Published in final edited form as:

Pediatr Cardiol. 2008 July ; 29(4): 706–712. doi:10.1007/s00246-007-9152-5.

Morphological Studies of Pulmonary Arteriovenous Shunting in a Lamb Model of Superior Cavopulmonary Anastomosis

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Abstract

We sought to identify and characterize the abnormal vascular structures responsible for pulmonary arteriovenous shunting following the Glenn cavopulmonary shunt. Superior cavopulmonary shunt is commonly performed as part of the staged pathway to total cavopulmonary shunt to treat univentricular forms of congenital heart disease, however, clinically significant pulmonary arteriovenous malformations develop in some patients after the procedure. The causes of pulmonary arteriovenous malformations and other pulmonary vascular changes that occur after cavopulmonary shunt are not known. Using a juvenile lamb model of superior cavopulmonary anastomosis that reliably produces pulmonary arteriovenous malformations, we performed echocardiography and morphological analyses to determine the anatomic site of shunting and to identify the vascular structures involved. Pulmonary arteriovenous shunting was identified by contrast echocardiography in all surviving animals ($n = 40$) following superior cavopulmonary anastomosis. Pulmonary vascular corrosion casts revealed abnormal tortuous vessels joining pulmonary arteries and veins in cavopulmonary shunt animals but not control animals. In conclusion, unusual channels that bridged pulmonary arteries and veins were identified. These may represent the vascular structures responsible

for arteriovenous shunting following the classic Glenn cavopulmonary shunt. Detailed analysis of these structures may elucidate factors responsible for their development.

Keywords

Heart defects; Congenital pulmonary circulation; Arteriovenous malformation

The classic Glenn surgical superior cavopulmonary anastomosis (CPA) is commonly performed as part of the staged Fontan pathway to treat univentricular forms of congenital heart disease [6,7]. Up to 60% of these patients develop pulmonary arteriovenous shunting (PAVS), which may lead to progressive and clinically significant hypoxia [1–3,11]. Although PAVS is believed to occur via pulmonary arteriovenous malformations (PAVMs) that develop following creation of a Glenn shunt, the vascular structures responsible for PAVS have not been clearly characterized. It is commonly believed that PAVMs develop because of the absence of a putative hepatic venous ‘factor’ in the pulmonary circulation of these patients [9,15,17]. Identification and morphologic analysis of the structural components responsible for PAVMs may provide a better understanding of abnormal vascular developmental processes in these patients and facilitate isolation of putative circulating etiological substances. Using a juvenile lamb model that reliably produces pulmonary arteriovenous malformations, we performed echocardiographic, angiographic, and morphologic analyses to identify and characterize the vascular structures involved in PAVS following the Glenn shunt.

Materials and Methods

These studies were performed in compliance with animal welfare regulations of the University of California—San Francisco and USDA guidelines. Furthermore, these studies conformed to the “Position of the American Heart Association on Research Animal Use,” adopted by the American Heart Association on November 11, 1984, and were approved by the University of California—San Francisco Animal Research Institutional Review Committee (IACUC).

Animal model

Forty Western lambs were studied. The lambs (4–6 weeks of age) were sedated with ketamine HCl (10 mg/kg IM) and anesthetized with continuous 1%–2% isoflurane using mechanical ventilation. Animals underwent midline sternotomy and extensive mobilization of the right pulmonary artery and superior vena cava, and were heparinized (300 IU/kg). Passive superior cavoatrial bypass was accomplished using two venous cannulae prior to creation of the Glenn shunt in CPA animals (n = 23). The superior vena cava was then transected and the cardiac end was oversewn with 6-0 monofilament suture. The right pulmonary artery was transected flush with the main pulmonary artery after applying vascular clamps and the proximal stump of the right pulmonary artery was over-sewn with 6-0 monofilament suture. The superior intercostal vein (equivalent to the azygos vein in humans) was ligated and divided. The distal right pulmonary artery was then anastomosed to the superior vena cava in end-to-end fashion using 7-0 monofilament suture (Fig. 1). Following completion of the anastomosis, bypass cannulae were removed and passive blood flow was established from the superior vena cava to the right pulmonary artery (Glenn shunt). Blood from the inferior vena cava continued to drain into the right atrium and was directed to the left lung in normal antegrade fashion. Following closure of the surgical site animals were allowed to recover from anesthesia and then extubated. Age-matched control animals (n = 17) underwent sternotomy, mobilization of the right pulmonary artery and superior vena cava, and temporary (30-min) occlusion of the right pulmonary artery prior to chest closure and extubation. Each animal underwent a single initial surgical procedure and a single subsequent terminal study to detect the presence of PAVMs. The interval between surgery and terminal study ranged from 1 to 27 weeks. At the

conclusion of each terminal study, animals were euthanized with a lethal dose of pentobarbital and the heart was examined by gross dissection.

Contrast echocardiography

At the time of initial CPA or control surgical procedure and terminal study, animals (n = 40) were anesthetized and heparinized as described above and two-dimensional cross-sectional epicardial contrast echocardiography was performed using a diagnostic ultrasound system (Aloka Co. Ltd., Tokyo) and recorded on videotape. A vigorously agitated mixture of saline and blood was used as echogenic contrast solution [20]. With all four cardiac chambers visualized, 2–5 ml of contrast solution was rapidly injected into the pulmonary circulation via an indwelling catheter in the right internal jugular vein (superior vena cava injection). The presence of bubbles in the left atrium within three cardiac cycles of injection indicated PAVS (Figs. 2c and d). Although the presence of PAVS was detected by contrast echocardiography, quantitative estimates of PAVS were not performed. Contrast agent was also injected into the inferior vena cava of CPA animals to test for intrapulmonary shunting in the left lung.

Bronchial arterial mapping

After euthanasia, the lungs, heart, and thoracic aorta were removed en bloc from 10 CPA and 10 control animals. India ink (diluted 1:10 in normal saline) was injected directly into the origin of the bronchial artery and allowed to fill the bronchial circulation.

Vascular corrosion casting

Corrosion casting methods previously reported by Schraufnagel et al. [14] were modified [13] to decrease the overall complexity of the cast to facilitate identification of PAVMs. Polystyrene microspheres (10- μ m diameter; 2×10^6 spheres/kg body weight; Bangs Laboratories, Inc., Fisher, IN) were suspended in 20 ml saline and gently infused directly into the right pulmonary artery over 5 min in 11 CPA and 9 control animals. After euthanasia, the heart and lungs were removed en bloc and the left atrial appendage was amputated to allow drainage of casting material. Resin (Mercox/catalyst, 10/0.3; Ladd Research Industries, Burlington, VT) was prepared according to the manufacturer's instructions and infused through an indwelling right pulmonary arterial catheter at a constant pressure (80 mm Hg) until it was seen returning to the left atrium. After polymerization was complete (approximately 4 h) the specimen was transferred to a bath containing 30% NaOH at 40°C. After 72 h the specimen was washed in a gentle-flow water bath at 100°C to remove digested tissue. The cleaned cast was then air-dried and examined under a dissecting microscope (Stemi 200-C; Carl Zeiss, Inc., Germany).

Statistics

Groups were compared using unpaired *t*-tests for continuous variables or Fisher exact tests for dichotomous variables.

Results

All CPA animals exhibited a positive contrast echo examination consistent with PAVS when examined 5 or more weeks post CPA. PAVS developed in the right lung (Glenn shunt) but not the left lung (antegrade inferior vena cava blood) of CPA animals. PAVS was not detected by contrast echocardiography in any animal at the time of initial (presurgical) CPA or control surgical procedure. One CPA animal developed PAVS by 5 weeks and all CPA animals studied after 6 weeks showed evidence of PAVS (Fig. 3). No control animals developed PAVS when examined up to 27 weeks after surgery. Postmortem heart dissection did not reveal atrial or ventricular septal defects in any of the animals.

Vascular morphology

The surfaces of both lungs of control animals were grossly normal when examined at the time of each terminal study (2–20 weeks). Prominent tortuous vessels were observed covering the surface of the right lung (Glenn lung) but not the left lung (control lung) in all CPA animals examined at the time of terminal study (2–27 weeks) (Fig. 4). However, direct dye injection studies demonstrated that these abnormal surface vessels originated from bronchial arteries and were, therefore, derived from the systemic rather than pulmonary circulation. Although this unique vascular pattern was observed in all CPA animals, there was no clearly observable increase in surface vessel abnormalities over time.

Stereomicroscopic analysis of pulmonary vascular corrosion casts of the right (Glenn) lung identified multiple unique direct pulmonary arteriovenous communications (Figs. 5 and 6) in the central areas of the right lung in animals 12 weeks or more after CPA. These abnormal tortuous structures had a minimum lumenal diameter of 15 to 20 μm and joined individual pulmonary arteries to several pulmonary veins (Fig. 6). Direct arteriovenous communications were not observed in the left lung of CPA animals or either lung of control animals examined at any time point.

Discussion

This animal model of Glenn superior cavopulmonary shunt reliably produces echocardiographic evidence of PAVS as early as 5 weeks after surgery. By creating a unilateral CPA in this model we were able to compare physiologic and morphological changes between right (Glenn) and left (control) lungs in each animal. Using calibrated microspheres to embolize normal pulmonary capillary beds, we created corrosion casts of remaining pulmonary vascular structures to identify numerous central pulmonary arteriovenous malformations. These abnormal vascular channels are at least 15 μm in diameter and completely bypass alveolar capillary beds (Fig. 7). Prominently tortuous subpleural vessels were observed in the right (Glenn) lung of all CPA animals, whereas the surfaces of the left lung of CPA animals and both lungs of control animals were normal. Surface vascular changes appeared to develop before echocardiographically detectable PAVS. Corrosion casts did not reveal vascular abnormalities in peripheral lung, suggesting that these abnormal surface vessels are not involved PAVS. In sheep, a typically single bronchial artery originates from the thoracic aorta and bifurcates to supply the left and right bronchial circulation. Bronchial arterial mapping demonstrated that the unique subpleural vessels covering the right (Glenn) lung were directly supplied by the bronchial artery. Contrast ink also filled surface bronchial arteries in the left (control) lung of CPA animals and both lungs of control animals, however, the observed vessels were small and significantly less prominent than in the CPA lung. It is unlikely that the dilated bronchial artery complex observed in CPA animals contributes to PAVS. Similar changes in subpleural bronchial vessels have been reported after experimental pulmonary artery banding [8], which is not associated with PAVS. Such vascular remodeling presumably occurs as a compensatory mechanism to increase bronchopulmonary blood flow. It is likely that similar compensatory vascular changes occur after CPA. Recruiting additional systemic blood flow may provide a source of putative hepatic factor [3] necessary for normal pulmonary vascular development. It is intriguing that CPA induces changes in both the bronchial and the pulmonary arterial circulations while PA banding does not.

Abnormal, thin-walled parenchymal vessels have been reported in the lungs of patients [2, 17] and experimental animals [19] following CPA. However, it is unclear whether these vascular structures are of pulmonary or systemic origin or whether they mediate PAVS. Similar dilated, thin-walled vessels arising after pulmonary artery banding appear to represent collateral bronchial arteries [8]. Clinical studies using Tc-99m microaggregated albumin total-body imaging have demonstrated that abnormal vascular channels measuring < 20–60 μm are

responsible for non-CPA intrapulmonary shunting. Direct pulmonary arteriovenous communications identified by corrosion casting in this study generally appear to have a larger luminal diameter than the CPA-induced abnormal vascular structures described previously [2,17,18].

All CPA animals in this study examined after 6 weeks developed PAVS. Early clinical reports suggested that only 25% of patients develop PAVS following Glenn CPA [3]. Subsequent reports indicate that the prevalence of PAVS in these patients may be higher [1,10]. Using radionuclide-labeled microspheres, Vettukattil et al. found that all patients developed some degree of PAVS following CPA [21]. It has been suggested that children undergoing Glenn CPA at a younger age are more likely to develop fulminant PAVS [1]. The relatively young age of our study animals at time of CPA supports this assertion. Clinical observations and our data suggest that PAVS progresses over time in susceptible individuals. It is unknown why earlier CPA increases the likelihood of early, fulminant PAVS. We have previously shown that PAVS is normally present in late gestation fetal and early neonatal lambs but then disappears during the later neonatal period [12]. The presence of direct arteriovenous communications in the normal fetal pulmonary circulation suggests that these channels may play a role in normal lung development. Small, poorly perfused “supernumerary” arteries have been identified in the normal pulmonary circulation and are believed to function as recruitable sources of pulmonary blood flow when oxygenation demands are high [5,16]. Recently, exercise-induced PAVS has been described in otherwise normal, healthy humans [4]. Taken together, these findings support our belief that PAVS associated with CPA or other processes affecting the developing pulmonary circulation may reflect regression of pulmonary vascular remodeling to an earlier (fetal) developmental state.

The results of this study may facilitate future studies designed to isolate the abnormal vascular structures responsible for PAVS and elucidate the unique cellular and molecular changes associated with their development. Characterization of these changes may assist us in determining the nature of the circulating splanchnic/liver-derived mediators of angiogenesis contributing to the development of PAVMs.

Acknowledgments

This work was supported in part by National Individual Research Service Award 1 F32 HL10108-01 and grants from the UCSF School of Medicine (REAC) and Academic Senate.

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Fig. 1.

Experimental Glenn cavopulmonary shunt. Anatomy of the heart and great vessels (**A**) showing the right atrium (RA), left atrium (LA), ascending aorta (Ao), main pulmonary artery (PA), left pulmonary artery (LPA), right pulmonary artery (RPA), and superior vena cava (SVC). Following completion of the superior cavopulmonary shunt (**B**), blood from the SVC drains directly into RPA, whereas blood from the inferior vena cava continues to flow into the RA and is directed to the LPA in normal antegrade fashion

Fig. 2.

Pulmonary arteriovenous shunting after Glenn shunt. Contrast echocardiogram performed 12 weeks after Glenn superior cavopulmonary shunt to the right lung demonstrating pulmonary arteriovenous shunting. Sequential four-chamber images following injection of contrast into the inferior vena cava (control; **A, B**) demonstrate contrast opacification of the right atrium (RA) and right ventricle (RV) but not the left atrium (LA) or left ventricle (LV). Sequential images after contrast injection into the superior vena cava (Glenn; **C, D**) demonstrate progressive opacification of the LA and LV without opacification of the RA and RV, indicating the presence of arteriovenous shunting involving the right lung

Fig. 3.

Time course of development of pulmonary arteriovenous shunting (PAVS). Contrast echocardiography evidence of PAVS was present in all animals that underwent Glenn superior cavopulmonary shunt (CPA; filled circles) by 7 weeks, whereas no animals in the control group (open circles) developed PAVS

Fig. 4. Glenn shunt-induced vascular changes involving the lung surface. Prominently dilated and tortuous subpleural vessels cover the surface of the right lung (**A**) but not the left lung (**B**) in an animal examined 12 weeks after unidirectional Glenn superior cavopulmonary shunt

Fig. 5.
Resin casts of pulmonary arteriovenous malformations. Stereomicroscopic images of corrosion casts of the right lungs of animals 12 weeks after Glenn superior cavopulmonary shunt demonstrating direct vascular communications between pulmonary arteries and veins (*) at low (**A, C**) and high (**B, D**) magnification

Fig. 6.

Stereomicroscopic image of pulmonary arteriovenous malformations. Image of a vascular corrosion cast prepared from the right lung 12 weeks after Glenn superior cavopulmonary shunt. Casting of capillaries has been prevented by selective microsphere embolization prior to resin injection (see Materials and Methods). Unique vascular structures measuring $<15\ \mu\text{m}$ directly join a pulmonary artery (A) with several pulmonary veins (V). Scale bar = $200\ \mu\text{m}$

Fig. 7. Angiogram showing pulmonary arteriovenous malformation. Three-dimensional reconstructed image of a selective computed tomography angiogram in an animal after Glenn superior cavopulmonary shunt demonstrating a pulmonary arteriovenous malformation (red arrow) communicating with a pulmonary artery (PA) and a pulmonary vein (PV). An intraluminal marker (*) was used to localize the abnormal vascular structure