SURGERY FOR CONGENITAL HEART DISEASE

NEW-GENERATION VALVED CONDUIT: AN EXPERIMENTAL STUDY

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Objective: An ideal valved conduit to repair complex congenital heart defects is yet to be developed. In this study we have evaluated the merits of our newly developed calcification-free biologic valve incorporated in a compatible conduit of biologic origin in an animal model. **Methods:** Porcine aortic valves and main pulmonary arteries were cross-linked in glutaraldehyde, followed by coupling to partially degraded heparin through an intermediate surface-bound substrate containing amino groups. Because commercially available valves are treated only with glutaraldehyde, control aortic valves and main pulmonary arteries were cross-linked in 0.625% glutaraldehyde. Valved conduits were fabricated from main pulmonary arteries, which were sewn to the aortic and ventricular ends of aortic valves. Valved conduits were examined for calcification and other pathologic changes after being implanted in the descending thoracic aorta in juvenile sheep for 5 months. **Results:** Severe calcification was noticed in all layers of cusps (calcium, 231.86 ± 17.90 mg/gm) and aortic wall (calcium, 123.24 ± 24.72 mg/gm) of aortic valves and main pulmonary arteries (calcium, 135.43 ± 26.63 mg/gm) of valved conduits treated with 0.625% glutaraldehyde. Cusps (calcium, 1.28 ± 0.22 mg/gm) of the aortic valve of heparin-bonded conduits did not calcify at all. Only sparse calcific deposits were noticed in the medial layer of the aortic wall (calcium, 25.90 ± 22.79 mg/gm) of aortic valves and main pulmonary arteries (calcium, 9.64 ± 10.79 mg/gm) of the valved conduits coupled to heparin. **Conclusion:** Heparin coupling is effective in preventing calcification of glutaraldehyde cross-linked valved conduits implanted in the systemic circulation of juvenile sheep. (J Thorac Cardiovasc Surg 1997;114:218-23)

Repair of complex congenital cardiac anomalies often requires the use of extracardiac conduits. Various types of conduits, such as irradiated and frozen homografts, Dacron conduits containing glutaraldehyde-treated porcine aortic valves (AVs), mechanical valves incorporated in Dacron tubes, and more recently cryopreserved aortic or pulmonary homografts, have been used. Early calcification

and degeneration are the main causes of failure of irradiated and frozen homografts and glutaraldehyde-treated porcine xenografts. Pseudointimal proliferation is the main cause of obstruction of Dacron conduits. In 1966, Ross and Somerville¹ used an aortic allograft to repair pulmonary atresia with a ventricular septal defect. Since then, allograft valved conduits have been the best available biologic substitutes for children.²⁻⁴ However, early degeneration and progressive calcification have limited the durability of the allograft valves in pediatric patients.^{2, 5-8} These facts prompted us to develop a calcification-free biologic valve incorporated in a compatible conduit of biologic origin.

Materials and methods

Groups 1 and 2. Porcine AVs and main pulmonary arteries (MPAs) were cross-linked in glutaraldehyde

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(Glutaraldehyde EM 25%, TAAB Laboratories Equipment Ltd., Reading, United Kingdom) in normal saline solution (pH 7.4) with gradually increasing concentrations of glutaraldehyde from 0.1% to 0.25% in normal saline solution at 37° C for 1 month. After glutaraldehyde treatment, subannular tissues of the AV were trimmed to approximately 2 mm from the base of the leaflets, and the MPA grafts were sewn to the ventricular and aortic ends of the valves with 5-0 and 4-0 polypropylene, respectively (arrangement: MPA-AV-MPA). Glutaraldehyde-treated valved conduits were coupled to 0.1% partially degraded heparin (depolymerized by deaminative cleavage with nitrous acid). For covalent binding of heparin to these valved conduits, an intermediate surface-bound substrate containing amino groups was used. This intermediate surface-bound substrate containing amino groups was prepared by coupling 0.1% chitosan (Sigma Chemical Co., St. Louis, Mo.) and 0.015% gentamicin sulfate (Schering-Plough, Osaka, Japan) to free aldehyde groups of glutaraldehyde already bound to the valved conduit. The partially degraded heparin was coupled with stable covalent bonds to aminated surfaces of glutaraldehyde-treated valved conduits by reduction with sodium borohydride at pH 8.4. Valved conduits without heparin and with heparin bonding were grouped as follows: group 1 (treatment with glutaraldehyde, chitosan, and gentamicin) and group 2 (treatment with glutaraldehyde, chitosan, gentamicin, and heparin).

Group 3. The AV and MPA were cross-linked with 0.625% glutaraldehyde in phosphate buffer solution (0.067 mol/L, pH 7.4) at 2° to 4° C for 24 hours followed by preservation in 0.2% glutaraldehyde in the same buffer at 2° to 4° C for more than 4 weeks. Valved conduits treated with 0.625% glutaraldehyde were fabricated like valved conduits of groups 1 and 2.

Implantation in sheep. Valved conduits of different groups were inserted in the descending thoracic aorta of 21 ($n = 7$ for each group) 6-week-old juvenile sheep weighing 10.5 to 12 kg. All animals received humane care in compliance with the "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research, and the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 80-23, revised 1985). Surgical technique of implantation of the valved conduits was similar to that described elsewhere.⁹ In brief, recipient sheep were preanesthetized with intramuscular ketamine (15 mg/kg). After additional anesthesia with intravenous pentobarbital sodium (30 mg/kg), sheep were endotracheally intubated and a 0.1 mg/kg dose of pancuronium was administered. Ventilation was maintained with a mixture of room air and oxygen. Under aseptic conditions, a left thoracotomy incision was performed through the fifth intercostal space. The descending thoracic aorta, distal to the subclavian artery and 4 to 5 cm in length, was mobilized, proximally crossclamped, and cut at the middle of the mobilized part. After heparinization (100 units/kg), a temporary apicoaortic shunt was established with a piece of heparin-coated polyvinylchloride tube. Elastic recoil of the transected aorta permitted a valved conduit 5 cm long (internal diameter 16.5 ± 1.5

mm) to be inserted. Proximal and distal ends of the valved conduit were anastomosed to the cut ends of the descending thoracic aorta in end-to-end fashion with continuous 5-0 polypropylene, and the shunt was discontinued. The chest tube was removed immediately on completion of the skin suture. Intravenous cefazolin 2 gm (Cefamezin, Fujisawa Pharmaceuticals, Osaka, Japan) and amikacin 100 mg (Banyu Pharmaceuticals, Tokyo, Japan) were routinely administered before the operation. Amikacin 100 mg was repeated twice daily for 5 days after the operation. Five months after implantation, the valved conduit with part of the adjacent aorta was removed en bloc, grossly examined, and incised longitudinally. A longitudinal section of the valved conduit was preserved in 10% formalin solution for histologic study. Histologic sections were stained with hematoxylin and eosin and von Kossa stains for general morphology and calcification study. Remaining portions of the cusps, the aortic wall of the porcine AV, and the MPA of the explanted valved conduit were carefully excised and individually lyophilized for estimation of calcium content by atomic absorption spectroscopy, as mentioned elsewhere.⁹ Statistical analyses were carried out with two-tailed unpaired *t* tests. Significant differences were considered to exist at the $p < 0.01$ level.

Results

General assessment. Paraparesis developed in one sheep of group 1 as a result of excessive division of intercostal arteries. This sheep was killed electively 3 months after the operation so that the pathologic changes of the implanted valved conduit could be observed. Histopathologic examination showed severe calcification of the valved conduit. Another sheep of this group died 12 hours after the operation of intraoperative bleeding. One sheep of group 2 died suddenly 21 days after the operation. This animal had ventricular tachycardia, which was successfully managed, after the aorta was unclamped. Postmortem examination revealed no disease of systemic organs, and the valved conduit was completely free of thrombus. Histochemically, calcification of the valved conduit was not identified. One sheep of group 3 died suddenly 3 months after the operation. This sheep had no other pathologic abnormalities of systemic organs except severe calcification of the implanted valved conduit. All these four sheep were excluded from our study. The surviving 17 sheep (group 1, $n = 5$; group 2, $n = 6$; and group 3, $n = 6$) did well through the planned 5-month implantation period, and their preoperative weights more than doubled over 5 months. Continuous pressure measurements showed no transconduit gradient in any sheep except in one of group 3, in which the transconduit gradient was 20 mm Hg. Seventeen valved conduits were examined morphologically after having been implanted for 5

Fig. 1. Gross view of a longitudinally sectioned valved conduit treated with 0.625% glutaraldehyde (group 3), implanted in a juvenile sheep for 5 months, demonstrates severe calcification of cusps and conduit wall. Surface thrombi (*dark*) in one sinus of Valsalva (*middle*) and at both anastomotic sites of the valve with the MPA can be seen. Note that flattening caused fracture of the conduit wall and cusps.

Fig. 2. Longitudinal section of a heparin-coupled glutaraldehyde-treated valved conduit (group 2) implanted in the descending thoracic aorta of a juvenile sheep for 5 months. Grossly, in comparison with Fig. 1, no pathologic changes of cusps and conduit wall can be noticed.

months. Valved conduits of groups 1 and 3 were hard and tenaciously adherent to the left lung and adjacent parts of the ribs and vertebrae, whereas valved conduits of group 2 were soft and adhered only to the lungs. All cusps of explanted valved conduits of groups 1 and 3 were rigid, friable, thick, and completely calcified (Fig. 1). The flexibility of the conduit walls of the valved conduits of both groups 1 and 3 was totally lost. Flattening of the incised conduits caused fracture of completely calcified walls and detachment of calcific plaques (Fig.

Fig. 3. Transverse section of a heparin-bonded glutaraldehyde-treated valved conduit (group 2) implanted in a sheep for 5 months. No thrombi and fibrous peel can be seen on the outflow surface of leaflets of the valve.

1). In contrast, the conduit walls of the valved conduits of group 2 were soft and flexible, and flattening of the incised conduits did not cause any change (Fig. 2). Cusps of valved conduits of this group were absolutely unchanged (Fig. 3). Neither dissection nor aneurysmal dilation was detected elsewhere in any conduit of any group.

Histologic findings. Thinning of the conduit wall was not observed in any group. Severe calcification of intima, media, and adventitia was noticed both in the aortic wall and in the MPA of the valved conduits of groups 1 and 3. Fracturing of calcified intima and adventitia of the conduit (both MPA and aortic wall) was prominent in all explants of groups 1 and 3. Severe calcification of cusps was revealed in all valved conduits of these groups (Fig. 4). Diffuse calcification was noticed in the fibrosa, and the spongiosa was severely affected. In some specimens histologic sectioning caused separation of ventricularis from spongiosa. Ventricularis was less calcified than the other two layers. Only sparse calcific deposits were noticed in the medial layer of the aortic wall and MPA of the valved conduits of group 2. Calcific changes of the aortic wall of the valved conduits of this group were identified mainly above the level of the commissures. Basal ring and muscle shelf of the AV were completely free of calcification. Cusps of the valved conduits of group 2 had not calcified at all at 5 months (Fig. 5).

Calcium content. Calcium content of the cusps (calcium = 186.58 ± 35.20 mg/gm dry tissue weight) of valved conduits of group 1 did not significantly differ from that of the cusps (calcium = 213.86 ± 17.90 mg/gm; $p = 0.05$) of valved

Fig. 4. Morphologic features of calcification of a cusp of valved conduit from group 3, in place for 5 months. Both the cusp (*top*) and aortic wall (*bottom*) are calcified. Dense calcific deposits damaged the spongiosa of the cusp completely. Histologic sectioning probably promoted detachment of the spongiosa itself. (von Kossa stain; original magnification $\times 50$.)

Fig. 5. Histologic appearances of a cusp of a valved conduit from group 2, implanted in the descending thoracic aorta in a juvenile sheep for 5 months. No calcific deposits were observed in the cusp (*top*) or in the aortic wall (*bottom*, below the level of the commissures) of the valve. (von Kossa stain; original magnification $\times 50$.)

conduits of group 3. The calcium content of the cusps of valved conduits of group 2 was only 1.28 ± 0.22 mg/gm. This value was significantly lower than that of group 1 or 3 ($p < 0.0001$). Calcium contents of the aortic wall (group 1: calcium = 111.94 ± 27 mg/gm; group 3: calcium =

123.24 ± 24.72 mg/gm) and the MPA (group 1: calcium = 122.87 ± 29.01 ; group 3: calcium = 135.43 ± 26.63 mg/gm) of valved conduits of both groups 1 and 3 were virtually identical. However, the calcium levels of the MPAs and aortic walls of valved conduits of group 2 were 9.64 ± 10.79 and

25.90 $\pm$ 22.79 mg/gm (group 2 vs group 1 or 3, p = < 0.0001), respectively.

Discussion

Our present experimental study yields the following conclusions: (1) Complete prevention of calcification of the cusps of glutaraldehyde-treated porcine AVs may be achieved by coupling of heparin to the valve; (2) heparin binding inhibits the calcification of the aortic wall of the glutaraldehyde-treated AV; (3) the proposed anticalcification treatment is more effective in the porcine pulmonary artery than in the aortic wall of the AV.

True aneurysms of pulmonary allograft patches, suture-line pseudoaneurysms of pulmonary allografts,¹⁰ and even rupture¹¹ occurred in clinical practice when pulmonary allografts were subjected to systemic pressure. While standardizing our experimental model, we used valves attached to Dacron conduits. Because the addition of a valve to the Dacron conduit appeared to accelerate pseudo-intima formation, as observed by other investigators,¹²⁻¹⁴ we have abandoned Dacron grafts and decided to use the porcine MPA as a conduit.

Distensibility is greater and the tendency toward calcification is reduced in MPAs in lambs.¹⁵ With our proposed anticalcification treatment, the MPAs of group 2 had much less tendency toward calcification than did the aortic walls of the same group. However, none of the MPAs of either group became distended in our study. Severe calcification was the main feature of pathologic changes of all conduits of groups 1 and 3. Neither pseudoaneurysm nor dissection was observed at any anastomotic site of valved conduits implanted in the descending thoracic aorta in juvenile sheep at 5 months.

Heparin has been known to be effective in preventing calcified atherosclerotic plaque formation in major arteries.¹⁶ Heparin has a potent antigrowth effect in smooth muscle cells,¹⁷ and it also binds to the surface of cells.¹⁸ This may alter permeability to ions necessary for growth, change confirmation of molecules to which it binds,¹⁹ or affect cell volume.²⁰ The exact role of heparin in the anticalcification process of bioprostheses still remains elusive. Conceivably, coupling of heparin to glutaraldehyde-treated grafts fills the intertropocollagen spaces, blocks the potential binding sites, modifies charges, and thus makes the prostheses impermeable to host plasma calcium.

Heparin is as effective in preventing calcification of the porcine pulmonary valve as of the porcine AV

(unpublished data). The heparin-bonded porcine pulmonary valve extended proximally with a pulmonary artery may also serve as a prospective valved conduit.

Sterilization of fresh homografts with antibiotics, as well as preservation or cryopreservation, has resulted in a resurgence in the use of aortic homografts.³ Concern has been expressed, however, about calcification of these homografts in the pulmonary position.²¹ The rate of calcification of homografts used for reconstruction of the right ventricular outflow tract appears to be accelerated in younger children and in patients with pulmonary hypertension.²² Although the cryopreserved pulmonary allograft appears to be the conduit of choice for reconstruction of the right ventricular outflow tract,³ the prevalence of aortic allograft fibrocalcification and valvular insufficiency warrants reexamination of the surgical options available for young children with congenital anomalies of the left ventricular outflow tract.²³ Because the homografts calcify much faster in high-pressure systems^{22, 23} than in the usual pressure of the pulmonary circulation, and the juvenile sheep is a good animal model for the study of accelerated calcification of cardiovascular bioprostheses,²⁴ we have designed our experiment to implant the treated valved conduit in the systemic circulation in juvenile sheep.

Preliminary studies show the satisfactory results with proposed chemical treatment in preventing calcification of porcine AV conduits implanted in the systemic circulation in juvenile sheep for 5 months. However, long-term study is necessary before the valved conduits can be recommended for use in patients.

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