Autologous pericardial pulmonary conduit with single point attached commissures in a sheep model

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Abstract

Objective: For the surgical treatment of congenital heart disease and in Ross procedure a valved conduit is frequently required. Since homografts are not readily available in every country, a reliable alternative is needed. We developed a novel technique to construct a valved pulmonary conduit with single point attached commissures (SPAC) in a simple and fast way from a small strip of autologous pericardium, molded and briefly treated with glutaraldehyde. Methods: Autologous pericardial pulmonary conduit was constructed intraoperatively and implanted in pulmonary position in a beating heart in six sheep. The prosthesis size was 31 mm for all sheep and the construction time (including 10 min glutaraldehyde treatment) was 19.0 ± 3.3 min. Implantation time and cardiopulmonary by-pass was 27.3 ± 4.5 min and 40.5 ± 7.7 min, respectively. The sheep were euthanized after 6 months (222.7 ± 5.8 days) postoperatively. Results: In all sheep, the autologous pericardial valve was immediately competent. At sacrifice, the pericardial valve was pliable and competent in all cases with SPAC well anchored to the pericardial conduit wall. The maximum transvalvular gradient at implant and at sacrifice was 3.3 ± 2.8 mmHg and 3.3 ± 2.0 mmHg, respectively. Conclusions: This novel autologous pericardial pulmonary conduit with SPAC can be reliably produced in a very short time intraoperatively before cardiopulmonary by-pass. The simplicity of construction, biocompatibility and freedom of stenosis or thrombosis makes this autologous pulmonary conduit especially useful for patients at locations where homografts are not readily available.

Keywords: Pulmonary conduit; Pericardium; Autologous; SPAC

1. Introduction

The utilization of extracardiac conduits for right ventricular outflow tract reconstruction has made possible the routine correction of complex congenital heart malformations [1,2]. The Ross procedure, in which the living pulmonary autograft is implanted in aortic position, likewise requires a right ventricular outflow tract reconstruction with a valved conduit [3–5] or a homograft [6]. Although the optimal conduit for the replacement of the pulmonary trunk and cardiac reconstructions is currently a pulmonary allograft [7,8], a homograft is not always available in all sizes required and is not available in many areas of the world [5]. This made surgeons look for alternatives to homografts [3,4,9,10]. Comparison between xenograft conduits and fresh autologous pericardial conduits showed the advantage of a fresh autologous pericardial conduit [11,2], although the fresh autologous valve is expected to retract soon, leaving a pericardial conduit with an incompetent valve [12,2]. Rapid deterioration of fresh autologous pericardial leaflets placed in the blood stream was demonstrated although the mechanism responsible is not fully understood [13]. It is obviously necessary to treat the autologous pericardial tissue with glutaraldehyde to increase the durability of valvular leaflets [14].

Our aim was to construct an autologous pericardial conduit treated briefly with glutaraldehyde, utilizing the smallest possible strip of pericardium. To achieve this goal, a new autologous pericardial conduit consisting primarily of a pericardial valve allograft with only the height of the valve unit was developed. If more autologous pericardium is available, the inflow and outflow portions can be extended to match the pulmonary artery and the right outflow tract. If necessary other biocompatible materials can be used to extend the valve unit with additional inflow and outflow portions. We
intended to use as little suture material as possible to allow maximum flexibility of the leaflets because sutures are known to be a nidus for tissue calcification [15]. This could be achieved by using a novel technique with single point attached commissures (SPAC) [16,17]. The valved conduit can be constructed quickly of autologous pericardium, which is molded and briefly treated with glutaraldehyde. The leaflets are molded in a shape that is closer to the natural valve, which was found to positively influence leaflet stress distribution and coaptation [18], and the conduit root is molded to incorporate sinuses of Valsalva, which are known to be critical for the durability of a valved conduit [19].

2. Material and methods

Six adult sheep (body weight 45 ± 2.1 kg) underwent implantation of a valved conduit in pulmonary position with a molded autologous pericardium prosthesis treated for 10 min with glutaraldehyde.

All animals received humane care in accordance with the Principles of Laboratory Animal Care formulated by the Animal Welfare Act in the Guide for Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health, Singapore (NIH publication No. 85-23, revised 1996).

The animals were pre-medicated with ketamine 1.0 mg/kg, Atropine 0.03 mg/kg, and propofol 4.0 mg/kg body weight. The ECG was monitored continuously with five leads. Arterial pressure was monitored through a pressure catheter in the axilla artery. Animals were intubated and ventilation was achieved with a volume-regulated respirator (North American Drager, Telford, Pennsylvania, USA) and oxygen at a flow of 4 l/min. Anesthesia was maintained with isoflurane at a gas level of 0.5–1.0% as needed. The animals were then placed in the right lateral decubitus position. The heart was exposed with a standard left thoracotomy through the fourth intercostal space.

2.1. Construction of autologous pericardial conduit

The mediastinal surface of the pericardium was dissected free of fat and connective tissue. A rectangular piece of pericardium extending from the left phrenic nerve to the midline was resected. The size of the pericardial strip was chosen according to the chosen mold size with approximately a width corresponding to the mold circumference and a height according to the required conduit length. The pericardium was placed on a wet towel, where further cleaning of its mediastinal surface was done.

Once cleaned, the pericardium was placed between two L-shaped molds that imitate the shape of the pulmonary conduit incorporating sinuses of Valsalva and the attached valve leaflets. The sandwiched pericardium was placed in a container with 0.5% buffered glutaraldehyde at room temperature for 10 min followed by rinsing in three separate bowls with ringer lactate for at least 10 min. The pericardium was then trimmed down along the mold-rim (Fig. 1). At the three commissural points of the later valve leaflets, a U-stitch was performed with a 4-0 prolene suture. Subsequently this suture was stitched through the vascular graft wall at a hole in the mold that corresponds to the commissure. These sutures were left loose (Fig. 2). The free ends of the pericardial strip were sutured together forming a vascular graft (Fig. 3). Subsequently the valve at the base of the graft was inverted by pulling on the still loose sutures at the commissures. The commissural sutures were tied outside of the conduit forming a vascular graft including a molded valve with SPAC (Fig. 4). An additional strip of pericardium was sutured to the inflow tract of the conduit to match the right ventricular outflow tract (Fig. 5).

2.2. Implantation of autologous pericardial conduit

Heparin 300 U/kg I.V. was injected as a bolus in preparation for cardiopulmonary by-pass with a target ACT of ≥480 s. A 16 Fr arterial cannula was inserted into the descending aorta and 32 Fr double stage venous cannula into the inferior right atrial appendage. After installing the cardiopulmonary by-pass, the pulmonary trunk was transsected, the native pulmonary valve was removed and a right ventricular vent was inserted.

Fig. 1. Molded autologous pericardial strip, treated with 0.5% buffered glutaraldehyde. The upper part will form the conduit tube including the sinuses of Valsalva, whereas the lower part will form the valve leaflets.

Fig. 2. Placing U-stitch sutures at the leaflet commissures and the trunk that will later connect the upper part of the leaflet commissures with the corresponding point at the conduit tube forming the single point attached commissures (SPAC).
The inflow portion of the conduit was sutured to the right ventricular outflow tract and the outflow portion of the conduit to the distal pulmonary trunk using a running 4-0 prolene suture.

After implantation of the conduit the right ventricle and pulmonary conduit was de-aired by filling the chambers before closing the incisions and all knots were tied over pericardial pledgets.

The animals were weaned off by-pass. Once normal hemodynamic had returned, an epicardial 2D color echocardiographic study was performed to assess valve competence. Simultaneous pressure readings were taken using Millar pressure transducer control unit (TCB 600) and MikroTip® Pressure Transducer Catheters SPC 330A (Millar Instruments, Inc., Houston, TX, USA) in the pulmonary trunk approximately 15 mm above commissural level and in the right ventricle to determine the transvalvular gradient.

Intercostals nerve block with 0.25% bupivacaine was given for postoperative analgesia. The chest was closed in layers after insertion of a thoracic drain tube that was kept for a few hours. The animals were awakened immediately, extubated and transferred to the holding pen. All animals received antibiotic coverage with ceftriaxone-sodium 3 mg/kg and gentamicin 80 mg I.M. for 3 days.

2.3. Sacrifice

At the time of sacrifice, under general anesthesia the thoracotomy was re-entered. An epicardial echocardiogram was recorded as well as simultaneous right ventricular and pulmonary trunk pressures. Under full heparinization the heart was excised, with the pulmonary trunk transected distally of the distal anastomosis. The right ventricle was opened and the macroscopic findings recorded and photographed. The block comprising right ventricular outflow tract, the pericardial conduit and the distal anastomosis were harvested.

The harvested specimens were preserved in 10% phosphate buffered formalin (Sigma Aldrich, USA) for 2 days. Specimens were processed in a Tissue Processor (Leica TP1020, Germany) where they underwent dehydration through serial bath treatments, containing ascending concentration of ethanol, and finally being impregnated in a hot wax bath prior to blocking in wax. The tissue blocks were then sectioned into 7 μm thick slices using a rotary microtome (Leica RM2135, Germany) in longitudinal sections of the pericardial conduit, a longitudinal section through the middle of each cusp and a transverse section crossing each commissure and collected on slides. Representative sections were stained with hematoxylin and eosin (H&E) for general tissue and cellular morphology.

3. Results

The prosthesis size was 31 mm for all sheep and the construction time (including 10 min glutaraldehyde treatment) was
19.0 ± 3.3 min plus rinsing for 10 min in saline before implant. Implantation time and cardiopulmonary bypass was 27.3 ± 5.4 min and 40.5 ± 7.7 min, respectively. Epicardial echocardiography revealed in all six sheep that the valves were immediately competent and no remaining regurgitation was recognized. All six sheep survived until sacrifice at 6 months (222.7 ± 8 days) postoperatively. In all sheep the pericardial vascular graft as well as leaflets appeared macroscopically pliable and free of pannus-growth or calcification. At sacrifice cusp mobility was excellent in all conduits. Epicardial echocardiography at sacrifice showed either no or trivial central regurgitation in all sheep (Figs. 6 and 7). Macroscopically all single point attached commissures appeared safely anchored at the pericardial conduit wall covered with fibrous tissue. The pericardial leaflets remained separated up the commissural point.

Transvalvular gradient was acquired by inserting Miller pressure catheter in the pulmonary conduit approximately 1.5 cm distal of the pulmonary valve and in the right ventricle after cardiopulmonary by-pass, as soon as the circulation was stable and at the time of sacrifice. The maximum transvalvular gradient at implant and at sacrifice was 3.3 ± 2.8 mmHg and 3.3 ± 2.0 mmHg, respectively.

4. Histology

The specimens stained with H&E showed a normal pericardium with intact collagen and elastin structure. There were no thrombi or endothelium present on the pericardial tissue. A few mononuclear cells were scattered throughout the pericardium. The commissures appeared intact without tears.

Longitudinal sections of the specimens showed that the collagen and elastin fibers were intact throughout the entire length of the pericardium and similar in appearance to a control slide of fresh pericardium. In all cases a thin discontinuous fibro connective tissue was present at the intravascular side, especially at the area of the single point attached commissures but no endothelia like layer was seen on the intravascular side of the pericardium. The lungs showed normal pulmonary architecture without pathological features and signs for pulmonary thromboembolism.

5. Discussion

Commonly homografts are used to replace the autotransplant pulmonary root in Ross procedure [6] or for the correction of congenital malformations in neonates and infants [1,2]. Although homografts are considered to be the conduit of choice their use has been restricted by their limited availability [7].

Fresh autologous pericardial pulmonary conduit showed excellent immediate results but early valve insufficiency was disclosed in most of the patients [2,12]. The early incompetence of the fresh pulmonary conduits can be explained by the fast degeneration of freely floating autologous pericardial tissue in the bloodstream [13]. The reason for the accelerated structural degeneration of living autologous pericardial leaflet is not known although the elegant experiment by Cheung et al. [13] revealed that a floating flap of fresh autologous pericardium in the bloodstream is retracted and actually disappears completely after 30 days whereas autologous pericardium in which the cells were killed does not experience this fast degeneration.

According to these findings it is essential to kill the pericardial cells if the pericardium placed in the bloodstream is supposed to work as a valve leaflet for more than 30 days [14]. Frequently glutaraldehyde is used as a cell-toxic chemical while it cross-links the pericardium in an advantageous way [20].

Long-term studies of autologous pericardial valves in the aortic position in humans showed an acceptable durability of autologous pericardium treated for 10 min in 0.5% glutaraldehyde at room temperature. The results of autologous pericardial valves in aortic position are at least as good as commercially available bioprostheses [21].

Autologous pericardial conduits in pulmonary position are expected to perform as good as a pulmonary homograft [12].
Several attempts were made to construct a hand-fashioned autologous pericardial valved conduit intraoperatively [2,12,10,22–25]. The methods were time-consuming and not always reliable. We designed a novel technique to construct a valved pulmonary conduit with SPAC [16,17] in a simple and fast way of autologous pericardium, molded and briefly treated with glutaraldehyde. The pericardial valve leaflets were molded to be closer to the natural valve shape, which was found to positively influence leaflet stress distribution and coaptation [18], and the conduit root was molded to incorporate sinuses of Valsalva, which are known to be critical for the durability of a valved conduit [19].

Previous studies had shown the reliability and force distribution at SPAC [17]. Preceding in-vitro studies in a pulse-duplicator with a pericardial conduit made of molded, and briefly treated sheep pericardium, confirmed the reliability of the conduit and the valve leaflets with SPAC for a pressure of more than 200 mmHg without any regurgitation.

The immediate results in this animal study were exceptionally reliable due to the unique design of the conduit and the leaflets, and 6-month outcome demonstrated efficacy and durability of the autologous pericardial pulmonary conduit with SPAC. A selective clinical study will follow.

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References