Growth of Pulmonary Autograft in Swine
I.Feasibility of the Operation

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=Abstract=

신생돈을 이용한 이식 자가 폐동맥의 성장에 관한 연구
제1보, 수술의 적합성

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In order to test the hypothesis that the pulmonic valve, when used to replace the aortic root as a pulmonary autograft, will remain a viable anatomical structure and will grow and develop normally along with the host, we performed aortic valve replacement with the pulmonary autograft in 15 neonatal piglets. The weight of the donor was 9.3 ± 0.2kg, the recipient 9.6 ± 0.3kg. Measured diameters of pulmonic annulus were 14 ± 0.2mm for autograft and 14.2 ± 0.2mm for pulmonary artery homograft. Operation was performed under cardiopulmonary bypass with deep hypothermia (20℃) at low flow perfusion (70 ml/kg/min). The mean operation time was 227 ± 10min., bypass time 152 ± 7.6min. and aortic cross clamp time 73 ± 4.6min. 9 piglets survived more than 12 hours. One survived 12 days and died of pneumonia and the latest one survived in good condition and sacrificed at postoperative 6th week for cardiac catheterization and pathologic examination that revealed the viability and growing of the pulmonary autograft. Currently we are able to complete the operation with good preservation of cardiac function, and our postoperative care has evolved to the extent that we are now confident enough of having an acceptable percentage of long term survivors to undertake a definite study in this regard.

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Key words : 1. pulmonary valve
2. Transplantation, homologous
3. Transplantation, autologous
4. Viability

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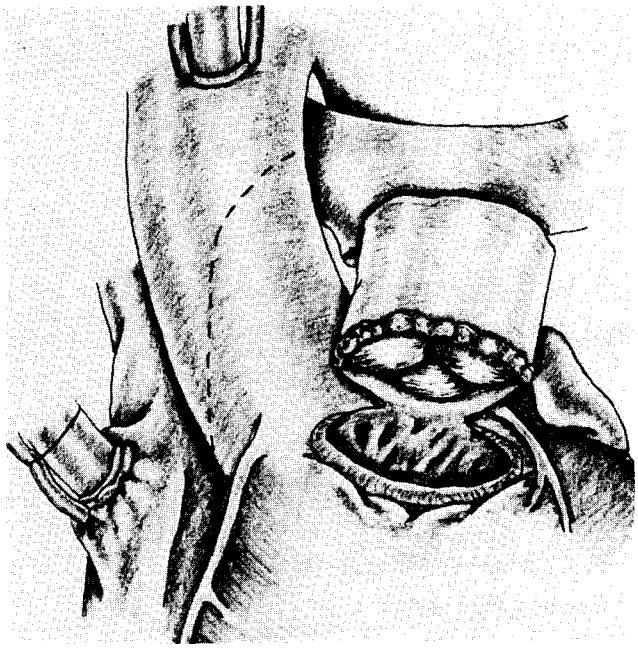

Fig. 1. The pulmonary homograft is harvested from the right ventricular outflow tract. Divide the distal pulmonary artery at the level of bifurcation of the pulmonary artery.

INTRODUCTION

Despite the numerous advances in mechanical and bioprosthetic valves, there still is no perfect aortic valve substitute for children[1~4]. Of the available alternatives, aortic homografts have the perfect natural design and freedom from thromboembolic complications, but also have the limited availability, more demanding technique to insert and limited long-term durability[5]. These homografts are not able to grow, and therefore may need to be replaced more than once before the child reaches maturity.

To overcome these disadvantages, living autologous tissue in the form of pulmonary autograft has been used in a few institutions[6~11]. Unlike the aortic homografts, pulmonary autografts are thought to be viable and potentially to grow. They may therefore offer the best hope for permanent valve replacement in growing children.

This study has been originally designed to verify the viability and growing of the pulmonary autograft along with the host in the piglet model.

METHOD

Six-to-eight week old piglets (Poland-China) of either sex were used as this experimental model, because they can grow rapidly within short period. The donor procedure began with premedication with ketamine (22mg/kg) and atropine (0.05mg/kg) intramuscularly. After this, the animal was transferred to the operating table and intubated, then started inhalation anesthesia with Isoflurane and ventilated with a Harvard ventilator (tidal volume 15cc/kg, rate 14-16 breaths/minute). Fentanyl (0.03mg/kg) was given initially and repeated as necessary for analgesia. After sterile surgical skin preparation, the donor heart was exposed through a midsternal incision. After heparinized (300 IU/kg IV), blood was collected for the future transfusion to recipient. The pulmonary artery (PA) was transsected at the level of the branched PA and the pulmonary root excised with 4mm rim of right ventricular (RV) myocardium proximal to the pulmonary sinuses (Fig. 1). We measured the diameter of this PA with Hegar dilator and placed in Ringer's lactate solution at 4℃. For the blood transfusion during and after the operation, one more pig was prepared. After adequate anesthesia of this blood donor, the jugular vein was exposed and 12 gage needle inserted to give heparin and to collect the blood.

For the recipient, the operative approach was the same as the donor except that a catheter was placed in the carotid artery for pressure monitoring and another in the external jugular vein. After open the pericardium, the animal was heparinized (300U/kg IV) and cannulated using two #20 venous cannulae through the right atrium directed into the superior and inferior vena cavae. An aortic cannula was placed high in the ascending aorta and cardiopulmonary bypass (100ml/kg/min) was initiated with left atrial venting. A pediatric membrane oxygenator primed with prepared whole blood was used and the perfusate was cooled to 15℃. With caring for the coronary arteries (first septal perforator is usually located very close to the dissecting plane of the pulmonary root) (Fig. 2), the PA was transsected at the level of the branch PA and the pulmonary root excised with 3 mm rim of RV myocardium proximal to the pulmonary sinuses like donor homograft preparation (Fig. 1). This PA with the valve was measured with

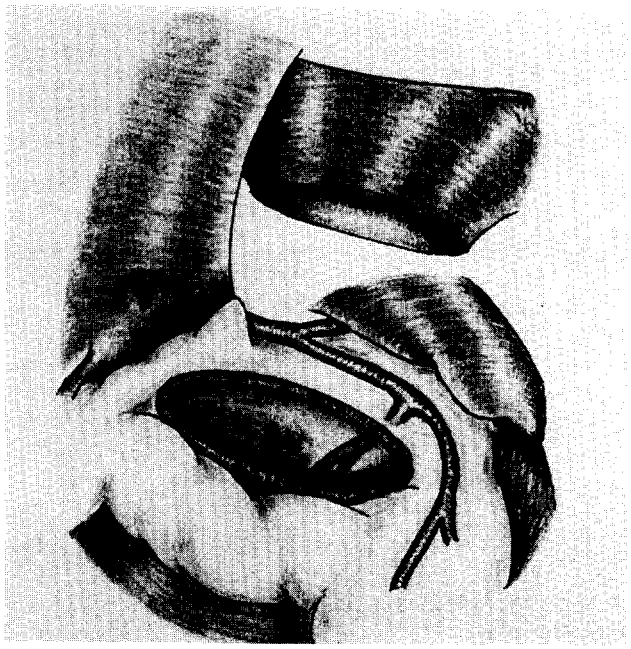

Fig. 2. The position of the first septal perforator. It is located very close to the dissecting plane of the pulmonary root

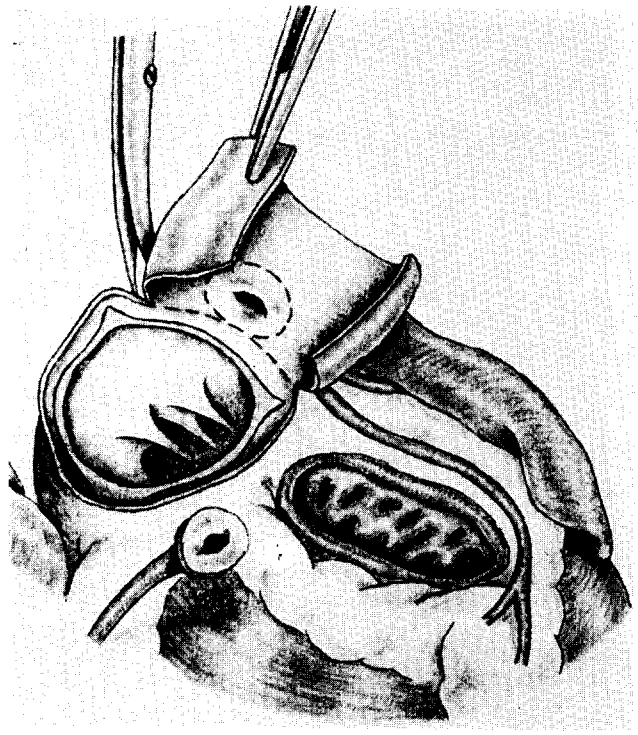

Fig. 3. Excision of the aortic wall with generous cuff of aorta surrounding the coronary arteries.

Hegar dilator and kept in the saline at 4℃. Crystalloid potassium cardioplegia (15ml/kg) was infused after aortic cross clamp and the aorta was divided proximal to the clamp. The aortic root excised leaving the coronary ostia with a small cuff of aortic wall to facilitate suturing (Fig. 3). After removal of the aortic valve leaflets, the procured autograft PA was sutured to the base of the excised aortic root using a continuous 5/0 prolene for the proximal anastomosis (Fig. 4). A 4-5 mm punch was used to create openings in the autograft sinuses for reimplanting the coronary arteries and a continuous 7/0 prolene was used for these anastomosis. The distal anastomosis was also done with 5/0 prolene. After released the aortic cross-clamp, reconstructed the RV outflow tract with a procured pulmonary allograft on the perfused heart during rewarming (Fig. 5). We used continuous 5/0 prolene for both sides. After rewarming, cardiopulmonary bypass was gradually discontinued. If spontaneous cardiac rhythm did not occur, internal defibrillator at 10 joules was used. Following decannulation, protamine sulfate was administered to reverse the heparin. Both pleural cavities were drained with chest tubes connected to Emerson suction and the wound was closed.

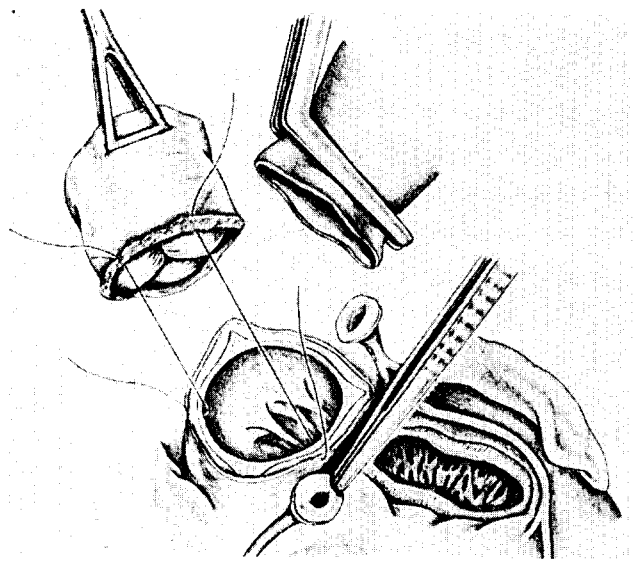

Fig. 4. Autograft root is positioned after the coronary arteries have been mobilized away from the aorta.

The recipient was recovered in the operating suite for 12 hours postoperatively while continuous arterial pressure

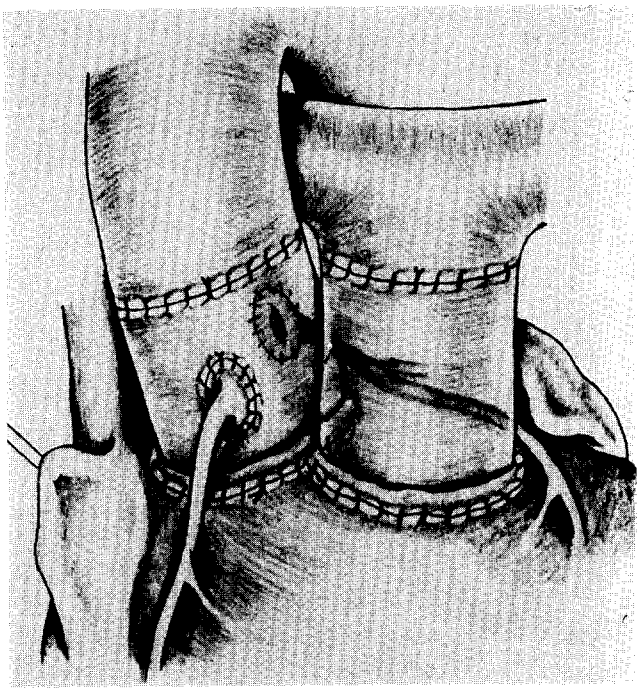

Fig. 5. The complated root replacement of aorta with pulmonary autograft, coronary reimplantation and right ventricular outflow reconstruction with fresh pulmonary homograft.

and ECG tracings were monitored. They were anesthetized by small doses of Isoflurane until full recovery of cardiac function had been achieved, then extubated the chest tubes and endotracheal tube. After removal of the arterial line, they were transferred to the animal recovery room. Animals were housed in an environmentally controlled cage and given bupernorphine HCl(0.01mg/kg) intramuscularly for analgesia and cefazoline 500mg for 7 days, and the animals were allowed food and water ad lib the day following surgery.

Originally we planned to follow up these pigs for more than 4 months after surgery for verifying the growing of the transplanted autograft, but we were not successful in this respect until this report and this is a preliminary report about the appropriateness of this type of pulmonary autograft experiment. We assure the feasibility of this operation and from this last case we started to succeed the long term survival of this pig model.

Table 1. The summary of these experimental animals

Case	Survival time	Extubation	Cause of death
1	0	no	metabolic acidosis(TF/R)
2	17hr	yes(4hr)	pneumothorax
3	0	no	bleeding from liver capsule
4	12hr	no	hypovolemia
5	25hr	yes(15hr)	pneumothorax
6	12hr	no	metabolic acidosis(TF/R)
7	12days	yes(12hr)	pneumonia, sepsis
8	19hr	yes(13hr)	accidental A-line bleeding
9	0	no	bleeding
10	0	no	hypovolemia
11	14hr	yes(12hr)	respiratory failure
12	33hr	yes(21hr)	respiratory failure
13	0	no	bleeding
14	0	no	hyperkalemia
15	good	yes(17hr)	–

hr; hour, TF/R; transfusion reaction
A-line; arterial line

RESULTS

We performed the aortic valve replacement with the pulmonary autograft in 15 neonatal piglets. The weight of the donor was 9.3 ± 0.2 kg, recipient 9.6 ± 0.3 kg and blood donor 23.3 ± 0.8 kg. Measured diameters of the pulmonary annulus were 14 ± 0.2mm for autograft and 14.2 ± 0.2mm for PA homograft. Homologous blood (200-600 ml) diluted with Ringer's lactate and/or Hespan were used for perfusion with deep hypothermia (20℃) at low flow performance (70ml/kg/min).

The mean operation time was 227 ± 10min. (265 ± 11min. for table death, 199 ± 4.8 min. for early survivor), pump time 152 ± 7.6 min. (171 ± 12.5 min. for table death, 139 ± 5.8 min. for early survivor) and aortic cross clamp time 73 ± 4.6 min. (75 ± 9.7min. vs. 72 ± 3.5min.).

Among 15 cases (table I), 6 died early postoperatively (40%) mainly due to technical problems associated with perfusion and anastomotic site bleeding. 9 piglets survived longer than 12 hours after operation and 7 of them were able to be extubated the endotracheal tube. 7 died within 2 days postoperatively. Apart from the technical errors, the early mortalities were associated with pneumothorax, res-

Table 2. Postoperative catheterization data of the last pig

LV pressure	110/17 mmHg
RV pressure	50/10 mmHg
Aorta pressure	100/75 mmHg
PA pressure	50/36 mmHg
RA pressure	18 mmHg
PA wedge pressure	22 mmHg

LV; left ventricle, RV; right ventricle
PA; pulmonary artery, RA; right atrium

piratory failure, severe metabolic acidosis probably due to the transfusion reaction and unexpected accident like arterial line bleeding. One survived 12 days and died of pneumonia, and the latest one survived in good condition and sacrificed at postoperative 6th week for cardiac catheterization and pathologic examination.

The follow-up cardiac catheterization data of this pig did not show any pressure gradients across the valves, which means there was no evidence of aortic or pulmonary regurgitation or stenosis (table II). Echocardiographic measurement of this pulmonary autograft at the aortic root was 16 mm, and 24 mm at the mid portion of this conduit. The annular diameter of the pulmonary homograf was 14 mm. After sacrificing this pig, we found that he grew up doubled in weight (from 8.9kg to 18.1kg), and both the new aortic and pulmonary valves were well coapted (Fig. 6). We could also see the clear suture site and leaflets. The pulmonary autograft enlarged from 14 mm to 16 mm at the annular level, to 24 mm at the mid portion and to 17 mm at the distal anastomotic site. The homograft also showed the increase in size from 13 mm to 17 mm at annular level, to 24 mm at the mid portion and to 18 mm at the distal site. The microscopic findings of this pulmonary autograft showed the well-preserved central cellular fibrous core, relatively plump, clear outlined, actively proliferating fibroblast and regularly arranged peripheral elastic areas in the cuspal stroma, which mean the viability of this autograft tissue (Fig. 7). The pulmonary homograft also maintained the viability, and much thinner fibrous core might suggest the less durability, but we need more long-term follow-up in this respect.

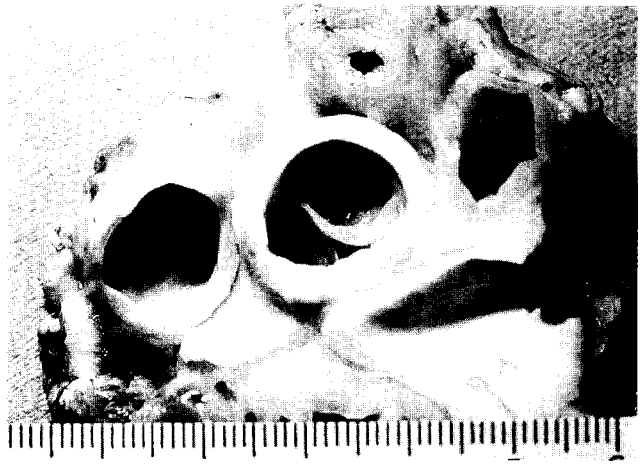

Fig. 6. The gross appearance of the new aortic and pulmonary valve showing well coapted cusp.

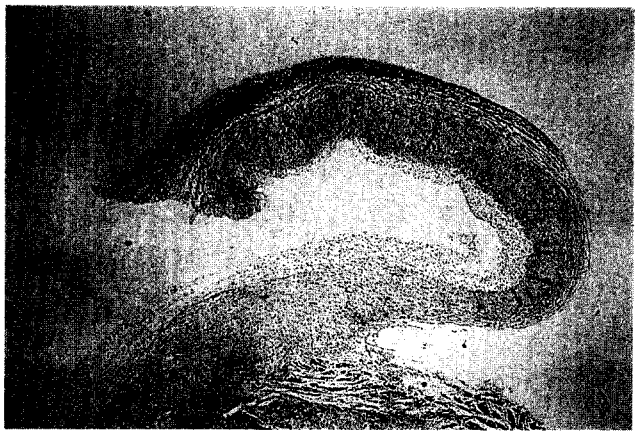

Fig. 7. Microscopic findings of the pulmonary autograft valve leaflet positioned at aortic root showing the well preserved central cellular fibrous core, actively proliferation fibroblast and regularly arranged peripheral elastic areas in the cuspal stroma.

DISCUSSION

Aortic valve replacement is one of the most significant advances in the treatment of cardiac disease in this century[12]. Attempts at aortic valve replacement had been sporadic until Starr et al[13] developed a mechanical ball-cage valve device in 1960. Since that time, there has been an ongoing search for a reliable aortic valve prosthesis that is not susceptible to infection and complications. From the

ensuing clinical and experimental research has evolved a variety of mechanical and prosthetic tissue valves, and the aortic homograft that has been either antibiotic-sterilized, irradiated, or cryopreserved in an attempt to preserve viability.

The pioneering work by Lower et al[14], Pillsbury et al[15] and Ross[6], has proved the feasibility and safety of the pulmonary autograft in the experimental model and patients. Our clinical data illustrates that this operation is technically feasible and safe in our hands[9~11, 16~18]. This pulmonary autograft operation is a little bit complicated procedure, a double valve procedure inspite of the single valve disease, but has so many advantages compared to the other valvular substitutes, such as appropriate size for the patient, excellent hemodynamics, non antigenicity and no need for anticoagulation. Besides these, unlike aortic homograft, this pulmonary autograft is thought to be viable and potentially to grow[7, 8, 17]. We hope that the pulmonary autograft in young patients would grow and maintain its structural and functional integrity indefinitely. At this point, we have to differentiate the actual growing from simple dilatation. We define the actual growing is when maintain the viability and increase in size, but simple dilatation is only increase in size without viability. Then we have to answer what the viability of the autograft is. There are several methods to prove the viability: morphological, proliferative, metabolic and mechanical method[19, 20]. In this experiment we applied the morphological method and we regarded the tissue viable when maintain the endothelial cell integrity and preserve the architectural composure, but amorphous non-cellularity in homogenous fibrous tissue is the evidence of the non-viability. In this point of view, both autograft and fresh homograft of our experiment were viable and seemed to grow actually, but we have to consider this was the early postoperative findings and even though we could not find the regurgitation or stenosis, there occurred some turbulence around the anastomosis which could dilate the vessels. We used a sibling piglet as a fresh homograft donor and this possible good histocompatibility could also make the homograft grow. Because of the potential for growth of the pulmonary autograft, some groups have proposed that a pulmonary autograft should be a primary consideration in the aortic valve diseases in pediatric population[16, 21, 22].

Conclusively, this type of definitive study needs to be undertaken to resolve the important issue of growth and viability of the pulmonary autograft[17]. This study would therefore attempt to address clearly the few pertinent remaining question marks surrounding the advisability of the pulmonary autograft as an aortic valve replacement in children. We chose the piglet as the experimental animal for being expected to grow from 10kg to over 80kg in the 4-6 months follow-up period[23, 24]. This will allow sufficient growth of the pigs to evaluate concomitant growth of the pulmonary autograft; harvesting the pulmonary autografts at the end of the follow-up periods, measuring the size and analyzing the tissue histologically.

The postoperative care of this series was evolved after much trial and error. Cullum described the extreme sensitivity of the porcine heart to ischemia[25], and at the end of the operation care of this animal is critical, because the pig recovering from anesthesia is very intolerant to a endotracheal tube and tachypnea is marked in the first 24 hours and resembles the neonatal respiratory distress syndrome. The loss of surfactant could be a contributing factor[23, 26].

Despite undesirable result until now, with our progression of the postoperative care of this animal we are confident enough of having an acceptable percentage of long term survivors to undertake a definitive study after this.

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=국문초록=

자가 폐동맥 판막으로 대동맥 판막 이식술을 시행했을 경우 이 치환된 폐동맥 및 판막이 해부학적으로 생명력을 유지하고 정상적으로 발육할 것이라는 것을 증명하기 위해 15 마리의 수용돈(9.6 ± 0.3 kg)을 저체온과 저관류 (70ml/kg/min) 하에 인공 심폐기를 이용한 실험 결과 9마리가 술후 12시간 이상 생존했으며, 평균 수술 시간은 227 ± 10분, 인공심폐기 가동시간 152 ± 7.6분, 대동맥 차단 시간 73 ± 4.6분 이었다. 한 마리는 12일 간 생존하다 폐렴으로 사망하였고, 한 마리는 6주 이상 양호한 상태로 생존하여 심도자 및 병리조직 검사를 통하여 치환 폐동맥판막의 조직 생명력 유지 및 이식편의 성장을 관측할 수 있었다. 본 연구진은 현재 심기능 보존 및 술후 관리의 꾸준한 발전으로 계속되는 실험에서 장기 생존율의 증가 등 좋은 성적을 기대할 수 있게 되어 제 I 보로서 보고하는 바이다.