Pulmonary Arteriovenous Malformations After the Bidirectional Glenn and the Role of VEGF

N. Sreeram.

Heart Center, University of Cologne, Germany
Background

- PAVMs first recognised during follow-up after classical Glenn shunt, in ipsilateral lung
- Early BDG part of standard surgical sequence in staged Fontan palliation
- Is the durability of the BDG Glenn limited by development of progressive cyanosis due to PAVMs?
- What causes the development of PAVMs in some children?
Physiology of PAVMs

- Intrapulmonary right to left shunt proximal to the gas exchange units
- Can become evident as early as a few weeks after BDG
- Lesions tend to progress with time

**METHODS OF DETECTION**

- Angiography
- Radionuclide study
- Contrast echocardiography: more sensitive, detect right to left shunts even in the absence of clinically evident PAVMs
Angiogenesis

versus

Recruitment of preexisting vessels
Angiogenesis

• Increased microvessel density in the lungs after BDG, even in the absence of clinical evidence of PAVMs

• Continuing angiogenic stimulus, early after BDG

• Development of symptomatic PAVMs associated with histologic transitions
Microvessel density

Starnes SL, 2000
Microvessel Density: rat model

Right Lung/Left Lung Ratio of Microvessel Density

R sqr=0.79, P=0.003

Months after Glenn

Starnes SL, 2002
5 month old,
increasing cyanosis 3 weeks after BDG
Native LPA
Recruitment and dilation of pre-existing vascular channels

- Rapid onset of PAVMs (72 hours) after Kawashima procedure
- Not true angiogenesis, but recruitment of preexisting vessels
14 year old, post Fontan, gradually worsening O2 saturations
Selective angiography
Many hours & devices later
25 year old; status post-Fontan for TA
Histology

• **Sparse data:**
  – Weber Osler Rendu complex *vs* BDG *vs* hepatic cirrhosis

• **2 children:**

• Greatly increased number of new vessels extending into the periphery of the lung

• Connections between pulmonary arterioles and vascular lakes proximal to the capillary level
Lung Biopsy Micrographs

Duncan BW, 1999
Freedom from PAVMs

Srivastava D, 1995
Possible Etiologic Mechanisms

• Develop whenever hepatic effluent does not perfuse pulmonary arteries directly

• Occur bilaterally after BDG and with isolated drainage of hepatic veins to common atrium (Kawashima procedure)

• Surgical redirection of hepatic venous flow to the PAs causes PAVMs to regress …
Effect of Incorporation of Hepatic Veins after Kawashima Operation

McElhinney, 2005
...but not always

Diffuse bilateral AVMs

Unilateral progression despite Incorporation of hepatic blood

Vettukattil J, 2002
Specific Angiogenic Factors: VEGF

- VEGF a potent stimulator of endothelial proliferation & angiogenesis
- Increased VEGF in SVC and systemic artery after BCPA
- Increased expression of VEGF & its receptor (flk-1/KDR) in lung biopsy specimens after BCPA
- Rat model of classical Glenn: progressive time-dependent increase in VEGF mRNA compared with control lung
VEGF mRNA expression for CPA versus control lung

Rat model classical Glenn Shunt

% Increase Over Control

250
200
150
100
50
0

2 months
8 months
12 months

Time after CPA

Mumtaz MA, 2005
Arterial oxygen saturation versus serum VEGF

VEGF = 1318 - 11.4 x SpO^2
r = -.62, p < .0001

Suda K, 2004
Clinical correlates: Hypothesis

- Systemic VEGF levels would fluctuate during the course of various surgical alterations in patients with univentricular hearts
- Each patient as his/her own control
Methods

• VEGF (1) in the OR, prior to BDG (no additional sources of pulmonary blood flow) (age 13.4 ± 6.1 months)

• VEGF (2) at cardiac catheterization, prior to completion of Fontan (non-fenestrated lateral tunnel/ extracardiac conduit TCPC) (age 3.4 ± 1.2 years)

• VEGF (3) at >1 month after Fontan completion
Results

- VEGF (1) vs VEGF (2) vs VEGF3
  - (24.4 ± 28.3 pg/ml) vs (112.4 ± 68.5) vs (48.8 ± 27.1)

- O₂ saturation levels
  - 82% (74-85%) vs 79% (65-87%) vs 97% (91-100%)

- Inverse correlation of O₂ saturation with VEGF levels poorest just prior to TCPC
Dodge-Khatami A, 2003
Group A = healthy controls; Group B = cyanotic patients; Group C = post biventricular repair; Group D = post Fontan completion

Suda K, 2004
Problems

• No significant relationship in VEGF levels between patients with abnormal vessels (arteriovenous or venovenous) and those without

• Most patients with hypoxemia secondary to structural heart disease do not develop PAVMs

• VEGF levels do not always normalise after Fontan completion

• Additional influencing factors: venous pressure (stasis model) and age
VEGF versus mean SVC pressure

VEGF = 55 + 29 \times \text{SVC}

r = .45, p < .01

Suda K, 2004
Other mediators of Angiogenesis: Endothelial Oxidative stress

• PA banding versus CPA:

• Both result in upregulation of VEGF1 and its receptor

• Only CPA associated with ↑ endothelial oxidative stress in affected vascular bed, with ↑ stress-associated factors HIF-1 α, hemoxygenase 1 (HO1) and glucose transporter 1 (GLUT1)

Malhotra SP, 2002
Other mediators of Angiogenesis: *Impaired vasoconstriction vs Direct Inhibition*

- ↓ ACE mRNA & protein expression in shunted lung after classical Glenn shunt
  - Within 1 week

- ↓ Angiotensin II in the right pulmonary vein
  - Within 1 week

- ↑ Angiotensin II receptor mRNA (type 1 & type 2) and protein expression
  - Within 1 week

- AV shunting uniformly detectable by bubble contrast echocardiography at 6 weeks after Glenn shunt

_Malhotra SP, 2001/2002_
Role of the liver

• Actively involved in the maintenance of pulmonary vascular integrity

• Formation of inhibitor(s) of angiogenesis
  – Hepatocyte conditioned media strongly inhibitory for endothelial cell proliferation

• Degradation of an angiogenic substance which would not be removed from the pulmonary circulation after BDG

• Hepatopulmonary syndrome and role of orthotopic liver transplantation
The Liver, Portal Hypertension & Disordered Angiogenesis

- PHT in 2% of patients with cirrhosis & portal hypertension (portopulmonary syndrome)
- Severe PHT may be reversible by liver transplantation
- Liver disease related PHT and PAVMs seem to be mutually exclusive
Candidate Factors

- Plasminogen: precursor of angiostatin
- Collagen XVIII: precursor of endostatin
- Both inhibit endothelial cell proliferation, angiogenesis and tumor growth
Candidate Factors

• ↑ plasma Endothelin -1 and ↑ endothelial NOS
  – in vivo: rat cirrhosis model (CBD ligation)
  – in vitro: bovine PA endothelial cells

• Pulmonary microcirculatory vasodilation associated with gas exchange abnormalities

Zhang M, 1999
Pulmonary angiogenesis in hepatobiliary syndrome

- *Rat cirrhosis model:*
  - VEGF-A
  - VEGFR-2
  - Angiogenesis, with increased microvessel density

*Zhang J, 2009*
Endothelial Notch4 gene activation and lung AVMs

- Essential for vascular morphogenesis and arteriovenous specification

- Embryonic expression of Notch4 gene largely restricted to endothelial cells

- Function in adult mammalian vasculature not established due to embryonic lethality associated with gain/loss of function mutations

*Uyttendaele H, 1996*
Embryonic AVMs

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<th>Control</th>
<th>Notch4*</th>
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<td>Vessels &gt; 50 μm</td>
<td>24.8 ± 11.5</td>
<td>48.8 ± 14.5*</td>
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<td>Airspace count</td>
<td>129.0 ± 16.5</td>
<td>94.7 ± 22.0**</td>
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t-test: *p=0.009 vs. control, **p=0.010 vs. control
Notch4* can be (re) expressed in young/ adult mammals

- Endothelial expression of constitutionally active Notch4* elicits reversible AVMs in adult mice

- Cessation of Notch4* expression reverses these pathophysiological effects
AV shunting in Notch4* mutant adult mice

Miniati D, 2010
Fluorescent agarose angiography

Controls vs. Notch 4* mutants

Miniati D, 2010
A final common pathway?

- Effects of VEGF & HIF-1 α are mediated by Notch signalling

- A central mechanistic role?