Analysis of hemodynamic performance of the bovine pericardium valved conduit implanted in aortic position in ovines

Análise do comportamento hemodinâmico de conduto valvado de pericárdio bovino, implantado em posição aórtica de ovinos

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Abstract

Objective: The need for replacement of the valve, ascending aorta and coronary reimplantation in patients where anti-coagulation is undesirable, is increasing. We assessed the hemodynamic performance of an aortic valved conduit made with bovine pericardium (AVCP) treated with glutaraldehyde in animals.

Methods: Therefore, AVCPs were implanted in eight young ovine and explanted after 150 days. Angiographic and hemodynamic study was performed at preoperative period and prior the explant. EchoDopplercardiograms were performed at day 30 and 150 of postoperative (test) and also in five non-operated ovines. After explanted, AVCPs were submitted to a macroscopical, radiological and histological assessment by optic microscopy.

Results: In the hemodynamic analysis the arterial and pulmonary capillary pressure increased (P<0.05) between day 0 and 150. In the echoDopplercardiographic analysis, the test group presented higher values in the diastolic and systolic diameters of the left ventricle (P<0.05). In the test group, between day 30 and 150, occurred an increase of weight, thickness of the left ventricle walls, maximum transvalvar gradient, medium transvalvar gradient, left ventricle diastolic diameter and a decrease in the ejection function (P<0.05). Two animals with endocarditis explain those differences, as we can see with the statistical analysis without this sample. Macroscopy showed calcification in variable degrees. Optic microscopy revealed data similar to literature with the use of glutaraldehyde treated bovine pericardium.

Conclusions: These data indicate that the AVCPs allow the performance of this kind of experiment in the proposed model and that the hemodynamic outcomes found are similar to physiological parameters.


Resumo

Objetivos: A necessidade de substituição da valva, aorta ascendente e reimplante coronariano em pacientes onde a anticoagulação é indesejável, é crescente. Avaliamos em animais o comportamento hemodinâmico de um conduto valvado aórtico feito com pericárdio bovino tratado pelo glutaraldeído (CVAP).

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INTRODUCTION

Since the first description of Bentall & De Bono [1] in 1968, the technique for combined replacement of the valve, aortic root and ascending aorta by a valved conduit has been developed and turned into an excellent treatment option for patients with annuloaortic ectasia as well as those with acute or chronic dissection presenting aortic dilatation of the aortic annulus with consequent valvular incompetence. Originally, synthetic arterial conduits coupled to a mechanical valve prosthesis have been used. Though this operation provide good long-term outcome and durability of the repair, it exposes patients to the risk of complications associated with the prosthesis and anticoagulation, such as thrombosis and bleeding.

Rates of 10.3% patients/year of thromboembolic events have been reported after elective use of such conduit [2]. For this reason, authors such as David et al. [3] and Yacoub et al. [4] developed techniques which preserves the native aortic valve and uses only the arterial conduit and, depending on the technique, the coronary ostia are reimplanted. These techniques may not always be applied and require a natural learning curve for even the most experienced surgeons. Other possibilities have been developed to avoid the drawbacks of systemic anticoagulation of mechanical valves, such as using an arterial conduit of bovine pericardium attached to a bovine pericardial bioprosthesis with support [5].

It has also been used aortic homografts or autografts [6,7], which show excellent hemodynamic performance and consistent results in the long-term, but come up against limited availability of homografts and thus in less clinical applicability. It has emerged, with the advent of prosthetic heart valves without support [8], the possibility of using an aortic valved conduit made completely unsupported with heterograft in order to obtain greater similarity with the anatomy, tissue consistency and ease of surgical manipulation found in homografts. These valved conduits use a porcine aortic valve prosthesis treated by glutaraldehyde unsupported associated with a vascular graft made from bovine pericardium preserved by glutaraldehyde [9,10].

Several publications have shown that the pericardium has advantages over the porcine aortic valve in relation to resistance, as Vesely & Mako [11], durability, as Gao et al. [12] and hemodynamic performance as Jin & Westaby [13].

On this basis we decided to perform this study that aims to evaluate in an animal model, the hemodynamic performance of an aortic valved conduit made completely with bovine pericardium treated with glutaraldehyde, both the tube as the valve, and also to report the anatomic findings, radiological and histological findings. This conduit from now will be called AVCP.

METHODS

The experiment was performed at the Research Center of Laborcor Laboratórios Ltda. (Belo Horizonte, Brazil) and was performed with the approval of the Ethics Committee of the Heart Institute and the Clinics Hospital of the Faculty of Medicine, University of São Paulo, following the guidelines of the American Association for Accreditation of Laboratory Animal Care and Brazilian College of Animal Experimentation.

Sample Selection

Test groups: eight animals that received AVCP. The sample consisted of eight selected Santa Inês sheep (Ovis Aries), from 4 to 6 months of age, females or castrated males weighing between 26 and 35 kilograms, considered free from disease after a general clinical examination and a quarantine period of 15 days upon receipt of health certificate provided by the veterinarian. Control group: five animals considered healthy by clinical veterinary surgeon that did not undergo...
any type of intervention, with the same characteristics as race, weight and age of those who composed the test group at death.

The Control Group was used for comparison of Doppler echocardiographic parameters. The sheep was chosen as the experimental test to be used for the following reasons: the size of the animal allows technically the completion of implantation of aortic valve prostheses in human clinical size, the sheep is an established model for evaluation of preclinical safety and efficacy of prosthetic heart valves, the sheep is relatively easy to be maintained for long periods.

Follow-up

After surgery, the animals were sent to the sector of immediate postoperative recovery, which is located inside the operating room. After the first week they were transferred to the bioterium, where they remained under observation until complete daily exposure time of 150 days set out for sacrifice. Doppler echocardiographic studies were performed at 30 days after surgery and preceding the sacrifice. The hemodynamic and angiographic evaluation were performed after 150 days of observation and immediately before sacrifice.

Preparation of the prostheses

The making of AVCP consisted of a tube and a valve with three separate leaflets and a border suture implantation without support, made fully in pericardium treated by glutaraldehyde at 0.65%. At the height of the commissures, a polyester strip reinforces the structure for attachment of the leaflets, as illustrated in Figures 1 to 3. AVCPs were produced with 17, 19, 21 and 23 mm external diameter, measured at the edge suture implant. This way of preparing the AVCP was based on the proposed by Khaghani [14], only modified to leaflets in relation to the project in its leaflets with a single layer of pericardium that was sutured to the commissural posts. Because we find that there was need of the leaflets had greater height and that this may interfere with the gradients, the blade was sectioned into three pericardial leaflets with less time and they were sutured to the tube.

Preparation of experimental animals

The animals were fasted (solid and liquid) for 24 hours in the 6 hours prior to surgery. Prophylactic antibiotic therapy consisted of intramuscular injection of 1g of cephalothin, six hours before surgery. Fifteen minutes before induction of anesthesia was administered 1 mg of atropine intramuscularly.

The anesthesia was induced by intravenous administration of 12.5 mg/kg of thiopental sodium.

The external jugular vein was catheterized with a Swan-Ganz 7Fr used for measurement of hemodynamic parameters and for the administration of drugs and electrolyte solutions. As a result, the animal was monitored to obtain continuous path of electrocardiography and rectal temperature. Measurements of central venous pressure, pulmonary arterial, pulmonary capillary and cardiac output were performed by thermodilution. Muscle relaxation was obtained by administration of 100mg of intravenous succinylcholine, when the animal reached anesthetic depth. 250 mg of methylprednisolone and 1 g of cephalothin were administered intravenously. The animal was intubated and an orogastric tube was introduced, and through it were administered 150 ml of aluminum hydroxide. Mechanical ventilation was established with a volume of 12 ml O$_2$.kg-1 at a frequency of 12 cycles per minute. Anesthesia was maintained with administration of 1.5% halothane inhalation and 100mg suxamethonium (EV).

Surgical technique

The surgical protocol used was the same as previously described by Grehan et al. [15]. A left thoracotomy was performed through the fourth intercostal space and left internal mammary artery artery ligated and sectioned. The pericardium was opened longitudinally toward the apex of the heart. Thereafter, it was initiated the dissection of the aortic root for individualization of coronary ostia. The pulmonary artery was separated from the aorta and individualized to facilitate exposure of the aortic root.

Thereafter, heparin was administered at a dose of 350 units/kg intravenously. After cannulation of the descending aorta and right atrium, cardiopulmonary bypass (CPB) was established using a membrane oxygenator. The left atrium was cannulated for decompression. The arterial flow was maintained between 50 and 70ml/kg/min and gas at 0.5 l / min a mixture of 95% of O$_2$ and 5% of CO$_2$. The body temperature was lowered to 28°C. The aorta was clamped across and administered crystalloid cardioplegia through antegrade via at its root, with an initial dose of cardioplegic solution of 15 mL/kg cold crystalloid solution at 4°C, and the cardioplegias were repeated every 20 minutes, but the maintenance doses were 10ml/kg of blood cardioplegic solution into the coronary ostia.

We used the following cardioplegia solution: 500 ml of saline solution at 0.9% (or blood from the CPB machine for maintenance), 5 ml of magnesium sulphate, 5 ml sodium bicarbonate 8.4%, 5ml of 2% lidocaine and 2.5 ml of potassium chloride.

Ceased the first dose of cardioplegia, it was started the operation with a total transverse aortotomy. The aortic valve leaflets were removed and the ascending aorta was excised, leaving a margin of 2 mm proximaly thereof. In the next step, we isolated and repaired the coronary ostia. Completing the preparation, we began suturing the AVCP.
in aortic annulus, which was performed with 4-0 polypropylene continuous sutures in the proximal part. Once this stage began the reimplantation of the coronary arteries, starting from the left coronary artery, which is performed with 6-0 polypropylene continuous suture; further, we proceeded the same way about the right coronary ostium. Finally, the procedure was finished with the anastomosis of the distal part of the AVCP on the distal aortic stump with 4-0 polypropylene continuous suture. At the beginning of this suture, rewarming of the animal was started. On that occasion 1 g of cephalothin and 125mg metilpredisolona were administered. Figure 1 shows the result after surgery. We performed the removal of air from the heart chambers and the aorta was unclamped, pending the recovery of heart rate.

For defibrillation, we used an electric shock of 15 Joules. Mechanical ventilation was initiated again and prepared the CPB disconnection. When reached the hemodynamic and respiratory conditions considered satisfactory, CPB was discontinued. Figure 2 shows the final aspect of the surgery. Hemostasis was performed. Withdrawal of the cannula from the right atrium, left atrium and aorta. By peripheral vein it was initiated continuous infusion of protamine at a dose of 1 ml per 1000 IU of heparin. Finally, the pericardium was closed at the bottom and passed a 30-Fr tubular drain at the sixth left intercostal space and performed the synthesis of the chest wall by anatomical planes.

After ceased the bleeding by the chest drain, it was withdrawn in the operating room. When the animal was able to regain effective spontaneous respiration it was extubated and transferred to the sector of immediate postoperative recovery, which is located inside the operating room.

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Postoperative care
After five hours of extubation, it was returned the water intake and diet with hay, and we administered 1 g of cephalothin IM and subcutaneous heparin 5,000 UI, which were administered every 12 hours, in the first 3 days.

Throughout the study period, three animals died, and one still in the operating room for uncontrollable bleeding at the site of aortic cannulation. The other two were found dead in the sector of postoperative recovery in the first days of observation. Necropsies performed on these two animals showed the presence of large amounts of blood clots and chest cavity. These three animals were replaced so that we could complete the study group.

Two-dimensional Doppler echocardiographic evaluation
Transthoracic Doppler echocardiographic studies were

Fig. 1 - Full sequence of the replacement of the ascending aorta and aortic valve by AVCP

Fig. 2 - Final aspect of the surgery
performed using an ATL Ultramark 6 Phillips, 30 days after surgery and preceding the sacrifice (Test Group). The images were obtained in transverse, apical and parasternal longitudinal sections in various planes, recorded at least three consecutive cardiac cycles, being considered average values, excluding the images that were not well defined. We determined the mobility and valve competence, the average and maximum gradient, valve area, diastolic and systolic left ventricular diameters.

**Angiographic and hemodynamic evaluation**

Angiographic assessment was performed after 150 days postoperatively, prior to the sacrifice of animals for explantation of the prostheses. After general anesthesia the animal was performed right heart catheterization, using a Swan-Ganz 7Fr catheter. We obtained the records from the central venous pressure, pulmonary arterial, pulmonary capillary and cardiac output by thermodilution. Mean blood pressure was obtained directly from the aorta, followed by the performance of aortography using catheter Pig-Tail 6Fr. The animals were sacrificed and then performed left retoracotomy and removed the heart - en bloc - and great vessels. The AVCP was released from surrounding tissues, removed, washed in saline solution, photographed and sent for macroscopic evaluation. The remains of the animals were incinerated.

**Macroscopic evaluation**

Macroscopic examination analyzed: functionality and technical aspects of surgery, integrity and myocardial tissues of AVCP, guidance, direction, mobility and thickness of the valves, presence of thrombosis, vegetation, calcification and fibrosis (pannus), diameter, length and AVCP configuration.

**Radiological assessment**

After macroscopic examination, the bioprostheses were fixed in aqueous 10% formalin and sent for radiological study. The grafts were subjected to radiological examination to determine the distribution and intensity of existing calcium deposits. For this, we used the Senographe DMR mammography unit (GE, Buc, France) with acceleration voltage of 22kV. After completion of the radiological study, the AVCPs were sent to the Laboratory of Pathology.

**Histological assessment**

By optical microscopy were assessed the endothelial cells, collagen matrix and tissue preservation. The AVCPs were cut longitudinally, picking up three samples of each graft, so that the three valve leaflets, their sinus and whole body of the conduit (from the inlet to the outlet) were studied. For staining, we used the techniques of hematoxylin-eosin, Masson trichrome and picrosirius.

**Statistical analysis**

Initially, all variables were analyzed descriptively. For quantitative variables this analysis was performed by observing the minimum and maximum values, beyond calculating averages, standard deviations and quartiles (25th percentile - P25, Median, 75 percentile - P75). For qualitative variables, we calculated absolute and relative frequencies. To compare the two groups we used the nonparametric test Mann-Whitney, since the assumption of data normality was rejected. For comparison of two moments in the study group used the nonparametric test of Wilcoxon. To test the homogeneity between the ratios we used the Fisher exact test. The tests were performed using software SPSS 15.0 for Windows. The significance level used for the tests was 5%.

**RESULTS**

**Doppler echocardiographic analysis between the Control and Test Group**

Two animals in Group Test, at death, presented endocarditis (Cases 2 and 7), aortic insufficiency and different degrees of left ventricular dysfunction; the others presented the valves functioning normally and with preserved left ventricular function. No animal showed evidence of stenosis. The comparison of test and control groups (in 150 days) is described in Table 1, in which one can observe that the groups differ in left ventricular diastolic diameter (LVDD) and left ventricular systolic diameter (LVSD). This group shows significantly higher LVDD and LVSD compared to the control group. However, if we repeat the statistical analysis without the animals with endocarditis, we observed that the difference in the LVDD ceases to exist (test versus control 38.20 43.17 P = 0.052).

The data from echocardiograms obtained in the study group in the 30 days and 150 days postoperatively are listed in Table 2 and it is observed that there is significant improvement in weight, as well as interventricular septum thickness, maximum gradient (Grad. Max.), mean gradient (Mean Grad.), posterior wall thickness, left ventricular wall, LVDD at the time 30 days to 150 days time, and decreased ejection fraction (EF). No significant change in LVSD and aortic valve area (VA). This same comparison, but without the presence of animals with endocarditis, shows us that disappeared the difference found between the left ventricular ejection fraction (0.69 to 0.67 30 days versus 150 days P = 0.129).

Aortography performed at 150 days postoperatively (pre-explantation) revealed valvular incompetence in two cases with endocarditis (25%). The comparison of the hemodynamic findings at the time of explant with those obtained preoperatively are listed in Table 3, which shows that there is significant increase in pulmonary artery pressure.
(PAP) and pulmonary capillary wedge pressure (PCWP) days from the moment 0 to 150 days time. No significant change in mean arterial pressure (MAP) and cardiac output (CO). Without the presence of samples with endocarditis, we found significant improvement in cardiac output (3392.50 to 3971.67 30 days versus 150 days \( P = 0.028 \)).

Calcification was found in 100% of the animals, usually related to the suture lines and less frequent in the body of the conduit. In the two animals with endocarditis, was observed presence of fibrinous vegetation on the left coronary leaflets with commissural tear between this and non-coronary leaflet. In these same animals, there was evidence of red thrombi in impaired leaflets and in sinotubular junction. In the body/root, there is a higher percentage of non-calcification than in other places.

For radiological analysis at all AVCPs evaluated there was calcification as a constant, principally affecting the suture lines and less evident in the body/root of the AVCP, as observed in a macroscopic evaluation. Optical microscopy showed that both the tube as the leaflets were kept with their original dimensions, with areas of fibrosis and calcification (sometimes with chondroid metaplasia and ossification) of variable intensity from case to case, especially around the inlet. Histologically, there was overall good retention of collagen bundles, especially on the leaflets with endothelialization with good tissue preservation of the sampling in the region of the sinus of Valsalva. The endothelial layer was found to be continuous throughout the internal structure. Thrombosis was not identified, nor signs of active inflammation or micro-organisms. The proximal and distal ends of the tube showed preservation of the structural layers, with areas of granulation tissue and fibrosis, often in relation to surgery. The outer layer of the prosthesis proved to be of irregular thickness, with areas of fibrosis.

In patients with endocarditis, the histopathological presentation showed, in the region of the sinus of Valsalva and the leaflets, areas of endothelial discontinuity associated with semi-occludent thrombi of fibrinoid pattern. Signs of active inflammation and micro-organisms.

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Table 1. Percentile values of 25 (P25), Median and percentiles 75 (P75) of the ECO variables (Control Group X Test Group).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n=5)</th>
<th></th>
<th>Test (n=8)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P25</td>
<td>Median</td>
<td>P75</td>
<td>P25</td>
</tr>
<tr>
<td>Weight</td>
<td>31.00</td>
<td>35.00</td>
<td>37.00</td>
<td>32.00</td>
</tr>
<tr>
<td>Thick. Sept</td>
<td>0.65</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>Max Grad.</td>
<td>2.70</td>
<td>3.00</td>
<td>4.00</td>
<td>2.65</td>
</tr>
<tr>
<td>Mean Grad.</td>
<td>1.45</td>
<td>1.50</td>
<td>2.15</td>
<td>1.68</td>
</tr>
<tr>
<td>AV</td>
<td>2.98</td>
<td>3.10</td>
<td>3.75</td>
<td>2.50</td>
</tr>
<tr>
<td>Thick. wall</td>
<td>0.65</td>
<td>0.70</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>LVDD</td>
<td>35.50</td>
<td>38.00</td>
<td>41.00</td>
<td>40.50</td>
</tr>
<tr>
<td>LVSD</td>
<td>23.00</td>
<td>25.00</td>
<td>26.00</td>
<td>28.25</td>
</tr>
<tr>
<td>EF</td>
<td>0.61</td>
<td>0.66</td>
<td>0.78</td>
<td>0.61</td>
</tr>
</tbody>
</table>

\( (*) \) descriptive level of probability of the Non-parametric Mann-Whitney Test. Thick. Sept. = Thickness of the interventricular septum. Max. Grad. = maximum aortic transvalvular gradient; Mean Grad. = Mean aortic transvalvular gradient. AV = aortic valve area; Thick. wall = Thickness of the left ventricular posterior wall. LVDD = Left ventricular diastolic diameter, LVSD = Left ventricular systolic diameter. EF = left ventricular ejection fraction
DISCUSSION

The need for use of valve grafts and valved arterial grafts have been growing throughout the world. The lack of mechanical and synthetic prosthetic materials that obviate the use of anticoagulation remains a reality [2]. Treatments for fixation and preservation applied to biological tissues used for making prostheses remain unsatisfactory. The structural deterioration, immune reaction and calcification of homo-and heterologous grafts [16] seems to be a threat over their durability. Moreover, the number of special patients who need a valve replacement or a valved arterial conduit on whom anticoagulation is undesirable, such as the elderly [17,18], has also increased.

Checking comparative echodopplercardiographic analysis between both Control and Test Group, as the analysis performed in the test group between 30 and 150 days, we noted that the statistically significant differences found and that could mean a poor hemodynamic performance of AVCP ceased with the withdrawal of the animals with endocarditis, supporting the hypothesis that the presence of endocarditis is leading to deterioration of left ventricular function. Similar data were obtained by Santos et al. [19].
On angiographic evaluation, we found the images obtained with aortography performed immediately after surgery and those performed immediately before the sacrifice of animals. Aortogram in two pre-explantation showed there was valvular incompetence, and in one was classified as severe and the other as moderate and corresponded to animals with endocarditis, which certainly was the causative agent of prosthetic dysfunction.

As in the Doppler echocardiographic analysis, hemodynamic values collected after induction and immediately prior to the explant show that the presence of endocarditis had a negative influence on the results, leading to worsening of cardiac output.

In summary, based on these data, we believe that the simple removal of the sample of animals with dysfunction by prosthetic endocarditis was striking in relation to cardiac output, ejection fraction and left ventricular diastolic diameter. We understand that the weight and height gain of animals (young sheep) in the period of follow-up of 150 days, justifies some of the differences found, such as septal and posterior wall of the left ventricle. It is possible that the same weight and height gain could have led to some degree of disproportion between the size of the bioprosthesis and hemodynamic demand of each animal, generating other differences. However, it is necessary that this should be studied more accurately.

Several studies [16,20-22] have shown that the process of structural deterioration of collagen matrix and calcification of bioprosthetic begin in commissural and annular regions, which are the areas of greatest tension and bending of the leaflets and also in the suture lines, where there is loss of continuity and integrity of biological tissue. Thus, it is easy to understand the findings of the macroscopic, where calcification was present in all explanted AVCPs, principally affecting the suture lines and was less frequent in the body of the conduit. Besides calcification, vegetation, thrombi and rupture of the leaflets were found only in animals with endocarditis.

The analysis of radiological findings is fully compatible with the macroscopic analysis and were expected.

Liao et al. [23] showed that the bovine pericardium treated by glutaraldehyde, when implanted in mice, maintains the amount of collagen practically unchanged after 90 days, unlike the porcine aortic valve. García-Páez & Jorge-Herrero [16] showed that in explanted bioprostheses in humans, calcium accumulates rapidly in the inner layers of tissue: in spongy layer of the porcine valve and in the fibrous layer of the pericardium and that this finding often compared with bone calcification, involves a multifactorial process mediated by the methods of preservation and by factors of the host. The findings of histological analysis in relation to optical microscopy were very similar to that described above.

For all the above facts, the study suggests that the AVCP studied here could have advantages over the conduits using arterial grafts and synthetic prostheses because it does not require anticoagulation. Even comparing with the AVCP with valved conduits using porcine aortic valve without support, there would still be the advantage that the pericardium shows better resistance to calcification and structural deterioration, as pointed out by the literature already cited. Despite homografts have greater resistance to structural deterioration, especially in the young and superior hemodynamic response, they still present a barrier to their low availability for use in larger scale.

From a technical standpoint, the use of AVCP in surgeries to replace the ascending aorta and aortic valve is a reproducible procedure and the flexibility and strength of bovine pericardium allow all the anastomoses are performed with continuous suture.

Regarding the possibility of AVCP come into clinical use, we believe that further studies are necessary with this device, for example, comparing the hemodynamic performance with other models. We also consider that the lack of an ideal method of tissue preservation, which provides long durability to heterologous tissue is a limiting factor for clinical use. Maybe in a very special group of patients like the elderly, this proposal can be collated. Surely we should await the results of studies using tissue engineering and tissue decellularization with repopulation with host cells.

CONCLUSIONS

The aortic valved conduit used in this study showed that it is technically feasible to arrange surgery for total replacement of the ascending aorta and aortic valve with reimplantation of coronary ostia in this animal model of experimentation.

REFERENCES


