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A Practical and Less Invasive Total Cavopulmonary Connection Sheep Model

Dongfang Wang, MD, PhD, Mark Plunkett, MD, Guodong Gao, MD, Xiaoqin Zhou, MD, Cherry Ballard-Croft, PhD, Hassan Reda, MD, and Joseph B. Zwischenberger, MD

Department of Surgery, Cardiothoracic Surgery Division, University of Kentucky College of Medicine, Lexington, KY 40536

Abstract

Our goal is to develop a less invasive total cavopulmonary connection (TCPC) sheep model for testing total cavopulmonary assist (CPA) devices. Thirteen sheep underwent a right 4th intercostal lateral thoracotomy. In series I (n=6), a polytetrafluoroethylene (PTFE) extracardiac conduit (ECC) was connected to inferior vena cava (IVC) and superior vena cava (SVC) by end to side anastomosis. The SVC/IVC remained connected to right atrium (RA). A PTFE graft bridged ECC to right pulmonary artery (RPA). Clamps between SVC/IVC anastomoses and RA diverted total venous blood to pulmonary circulation. In series II (n=7), temporary bypasses between SVC/IVC to RA allowed SVC/IVC to be cut off from RA for better RPA exposure. The ECC-SVC/IVC were end-end anastomosed and ECC-RPA side-side anastomosed for total SVC/IVC to PA conversion. In each series, one sheep died of bleeding. In 5 sheep in series I and 6 sheep in series II, the TCPC model was successfully created with significantly increased central venous pressure and significantly decreased pulmonary artery pressure/arterial blood pressure. Our acute TCPC sheep model has a less traumatic right thoracotomy with no cardiopulmonary bypass and less blood loss with no blood transfusion, facilitating future long-term CPA device evaluation.

Keywords

Failing Fontan Circulation; Heart Failure; Cavopulmonary Assist; Congenital Heart Disease; Sheep

INTRODUCTION

Total cavopulmonary conversion (TCPC) is a standard physiological corrective surgery for patients with complex single ventricle congenital heart defects, improving patients' quality of life by increasing arterial O₂ saturation.¹ In the TCPC procedure, a cavopulmonary shunt is created to connect the inferior and superior vena cava (IVC and SVC) directly to the right pulmonary artery (RPA). Therefore, the central venous pressure (CVP) will be elevated to drive total venous blood from the IVC/SVC directly to the pulmonary circulation.² The total

Corresponding Author: Dongfang Wang, M.D., Department of Surgery, University of Kentucky College of Medicine, 800 Rose Street, MN269, Lexington, KY 40536-0298, Phone: 859-323-6013, Fax: 859-323-1045, dnwang2@email.uky.edu.

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single ventricle heart functions solely as a left ventricle in the systemic circulation. TCPC separates the unsaturated venous blood from the systemic arterial blood, eliminating cyanosis. However, TCPC long term mortality remains imperfect, and over 20 years mortality tends to be high.^{3–5} Any increase in pulmonary vascular resistance will require further CVP elevation to compensate for reduced cardiac output, triggering a cascade of failing Fontan circulation.^{6–9}

Several cavopulmonary assist (CPA) devices are under development to actively move blood from the SVC/IVC through the TCPC connection to the pulmonary artery, reversing the pathophysiology of the failing Fontan circulation.^{10–12} A large animal TCPC model of failing Fontan circulation is needed for CPA device *in vivo* evaluation, but it is technically challenging. The published TCPC animal model usually requires cardiopulmonary bypass (CPB) and blood transfusion with significant surgical trauma.¹³ In this paper, we describe the development of a new TCPC sheep model characterized by: 1) less traumatic right lateral thoracotomy instead of median sternotomy, 2) no CPB requirement, and 3) no need of blood transfusion. Our sheep model simulates human TCPC and failing Fontan circulation, establishing a platform to test CPA devices.

MATERIAL AND METHODS

All animal studies were approved by the University of Kentucky IACUC (Institutional Animal Care and Use Committee) and were conducted in accordance with the Principles of Laboratory Animal Care (National Society of Medical Research) and the “Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23, revised 1996)”. A total of 13 adult female cross-bred sheep (35–45 kg) were used in this study.

Animal Preparation and Instrumentation

After anesthesia induction with ketamine (5 mg/kg, i.v.) and diazepam (0.25 mg/kg, i.v.), all sheep were intubated with an endotracheal tube (Hudson RCI, Triangle Park, NC), transferred to the operating room, and connected to the anesthesia machine (Narkomed 2B, DRAGER, Telford, PA). Anesthesia was maintained with 1–3% isoflurane, titrating normal range of heart rate (HR) and arterial blood pressure (ABP). Arterial/venous catheters (BD Medical Inc, Sandy, UT) were placed into right femoral artery/vein for pressure monitoring and fluid administration.

TCPC configuration in our sheep model

In humans, the TCPC procedure is staged from young age. In stage 1, the SVC is cut from right atrium (RA) and directly anastomosed to upper RPA by 1 year old. In stage 2, the IVC is connected to lower edge of RPA several years later. Our sheep model has to be finished in one stage in adult sheep. Additionally, the sheep’s ascending aorta is in upper and anterior position of RPA, blocking two direct end-side anastomosis of SVC/IVC to upper/lower RPA, respectively. Therefore, only one RPA anastomosis is feasible in adult sheep.

In the first 6 sheep, TCPC was configured in this animal model as follows (Fig. 1A): 1) an extracardiac conduit (ECC) was connected to SVC and IVC by end to side fashion; 2) A side

to side connection between ECC and RPA was created by a short bridge graft; 3) Clamping between SVC/IVC and RA diverted total venous blood through the ECC to RPA and pulmonary circulation, bypassing right heart. In this configuration, the SVC/IVC was still connected to RA. SVC-RA in front of RPA blocked RPA surgical exposure and made the RPA anastomosis very difficult.

In the following 7 sheep, the SVC was cut off from RA, allowing much better RPA exposure and much easier anastomosis. Two temporary bridges from SVC/IVC to RA were created to enable SVC/IVC to be cut off from RA without interrupting blood flow from SVC/IVC to RA. Therefore, ECC connected SVC and IVC by end to end anastomosis, and connected RPA by side to side anastomosis, creating TCPC (Fig.1B).

Surgical procedure

In the first series (n=6), a right lateral thoracotomy was performed through the 4th intercostal space. The pericardium was opened to expose SVC, IVC, RA and PA. A bolus of intravenous heparin (150 U/kg) was administered to achieve an ACT level of 200–300 seconds. A 16 G arterial catheter was placed into the PA through a purse string for PA pressure monitoring. The SVC was isolated and pulled over to the left side to access the transverse pericardial sinus, through which the entire RPA was exposed. A partial occluding clamp was applied on the RPA for a 2.0 cm incision. A 3 cm long bridge graft (OD 18 mm Polytetrafluoroethylene(PTFE)) was anastomosed to this incision. Another partial occluding clamp was applied on the SVC for a 3 cm incision. This SVC incision was anastomosed to a 10 cm ECC (18 mm OD PTFE graft). An oval hole was made on the ECC for anastomosis to the end bridge graft. The third partial occluding clamp was applied on the IVC for a 3 cm incision for anastomosis to the other end of the ECC. All four of these anastomoses were made in end to side fashion with 5-0 Prolene continuous running suture. The three partial occluding clamps were removed one by one, and all anastomoses were carefully checked for bleeding. A supplemental stitch was placed for hemostasis as necessary. Two cross clamps were placed on SVC/IVC between RA and SVC/IVC to convert total venous blood from vena cava to RPA-pulmonary circulation (Fig 1A).

In series II (n=7), after exposure/isolation of SVC and RPA, a purse string was placed on the SVC and RA, respectively for cannulation. After systemic heparinization as in Series I, a 24 Fr venous cannula was placed into SVC and RA, respectively through the purse string. The other ends of SVC/RA cannulas were connected to create a SVC – RA bypass, allowing SVC to be cut off from RA. The RA cut end was closed by continuous running suture. Consequently, the RPA was easier to expose and isolate. The RPA was partially occluded for a 2 cm incision. A 10 cm long PTFE ECC (18 mm) was chosen to connect SVC/IVC to RPA. A transverse oval hole (1.8 cm major axis) was made on ECC. Continuous running suturing with 5-0 Prolene was used for ECC-RPA side to side anastomosis. The SVC cut end was anastomosed to the ECC in the end to end fashion. The same temporary bridge was also created between IVC and RA, allowing IVC to be cut off from RA. The RA cutting end was closed, and the IVC end was anastomosed to the other end of ECC. After removal of the two temporary SVC/IVC-RA bridges, total venous blood flow was diverted into pulmonary circulation (Fig.1B & Fig 2).

Data Collection and Analysis

The femoral arterial/venous catheters and PA catheter were connected to transducers (Edwards Lifesciences, Irvine, CA) for pressure monitoring. Arterial blood pressure, CVP, and mean PAP (mPAP) were continuously monitored during surgery via a Phillips MP-50 monitor (Boeblingen, Germany). Systolic arterial blood pressure (sABP), mPAP and CVP were recorded to differentiate a change among the status of control (normal baseline) and failing Fontan circulation. All data were expressed as mean $\pm$ SE. A p-value < 0.05 was considered statistically significant. Differences between the time points were evaluated using the student's T test.

Autopsy

After the experiment was finished, all sheep were euthanized with Beuthanasia-D (1 mL/10 lb body weight). An autopsy was conducted to visualize RPA anastomosis, bridge graft, and ECC.

RESULTS

One sheep died of massive bleeding from a torn RPA anastomosis in each series. In the remaining 5 sheep of the first series, and 6 sheep of series II, TCPC was successfully created without CPB and blood transfusion. Total venous blood was diverted from IVC/SVC to pulmonary circulation.

However, this Fontan physiology could not maintain normal ABP, CVP, and PAP in the adult sheep. In series I, this TCPC model was characterized by elevated CVP, decreased PAP and decreased ABP (Figure 3). CVP was significantly elevated from 6.8 ± 1.6 to 14.4 ± 0.8 ($p=0.011$) to drive the blood from the vena cava to the PA. The PAP was decreased from 15.6 ± 0.9 to 11.2 ± 1.1 ($p=0.049$). sABP was decreased from 110 ± 7.1 to 57 ± 1.5 ($p=0.02$). Heart rate did not change. Since total venous blood from SVC/IVC was diverted to PA, only blood from coronary vein was ejected from right ventricle to PA. The coronary blood flow through the right ventricle was too low to generate a pulsatile PAP waveform.

The hemodynamic results in Series II were similar to Series I. After TCPC was established, CVP was increased from 4.5 to 17 mmHg, sABP was decreased from 94 to 46 mmHg and HR remained unchanged (Table 2).

In autopsy, the ECC, bridge graft, and their anastomoses were found well connected as designed without thrombosis. No damage was observed at RPA, SVC/IVC, and RA (inside/outside) in all 11 successful sheep.

DISCUSSION

TCPC was successfully created in 11 of total 13 adult sheep. This TCPC large animal model successfully mimicked failing Fontan circulation with increased CVP and decreased sABP/PAP. This practical TCPC sheep model was achieved through a less invasive lateral thoracotomy without CPB and blood transfusion.

Requirement of TCPC animal model with failing Fontan circulation

Continuous improvement and optimization of the Fontan procedure along with careful patient selection have significantly increased its survival rate, but the Fontan does still fail.^{3,4} Eventually, the patient that develops failing Fontan circulation may require cavopulmonary assist and heart transplantation.⁶ In recent years, research has been focused on the development of a cavopulmonary assist (CPA) device for bridge to heart transplantation or destination therapy.^{10,13–17} Our ultimate goal is to develop a practical and survivable TCPC animal model for testing CPA device prototypes.

A lateral thoracotomy instead of median sternotomy

The human chest is flat in coronal direction, therefore the shortest distance to reach/expose SVC-RA-IVC and RPA in TCPC surgery is through a median sternotomy. However, the sheep chest is flat in dorsoventral direction, and the shortest distance to reach/expose SVC-RA-IVC and RPA is through a right lateral thoracotomy. Furthermore, the lateral thoracotomy is less invasive than a median sternotomy, decreasing surgical bleeding.

Because the sheep sits on the sternum, the median sternotomy affects post-operative healing of the incision and may result in wound infection. Therefore, the lateral thoracotomy is less traumatic and more feasible for sheep survival study.

Avoidance of CPB and blood transfusion

CPB is usually applied in clinical TCPC procedure. However, CPB causes blood trauma/platelet activation, leads to blood dilution from circuit priming, and requires high level systemic heparinization (ACT>410 sec). Blood trauma, blood dilution, and high level systemic heparinization results in excessive intra-/post-operative blood loss. Blood transfusion is very complicated in sheep experiment and often associated with serious adverse complications in survival study. In our TCPC procedure, CPB was avoided because sheep have a long (10 cm) IVC above diaphragm, allowing easy placement of a partial occluding clamp on IVC for conduit (end) to IVC (side) anastomosis in series I, and easy IVC-RA temporary bridge establishment in series II. Lack of CPB, less invasive lateral thoracotomy incision, and careful anastomosis eliminated the need of blood transfusion in our TCPC sheep model.

TCPC configuration in sheep and the surgical technique challenges

In humans, the SVC and IVC are cut from the RA and connected to the RPA individually in different stages. A bidirectional Glenn that connects the SVC to upper RPA is performed first. Months to year(s) later, the IVC is connected to lower RPA, completing TCPC. In experimental sheep, we have to complete TCPC in a one stage procedure. The sheep anatomy is different from humans. Sheep have a deep-narrow chest, whereas the humans have a flat-wide chest. The SVC-RA-IVC in sheep is not in the same layer as the RPA as it is in humans. It is very difficult to directly anastomose SVC and IVC-ECC onto the upper/lower edge of the RPA individually as in human TCPC. The sheep RPA is in a deep position beneath aortic arch, pulmonary vein and RA/SVC. Therefore, in series I, we used an ECC to connect the SVC and IVC and a 2 cm short bridge graft to connect the conduit to the RPA. In series II, we cut off IVC from RA to expose RPA better for ECC-RPA anastomosis. Even

one anastomosis on the RPA is challenging. Good surgical technique is required for reliable RPA anastomosis. Bleeding from the RPA anastomosis is a disaster and attempting an additional stitch is very hard. Even though one stage TCPC on a normal adult sheep is very difficult, this paper demonstrated that it is possible and practical with careful preparation.

In human TCPC, the SVC and IVC are connected to the RPA by two separate end (SVC/IVC) to side (RPA) anastomosis. There is no blood flow restriction from the SVC/IVC to the RPA. In our sheep TCPC model, the SVC and IVC were connected to the RPA by one side-side anastomosis. Therefore, all the blood from the SVC and IVC flow to the RPA through a single anastomosis, resulting in flow restriction and failing Fontan circulation.

Limitations

Our TCPC was on normal sheep which is different from human single ventricle heart. The current model only mimics the pathophysiology of failing Fontan circulation to establish a platform for testing cavopulmonary assist devices and cannot be used for pathophysiology studies. Another limitation of the TCPC animal model is that, so far, it is an acute model. Nevertheless, our ultimate goal is to develop a survivable TCPC animal model.

Conclusion

A one stage adult sheep TCPC model can be achieved through a right lateral thoracotomy. This less invasive procedure eliminates the need for CPB support and blood transfusion, facilitating survivable TCPC sheep model for long term test of CPA device.

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Figure 1.

Illustration of TCPC animal model to mimic failing Fontan circulation. **A:** In Series I, an extracardiac conduit connects to SVC and IVC. A short graft is used as a bridge between the conduit and RPA. Clamping between SVC/IVC and RA diverts total venous blood to RPA and pulmonary circulation, bypassing right heart. **B:** In series II, the SVC/IVC are cut off from RA and connected to an extracardiac conduit (ECC). The ECC is connected to RPA to divert total venous blood to RPA and pulmonary circulation, bypassing right heart.

Figure 2.
Picture of sheep TCPC model (Series II).

Figure 3. TCPC induced hemodynamic changes (decreased sABP, increased CVP, and decreased mPAP), indicative of a failing Fontan circulation.

Table 1

Abbreviations

Abbreviation	Description
ABP	arterial blood pressure
HR	heart rate
CPA	cavopulmonary assist
CPB	cavopulmonary bypass
CVP	central venous pressure
ECC	extracardiac conduit
IVC	inferior vena cava
mPAP	mean pulmonary artery pressure
PA	pulmonary artery
PAP	pulmonary artery pressure
RA	right atrium
RPA	right pulmonary artery
sABP	systolic arterial blood pressure
SVC	superior vena cava
TCPC	total cavopulmonary connection

Table 2

Series II Hemodynamic Parameters

	Baseline	TCPC 5 min	TCPC 15 min
HR (bpm)	101.5±23.6	92.0±38.2	100.5±36.7
sABP (mm Hg)	94.0±8.1	46.5±10.5 *	52.0±12.9
CVP (mm Hg)	4.5 ±1.9	17.0±5.3 *	13.0±2.5

*
= p<0.05 vs Baseline