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Acute histological changes of the lung after experimental Fontan circulation in a swine model

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

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Summary

Background:

Histological changes of the lungs were studied after the establishment of a modified total cavopulmonary connection (TCPC) without the use of cardiopulmonary bypass (CPB) or other means of temporary bypass on a swine model.

Material/Methods:

8 open chest-anesthetized pigs Landrace x Large White pigs (mean weight 43kg, mean age 4.5 months) underwent TCPC by the use of an appropriate size Y-shaped conduit connecting the superior and inferior caval veins (end-to-end anastomosis) to the pulmonary trunk (end-to-side anastomosis). After sternotomy, a wedge resection of the lung parenchyma was performed at baseline. Hemodynamic stability was sustained after TCPC establishment and 2 hours later another wedge resection of the lung was performed (from the same anatomic area). Histological studies were conducted by hematoxylin and eosin staining.

Results:

All samples (n=8) at baseline were consistent with normal lung parenchyma. After the establishment of TCPC, all samples (n=8) revealed moderate mononuclear infiltration adjacent to pulmonary alveoli and bronchioles, findings compatible with bronchiolitis.

Conclusions:

In a normal swine model, 2 hours after the establishment of Fontan circulation without the use of CPB, pathologic examination of the lungs revealed bronchiolitis. Further research is needed to clarify these findings and the potential implications to the Fontan circulation, either immediate or long-term.

Key words:

experimental fontan operation • total cavopulmonary connection • beating heart surgery • extracorporeal circulation • histology • lung

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BACKGROUND

During the past 4 decades, the Fontan operation has been carried out for the surgical treatment of children with congenital heart disease in which repair into a 2-ventricle system was impossible [1].

Fontan operation can be performed by a number of variants; currently, the most acceptable methods are the extracardiac conduit and the lateral tunnel total cavopulmonary connection techniques, both being accepted for their hemodynamic supremacy [2,3].

Today, more than 40 years after the first Fontan operation, perioperative mortality has decreased significantly and has stabilized at around 5% [4]. Factors contributing to the improvement of the survival from the surgical procedure include the more energy efficient circulation obtained after the application of total cavopulmonary connection techniques [2]. Moreover, the limited duration of cross-clamping of the aortic root, along with the decrease in the use of extracorporeal circulation, have both contributed to better surgical outcome.

The establishment of the Fontan circulation on an experimental model is difficult, a fact ultimately supported by the few experimental studies reported in the English literature. In an experimental swine model, we evaluated the possible acute histological changes of the lung for a period of 2 hours after the completion of the surgical procedure, when the pulmonary blood flow was switched from normal circulation to Fontan circulation. The whole procedure was performed with a beating heart technique without the use of extracorporeal circulation support.

MATERIAL AND METHODS

We used 8 anesthetized Landrace × Large White male pigs, with a mean body weight of 43 ± 3.8 kg and at the age of 4.5 months. All animals received humane animal care in compliance with the European Directive 86/609 of the European Council and the Presidential Decree 160/91 of the relevant Greek legal framework. The protocol was approved by the Ethics Committee of the Biomedical Research Foundation (Permission Number A.03.1/5/10-05). All animals underwent a 5-day quarantine and acclimatization period in the Animal House of the Center for Experimental Surgery prior to placement on the study. The day before surgery, physical examination and a pre-anesthetic blood work was performed in each animal. Food was withheld from pigs for 12 hours prior to the induction of anesthesia, and its body weight was obtained before surgery to facilitate the drug dosage calculation.

Each animal was pre-medicated with an IM injection of ketamine (10 mg/kg, Imalgen, Merial), atropine (0.04 mg/kg, Atropine, Demo) and midazolam (0.4 mg/kg, Dormicum, Roche). Anesthesia was induced with an IV injection of 0.9 mg/kg of propofol (Diprivan 1% w/v, Astra Zeneca) and animals were intubated and attached to a veterinary anesthesia machine (MDS Matrix, Model 2000, USA). Anesthesia was maintained by a closed-circuit system, with inhalation of a mixture of 3–5% sevoflurane (Sevorane, Abbott) and oxygen at a rate of 12–15 breaths/min. An

anesthesia monitoring record was made every 15 minutes, and was maintained for the duration of the procedure and for 1 hour post-surgery, including heart rate, respiration and arterial blood pressure, initially with a non-invasive technique and later through an arterial line. Body temperature, end-tidal carbon dioxide, peripheral oxygen saturation, inhalation anesthetic level, tidal volume, and ventilator pressure were also recorded for the entire period of the study. The Passport 2 (Datascope Corp., USA) and the LSI-8R Life-Sense (MedAir, SWEDEN) monitors were used to obtain and record all data. Physical methods were also used to monitor the animals during surgery, including the assessing of eye reflexes, muscle tone, peripheral pulses, capillary refill time and mucous membrane color.

Through appropriate skin incisions, both femoral veins were recognized and dissected. Catheters 10F (Avanti +, Cordis, USA) were inserted into the jugular vein and into 1 of the femoral veins for the rapid administration of crystalloid and colloid fluids, when needed. Through a median sternotomy, the superior (SVC) and the inferior vena cava (IVC), the azygos vein, the ascending aorta (Ao) and the main pulmonary artery (PA) were carefully dissected.

Electrocardiogram and all hemodynamic parameters were recorded, including phasic aortic flow signals, systolic, diastolic and mean arterial and pulmonary pressures, mean inferior vena cava pressure, mean left atrium pressure, as well as cardiac output and heart rate.

At this time a wedge resection of the lung was performed (middle lung field area).

An IV bolus of heparin (100 IU/kg) was given before SVC was divided between 2 vascular clamps and the cardiac end was oversewn using a continuous 5-0 polypropylene suture. The other portion of the SVC was anastomosed end-to-end to 1 of the upper sides of a Y-shaped Dacron-type conduit with a 16mm diameter using continuous 6-0 polypropylene suture. During this phase of the surgical procedure, animals were transfused through femoral venous catheter with crystalloid and colloid fluids to retain normal arterial pressure. However, due to hemodynamic instability, 3 of the animals needed inotropic support with dobutamine (2 µg/kg/min). Inotropic support was discontinued after the completion of the anastomosis. The other upper side of the Y-shaped conduit was anastomosed end-to-side with the main pulmonary artery by use of a continuous 6-0 polypropylene suture.

The IVC was recognized and the conduit was tailored to an appropriate length in order to be ready for an end-to-end anastomosis. The vessel was then divided between 2 clamps and the distal end was anastomosed to the graft with a continuous 6-0 polypropylene suture. The proximal side of the vessel was oversewn using a continuous 5-0 polypropylene suture. Animals were again supported by infusion of crystalloid and colloid fluids through the jugular venous catheter, while the 3 pre-mentioned animals again needed inotropic support, which was discontinued after the completion of the anastomosis. The proximal remnant of the inferior vena cava, on the right atrium, was oversewn with the use of a continuous 6-0 polypropylene suture, while the pulmonary artery trunk proximally to its anastomotic site with the graft was ligated. On performing all anastomoses, careful de-airing

Table 1. Mean values of hemodynamic parameters at the baseline and after the Fontan circulation.

Parameters	Baseline	Fontan circulation
HR (bpm)	90.81±3.61	71.61±2.67*
MAP (mmHg)	43.30±2.07	27.83±1.28*
IVCP (mmHg)	13.13±1.37	25.83±1.64*
LAP (mmHg)	5.32±0.69	4.28±0.40
PAP (mmHg)	18.28±0.52	10.68±0.90*
CO (lt/min)	3.24±0.28	1.29±0.13*
PVR (Wood's units)	3.56±0.25	6.22±0.70*
SVR (Wood's units)	11.62±0.86	16.56±1.68

All values are given as means ±SE, (*p<0.05).

precautions were taken to avoid air embolism. A 12F diameter and 30 cm long vent-tube was introduced into the right ventricle to collect the coronary venous blood, which was directed into the pulmonary flow of the graft by the use of a small roller pump, preventing the influence of right ventricular contractility on left ventricular contractility [5].

Each animal was in a steady hemodynamic status without the support of inotropic drugs or fluids, and they were kept warm at 37°C. The mean time required for the accomplishment of the Fontan circulation was 38±17 min and the mean blood loss was 115±48 ml (volume accumulated in the suction device). The total amount of crystalloid and colloid infused was about 1.000 ml. After 2 hours from the Fontan circulation, another wedge resection of the lung was performed from the same anatomic area.

When the study was considered completed, animals were euthanized with a bolus dose of sodium pentobarbital (Dolethal, Vetoquinol) and a thorough necropsy of the great vessels and all cardiac cavities did not reveal the presence of clots.

The lung biopsy specimens were taken (1.5×1.5×1.5 cm) when the lung was inflated, and were fixed in 10% formaldehyde for 24 hours. Serial paraffin-embedded sections, 4-µm, were stained with hematoxylin and eosin. All samples were examined by light microscopy at different original magnification (×25, ×100, ×200).

RESULTS

The whole procedure was performed with a beating heart without the use of extracorporeal circulation support.

All hemodynamic data were processed by SPSS 10 (IBM Co, U.S.A.) and are expressed as mean ±SD. Results were evaluated using a paired Student's t-test and differences of all recorded data before and after the Fontan circulation were considered to be significant at the level of $p<0.05$.

After the establishment of total cavopulmonary connection, there was no need for inotropic or volume infusion support. All recorded hemodynamic data before and after Fontan circulation are presented in Table 1 [5].

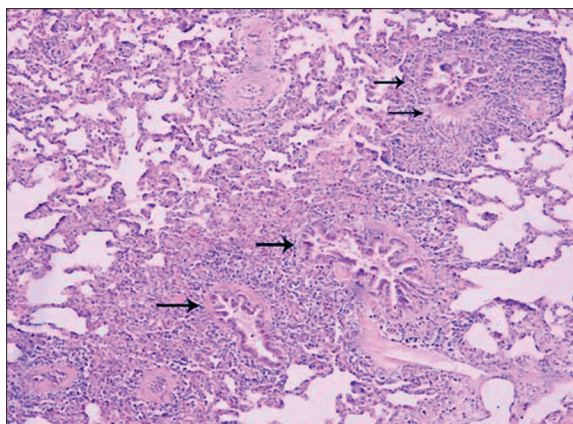


Figure 1. Lung biopsy specimen after the establishment of total cavopulmonary connection depicting mononuclear cell infiltration adjacent to pulmonary alveoli and bronchioles (arrows) (H&E, original magnification ×100).

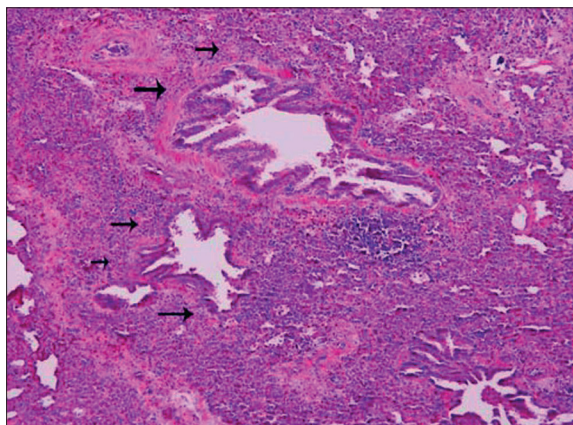


Figure 2. Lung biopsy specimen after the establishment of total cavopulmonary connection depicting mononuclear cell infiltration (arrows) (H&E, original magnification ×200).

The recorded hemodynamic parameters confirmed the Fontan paradox; thus, the existence of venous hypertension coexists with hypotension in the pulmonary artery [4].

All samples (n=8) at baseline were consistent with normal lung parenchyma. Two hours after TCPC completion, all samples (n=8) revealed mild-to-moderate mononuclear cell infiltration adjacent to pulmonary alveoli and bronchioles, findings compatible with bronchiolitis (Figures 1, 2). All samples were received from the middle lung field.

Mild congestion was observed in the vasculature of alveoli in 1 sample and another sample had mild alveolar hemorrhage.

DISCUSSION

Various types of cavopulmonary connections are unsuccessful, even when the selection criteria have been fulfilled based on preoperative hemodynamic status.

Hemodynamic status does not fully represent the condition of the pulmonary vasculature in patients with single ventricle physiology. Medial thickness of small pulmonary arteries,

which is an index correlated to mean pulmonary artery pressure, seems to be a strong predictive factor regarding the good or bad outcome after a cavopulmonary connection [6]. Furthermore, extension of muscle in peripheral pulmonary arteries was always present in cases of failure of the Fontan procedure, suggesting that histomorphometric study of distal intra-acinar arteries is useful in surgical decision-making before the Fontan procedure [7]. Selection of a single stage or 2-stage Fontan operation based on frozen section quick diagnosis intraoperatively has been discussed, and a 2-stage Fontan procedure is indicated when medial hypertrophy is recognized [6].

However, experimental studies showed that long-term non-pulsatile flow perfusion of the Fontan circulation can decrease the synthesis of endothelial nitric oxide synthase (eNOS) in vascular endothelial cells of the pulmonary vessels, increase the apoptosis of smooth muscle cells of the arteriole wall, and lead to arterial venous conversion and pulmonary vessel remodelling [8]. These data support the process of an endothelial dysfunction, which may be involved in distention and vascular structure remodeling due to the increase in pulmonary vascular resistance. Yin et al observed that the pulmonary arteriolar wall became thinner, endothelin-1 expression was weaker, and eNOS was stronger in the lung with chronic nonpulsatile flow, confirming endothelial dysfunction [9]. In addition, Adachi et al described their immunohistological analysis on main pulmonary artery tissue from a patient who had been palliated with a classic Fontan operation 23 years before. Data showed attenuation of muscular component, and disarray and fragmentation of elastic fibers in the pulmonary artery tissue [10]. These data suggest a chronic process and incorporate the contingency of an inflammatory process. Questions arise about when this process begins after the completion of the Fontan procedure and whether there are any acute changes that trigger this process.

Starnes et al demonstrated increased staining for vascular endothelial growth factor (VEGF) and its receptor in patients after cavopulmonary anastomosis, suggesting that VEGF may be a mediator of angiogenesis in the lungs of patients after cavopulmonary anastomosis [11]. VEGF is increased in smooth muscle cells and mononuclear inflammatory cells, and seems to play a major role in development of experimental obliterative bronchiolitis [12,13].

In this experimental model, 2 hours after TCPC completion, the lung tissue showed mild-to-moderate mononuclear inflammatory cell infiltration adjacent to bronchioles in all samples. Our study has a few limitations that should be considered. All animals used in the present study were healthy and free from any congenital heart diseases such as tricuspid atresia or univentricular heart condition. All experiments were acute, with a short survival period, and

were performed on animals with an open thoracic cavity. Long-term survival experiments are needed to understand the physiology of the Fontan circulation and the plethora of hemodynamic and histological changes arising from it.

CONCLUSIONS

In this acute experimental model, our data show an immediate inflammatory response. The question that still remains is if this mononuclear infiltration is a part of the cascade that leads to endothelial dysfunction of the pulmonary vasculature after the Fontan procedure. If so, does that procedure start at an early stage? Two hours after the establishment of Fontan circulation without the use of CPB, pathologic examination of the lungs revealed bronchiolitis. Further research is needed to clarify these findings and their potential implications to our understanding of the Fontan circulation, both immediate and long-term.

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