Increased expression of vascular endothelial growth factor messenger RNA in lungs of rats after cavopulmonary anastomosis

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Pulmonary arteriovenous malformations (PAVMs) are abnormal blood vessels that develop in a significant number of patients undergoing cavopulmonary anastomosis (CPA). PAVMs result in hypoxemia and decreased exercise tolerance from intrapulmonary right-to-left shunting. Histologically, PAVMs appear as numerous dilated and thin-walled vessels that extend far into the periphery of the pulmonary parenchyma.^{1,2} Vascular endothelial growth factor (VEGF) is an important mediator of both normal and abnormal vascular proliferation. We have demonstrated increased protein expression of VEGF and its receptor in the lungs of children after CPA.³ This study describes our attempts to measure VEGF messenger RNA (mRNA) expression in a rat model of PAVMs after CPA.

Methods and Results

Three male Sprague-Dawley rats (200-300 g) underwent a right superior cavopulmonary anastomosis (classic Glenn shunt) allowing the left (unshunted) lung of each animal to serve as a control as described previously.⁴ Rats were sacrificed at 2, 8, and 12 months after surgery, and VEGF mRNA was measured in the right (shunted) and the left (unshunted) lungs by relative quantitative reverse transcriptase polymerase chain reaction. This study received institutional approval (Animal Care and Use Committee, Children's Hospital and Regional Medical Center, Seattle, Wash). All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" (<http://www.nap.edu/catalog/5140.html>).

Reverse transcriptase polymerase chain reaction. A complementary DNA template was synthesized from total RNA collected from both the shunted and control lungs of each animal. After determination of the linear range of amplification for target specific primers to 18s ribosomal and VEGF mRNA, multiplex polymerase chain reaction products were electrophoresed on 6% denaturing polyacrylamide gel and visualized by rapid silver staining. The stained gels were scanned and densitometry was performed with intensity (pixel density) as a measure of optical density.

Both control and shunted lungs expressed mRNA for two isoforms, VEGF165 and VEGF189. Both isoforms were quantitated as a single value for total VEGF expression. The shunted lungs in each animal demonstrated a progressive time-dependent increase in VEGF mRNA when compared with the control lungs (Figure 1).

Discussion

CPA, a standard intermediate step preceding the Fontan procedure in the palliation of single-ventricle physiology, results in lack of hepatic venous effluent directly perfusing the pulmonary arteries. It has been hypothesized that pulmonary VEGF expression is upregulated after CPA because of the absence of hepatic-derived inhibitors of angiogenesis in the pulmonary circulation.⁵ We previously demonstrated increased pulmonary expression of VEGF and VEGF receptor, as determined by immunohistochemistry, in the lungs of children

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Figure 1. VEGF mRNA expression for CPA (right) versus control (left) lung. Each bar represents percentage increase of mRNA expression in CPA lung relative to control lung.

after CPA.³ This study provides the first corroboration of this finding of increased VEGF protein expression by demonstrating increased VEGF mRNA expression in a rat model of PAVMs after CPA. In addition, we previously demonstrated that the number of

pulmonary blood vessels demonstrate a time-dependent increase after CPA.² This study also suggests a possible role for VEGF in this phenomenon by demonstrating a similar time-dependent increase in VEGF mRNA after CPA. Although the exact role of VEGF has not been established by this preliminary study, these findings further support its importance in the development of PAVMs after CPA.

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A simple surgical technique for interventional transcatheter completion of the total cavopulmonary connection

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Since the initial description of interventional completion of total cavopulmonary connection (TCPC),¹ few surgical maneuvers to prepare the heart for subsequent transcatheter univentricular repair have been reported.^{2,3} Herein, we describe a simple technique that permits surgical preparation on the beating heart.

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Technique

The present technique evolved during experimentation in a porcine model. After approval by the Institutional Animal Care and Use Committee of the Research Institute in the Hospital for Sick Children, 4 Yorkshire pigs weighing 15, 20, 25, and 30 kg underwent a 2-staged procedure as described below. Superior vena cava (SVC) and inferior vena cava (IVC) were encircled with silk tapes in the first 3 animals to provide support for the covered stent (NuMed Inc, Hopkinton, NY). These support tapes were replaced with external stents (CP stent 8219, NuMed Inc) in the last animal.

Stage 1: Surgical preparation. Standard premedication, anesthesia induction, endotracheal intubation, muscle relaxation, and ventilation were achieved. Anesthesia was maintained with 1.5% to 2% isoflurane. The heart was exposed through a midline sternotomy. Heparin (300 IU/kg) was administered intravenously, and bicaval cannulation for cardiopulmonary bypass (CPB) was achieved. After institution of CPB, the caval veins were occluded by tourniquets, the right atrium (RA) was crossclamped below the sinus node, and the SVC was divided (Figure 1, A and B). A bidirectional cavopulmonary shunt was performed with the heart