discharge, the clinical status of the patient was determined and valve performance was assessed by color-flow Doppler echocardiography (TTE). Referring cardiologists performed clinical and echocardiographic follow-up at different time intervals according to their protocol, ranging from 1 to 3 year periods (mean 2.5 years). Patients with their homograft remaining in situ were contacted and submitted a standard questionnaire to complete clinical follow-up.

2.5. Microscopy

All explanted pulmonary homograft valves were examined macroscopically. Six out of eight valves explanted for tearing of cusp tissue underwent microscopic analysis. Only the cusp tissue was removed from reoperation, whereas the remnants of the sinus wall remained in the aortic root of the patient. The specimens were formalin (4%) fixed and paraffin-embedded. Sequentially radial tissue sections were cut (3 μm) of each semilunar cusp, and, whenever possible, the cross-section involved the site of the tear. Changes in cellular composition and tissue architecture were described, using routine hematoxylin and eosin (HE) staining. Elastin van Giesen staining was used to demonstrate elastic fiber structure. Two native pulmonary valves harvested at autopsy from middle-aged patients, and two unimplanted cryopreserved pulmonary donor valves from middle-aged patients served as controls.

3. Results

3.1. Clinical follow-up

Pulmonary homograft implantation was uneventful in all patients. Echo-Doppler study before discharge from hospital revealed no aortic regurgitation (AR) in 13, and mild AR in three patients, while the mean gradient was 7 mmHg (range 1–12 mmHg). From all 16 patients, the clinical fate of the pulmonary homografts is shown in Fig. 1. Ten patients (62.5%) were reoperated after a mean interval of 5.9 years (range 2.8–8 years). In eight patients (50%) rupture of one or more pulmonary homograft cusps was the reason for reoperation. All these eight patients suddenly became asymptomatic after a certain period of being without symptoms. In this group, no signs of endocarditis were present. Echo-Doppler analysis showed moderate to severe or severe aortic insufficiency in these patients, who were all reoperated within 3 months after the onset of symptoms and/or diagnosis, strongly suggesting acute cusp rupture.

Bacterial endocarditis was the cause of graft failure in the other two reoperated patients (12.5%), both with Strep-tococcus species. Both were cases of late endocarditis with a disease-free interval of 5.7 and 6.2 years. Vegetations were demonstrated with echocardiography (TTE) in both cases, with progressive destruction of the valve. After antibiotic treatment the homografts had to be replaced because of severe valvular incompetence. No annular abscesses or paravalvular leaks were observed.

Pulmonary homografts were replaced by mechanical prostheses in five, mechanical valved conduit (Bentall procedure) in one, Medtronic Freestyle® stentless bioprosthesis in two (root replacement in one), cryopreserved aortic homograft in one, and pulmonary autograft
(Ross procedure with root replacement) in another patient. One patient died 3 days after reoperation because of therapy resistant hypotension and cardiac insufficiency. In all other patients reoperation was uncomplicated and in the perioperative period no valve-related events occurred.

The mean clinical follow-up of patients \( (n = 6) \) with their homograft remaining in situ was 7.6 years (range 6–10 years). The mean echocardiographic (TTE) follow-up for this group was 5.8 years (range 3–8 years). Five patients were in NYHA validity class I at the time of latest follow-up. No AR was found in two patients, and trivial to mild AR was found in three patients. One patient is in NYHA class II–III, and echo-Doppler studies revealed moderate to severe AR. This patient is now scheduled for replacement surgery. No significant gradients were measured. For all 16 cases no correlation was found between early postoperative and late echocardiographic results, as shown in Fig. 1.

3.2. Macroscopy

Of the eight non-endocarditis valves, seven valves showed one or more tears or holes in one or two semilunar cusps, with no preference for right, left or non-coronary cusps. In one case, one of the leaflets had completely disappeared. At first sight, the cusp tissue looked normally pliable and thin, without any calcifications. In comparison with the reference tissues, subtle changes were observed. At the site of rupture and some other localized areas, the tissue appeared slightly thinner and more transparent than normal, whereas the remainder of the tissue was normal or slightly thicker and less transparent. No prolated of semilunar cusps or other mechanisms of valve incompetence were observed. No evidence of surgical or technical failure was found. The two endocarditis valves showed vegetations and the typical destruction caused by an infectious process, without evidence of mechanical rupture as an underlying cause.

3.3. Microscopy

Pathologic changes observed (Figs. 2 and 3) were remarkably similar in all the cusp tissues examined, and can be listed as follows: (a) blurring or complete disappearance of normal three-layered tissue architecture; (b) stretched appearance of the tissue, like in diastole, in comparison with the contracted state as observed in the control tissue; (c) collagen structure homogenized, instead of orderly arranged in bundles; (d) large areas where most or all cellular elements have disappeared and strongly decreased cellularity in the remainder of the tissue; (e) sparse or no endothelial cells; (f) few inflammatory cells with no signs of acute or chronic rejection; (g) thin layer of pannus formation, especially at the ventricular site on the proximal part of the cusp; (h) decreased thickness of cusp tissue, indicating reduction of extracellular matrix substances, in decellularized zones, and normal or increased thickness in areas where connective tissue cells are observed (both findings in comparison to control tissue samples); (i) the site of tissue rupture was always found in one of the thinner areas; (j) in these areas, Elastin van Gieson staining demonstrated strong degeneration of elastic fiber structure.

4. Discussion

The decision to start implanting cryopreserved pulmonary homograft valves in the aortic position seemed logical at that time, as has been explained in Section 1. In the same period several groups started using pulmonary homografts as an aortic valve substitute in young adults and middle-aged patients similarly \cite{10,12,14,15,18–21}, and some reported early graft failures due to tissue degeneration as well \cite{10,12,18–20}. Bacterial endocarditis and technical problems were also identified as important etiological factors for early graft failure.

It is known that freehand insertion of pulmonary homografts, in comparison with root replacement and even in comparison with freehand aortic homografts, is technically more demanding and more susceptible to technical errors, like mismatching and disturbance of valve geometry. By
some this is considered to be an important cause of early failure of pulmonary homograft valves that have been used as an aortic valve substitute [10,14]. The same applies to pulmonary autografts that have been inserted in the subcoronary position in the Ross procedure [5,6]. Our short-term results showed that the procedure is technically feasible,
with good postoperative echo findings and low postoperative mortality and morbidity. It is therefore unlikely that in this series technical aspects played an important role in the etiology of early graft failure. This statement is supported by the observation that there was no correlation between early postoperative and mid-term echocardiographic results. Furthermore, at reoperation no evidence of technical failure was observed.

The pathologic changes we observed in this series were strikingly similar in all examined grafts. Our observations (h–j) indicate that in pulmonary homografts, the disappearance of cellular elements in the tissue is combined with a reduction in quantity of the collagen and elastic fiber network, resulting in a loss of its biomechanical properties. This kind of tissue degeneration has not been described in viable and non-immunogenic pulmonary autografts, where tissue cellularity and architecture is largely preserved [6]. In the pulmonary homografts we examined, no signs of acute or chronic graft rejection were observed. It is therefore likely that the disappearance of viable connective tissue cells is responsible for degeneration of the valve matrix, which leads to fatigue rupture at the most deteriorated site and/or the site most (abnormally) stressed. Besides the higher systemic pressure, abnormal stress on the cusp tissue can also be caused by slight distortions in valve geometry, which practically always occur and which are not necessarily the result of technical errors. It can explain, however, why one graft fails more early than another and why rupture not always occurs at the same site or in the same cusp. The influence of different implantation techniques could not be studied in this series because a uniform technique was used.

In comparison with cryopreserved aortic homografts, intrinsic differences in the natural design of cryopreserved pulmonary homograft valves can be assumed to be responsible for the observed accelerated wear and tear. The tissue architecture of a pulmonary valve is comparable to that of an aortic valve, although pulmonary valve cusp tissue is originally thinner and has less elastic fibers in the ventricular layer [13, 14]. Although observations (a–g) are consistent findings in cryopreserved aortic homograft valve explants as well [16, 17], these characteristic changes seem to lead to a different mode of failure. The thicker extracellular matrix of the aortic homograft seems to hold longer after which other, atherosclerotic-like degenerative changes affect the valve tissue. Cryopreserved aortic homograft valves degenerate typically by thickening, stiffening, calcification, and deformation of the valve tissue, often leading to stenosis of the valve at a later stage. Early graft failure due to tearing of cusp tissue is a rare finding in aortic homograft valve pathology. Thus, the fiber structure of the pulmonary homografts is less resistant to tear and wear than the more robust fiber skeleton of the aortic valve homografts.

In conclusion, our disappointing mid-term results and consistent histopathologic findings form a strong argument against the further use of cryopreserved pulmonary homografts as an aortic valve substitute.

References

[18] Caudino M, Van Geldrop T, Daenen W, Kalmar P, Goffin Y. Are pulmonary homografts subjected to pulmonary hypertension more appropriate for aortic valve replacement than normal pulmonary

