Abstract

Cyanosis and the cavopulmonary anastomosis (CPA) are associated with pulmonary arterio-venous malformations (PAVMs) in single ventricle physiology. Vascular endothelial growth factor (VEGF) may be a marker of abnormal angiogenesis in this setting. Plasma VEGF levels were measured in 14 patients undergoing the surgical pathway leading to total cavopulmonary connection (TCPC). Venous blood samples were taken before and then months after CPA (n = 6), and immediately before TCPC and 1 month thereafter (n = 9). Corresponding arterial saturations were correlated with VEGF levels at each time frame. In six patients, pre-CPA plasma VEGF levels rose from a mean of 24.4–112.4 pg/ml (p < 0.03) just prior to completion of TCPC. In nine patients, VEGF levels diminished from 115.7 to 48.9 pg/ml (p < 0.05) after TCPC. VEGF levels were disproportionately elevated to arterial saturations most notably after CPA (r² = 0.002), suggesting an additional angiogenic stimulus besides cyanosis. Plasma VEGF levels fluctuate during the single ventricle surgical pathway, with maximal levels after CPA, and regression after completion of TCPC. High VEGF levels are disproportionate to hypoxia after CPA, potentially incriminating the absence of hepatic flow to the lungs as an abnormal angiogenic stimulus. Measuring VEGF in venous blood may serve as a biochemical marker of angiogenesis after CPA.

Keywords: Vascular endothelial growth factor; Single ventricle; Angiogenesis

1. Introduction

Abnormal angiogenesis complicates the clinical course of children with cyanotic heart disease. These blood vessels may be systemic venous collaterals, multiple aortopulmonary collateral arteries, or pulmonary arterio-venous malformations (PAVM). The latter are seen frequently after the various surgical stages of the single ventricle pathway, and singularly so after the classic and bidirectional Glenn operation without antegrade ventricular–pulmonary artery (PA) flow, or the Kawashima operation [1], procedures that exclude hepatic blood flow from the pulmonary vascular bed. Curiously, unilateral PAVMs are ipsilateral to a classic Glenn [1], while they are bilateral after bidirectional Glenn and Kawashima procedures [2]. Conversely, these vascular malformations tend to diminish or disappear after conversion of a superior cavopulmonary anastomosis (CPA) to a total cavopulmonary connection (TCPC) [2,3], whereby both chronic hypoxia is corrected, and hepatic blood flow to the lungs is restored.

Vascular endothelial growth factor (VEGF) acts as a specific potent mitogen on vascular endothelial cells, is implicated in wound healing, enhanced vascular permeability, tumor vessel neoproliferation, and capillary vasodilation through endothelial release of nitric oxide. Hypoxia [4,5], anemia, and ischemia are strong upregulators of angiogenesis that occurs, amongst other phenomena, through increased VEGF production.

Measuring serial plasma levels of VEGF in patients going down the single ventricle pathway was thought to give valuable insight as to the ‘angiogenic or vasculogenic potential’ of any given patient. It was hypothesized that...
systemic levels of VEGF would fluctuate during the course of the various surgical alterations in univentricular physiology, with a patient as his/her own control.

2. Materials and methods

Approval to undertake the study was obtained from the Hospital Research Ethics Committee, and informed consent was obtained from the parents of all patients. Peripheral venous blood (6 ml) was collected from 14 patients undergoing surgical palliation via the single ventricle pathway. Six infants and children had samples taken in the operative room just prior to a cavopulmonary shunt (VEGF1), followed by samples at cardiac catheterization or at clinical follow-up, immediately before completion of TCPC (VEGF2). In nine other children, a first sample was taken on the operative table prior to completion of TCPC (VEGF2), and a second sample at least 1 month after completion of Fontan (VEGF3). For all VEGF2 values, there were no additional surgical sources to pulmonary blood flow other than the CPA. These VEGF2 samples hypothetically represented the maximum VEGF levels for each child, being taken after the longest interval with cavopulmonary shunt physiology, with the longest ongoing state of chronic hypoxia in combination with an absence of anterograde pulmonary flow from the hepatic veins. Indeed, it is our policy to disconnect the pulmonary arteries from the pulmonic outflow, and therefore no patients had native forward ventricular–PA flow. At the time of the final plasma sample at least 1 month after separation of the circulations, eight patients had a non-fenestrated completion of TCPC (lateral tunnel; n = 3 and extracardiac conduit; n = 5). No patients had angiographically detectable PAVMs at any interval of their single ventricle physiology.

Venous blood samples were allowed to clot for 30 min, centrifuged at 3000g for 30 min at 4 °C. Plasma was removed and stored at −70 °C. VEGF levels were measured with a Quantikine VEGF kit (R&D Systems, Minneapolis, MN, USA). The expected limit of detection of VEGF was 9 pg/ml.

Aortic saturations measured at cardiac catheterization or upon arterial blood gases were correlated with plasma VEGF levels at each corresponding time frame.

3. Results

Median age at the time of CPA (VEGF1 samples taken just prior) was 13.4 ± 6.1 months. Median age at the time of completion of TCPC, before which VEGF2 samples were taken, was 3.4 ± 1.2 years, and the third sample (VEGF3) was 1 month after this age. Mean plasma VEGF levels were 24.4 ± 28.3 pg/ml prior to CPA in the six infants and children studied, and rose to 112.4 ± 68.5 pg/ml during the CPA physiology interval, measured just prior to completion of TCPC (P < 0.03). In nine children, mean VEGF levels were 115.7 ± 116.9 pg/ml immediately before TCPC, during the maximal interval of CPA physiology, and diminished to 48.9 ± 27.1 pg/ml when measured at least 1 month after TCPC (P < 0.05). This did not occur in two patients, where VEGF levels remained elevated, despite restoration of hepatic venous flow to the lungs and an absence of fenestration. Details of VEGF levels are given in Table 1.

Oxygen saturation levels were a mean of 82% (range 74–85%) before CPA, diminishing slightly to a mean of 79% (range 65–87%) just prior to completion of TCPC, and averaged 97% (range 91–100%) at the time of the last VEGF sample. VEGF levels were inversely proportionate to oxygen saturation before CPA (VEGF1; r2 = 0.43) and after TCPC (VEGF3; r2 = 0.1). Inverse correlation with corresponding VEGF levels was poorest just prior to TCPC (VEGF2; r2=0.002), with disproportionately elevated plasma VEGF levels after CPA (Fig. 1).

4. Discussion

PAVMs are identified in 21–60% of patients [2] after a cavopulmonary anastomosis, and tend to disappear after completion of TCPC [3]. These abnormal vascular structures contribute to morbidity and perhaps mortality, through right-to-left shunting and cyanosis [3,8], and may be demonstrated by bubble-contrast echocardiography as early as 6 weeks after CPA. Their etiology is yet unknown, and probably multifactorial.

The minimal interval of 1 month after completion of Fontan to measure VEGF levels was chosen on the basis of a study demonstrating high VEGF levels in normal adults subsequent to altitude training, which normalized after 1 month of their return back to sea level [6]. The fact that VEGF levels normalized in our patients, but remained higher than the levels prior to CPA may be explained either by the insufficient interval after TCPC before remeasuring...
levels, or by other unsuppressible angiogenic factors yet unknown. Also, VEGF levels remained high in two patients without a known anatomic substrate for cyanosis, for which we have no explanation.

Hypoxia is a well-known upregulator of VEGF secretion [4,5], and elevated serum levels of VEGF have been detected in children with cyanotic heart disease, as compared to acyanotic children [7]. A correlation between increased neovascularity in the lung and angiogenic proteins has been suggested by multiple elegant studies [7–9]. Increased staining for both VEGF and its receptor flk-1/DR were found in lung specimens of 13 children having undergone a bidirectional Glenn operation [8], four of which had angiographic evidence of PAVMs prior to completion of Fontan. The adhesion molecule CD31, present at intercellular junctions, has been found in decreased density in the lungs of children after CPA [9]. This results in basement membrane and endothelial discontinuity in PAVMs, and is speculatively one explanation for the capillary leak syndrome and prolonged pleural effusions after Fontan operations. These studies imply that virtually all children undergoing CPA are at risk of continuous angiogenic stimulation and subsequent PAVM development, regardless of symptoms or angiographic data. The combined findings suggest a labile angiogenic entity to be implicated in the integrity of the pulmonary microcirculation. Such a substance would presumably be degraded by the liver, and would be present in higher concentrations in the lungs after CPA [3,8], possibly with measurable systemic spillover, as is suggested by our results. However, VEGF may be only one of the pieces in the puzzle, and the source of high systemic VEGF remains purely speculative. In a lamb model creating a classical Glenn shunt, Malhotra et al. [10] reliably reproduced PAVMs and demonstrated an impairment of the pulmonary angiotensin system, with a reduction in angiotensin-converting enzyme, resulting in decreased circulating levels of the vasoconstrictor angiotensin II. Furthermore, Marshall et al. [4] partially purified a substance derived from hepatocyte-conditioned media that is inhibitory for the proliferation of cultured endothelial cells. This substance would be adequately cleared or degraded, once hepatic venous flow to the pulmonary circulation is restored, such as that after a completion of Fontan procedure, thus keeping pulmonary angiogenesis in check [4,11].

The implications of this study could be twofold. It is indisputable that CPA remains a valuable adjunct and is sometimes an inevitable palliative stage of the single ventricle pathway [12]. However, if the biphasic rise and fall of VEGF levels can be correlated with increased abnormal angiogenesis and PAVM formation in the lung during CPA physiology, there could be a strategic reconsideration with regard to leaving an isolated CPA circulation in place for too long. In those children among whom this step is necessary in the staged conversion towards completion of TCPC, it could be argued that the CPA stage should be reduced to as minimal a time as possible, so as to reduce the interval in which hepatic venous blood is excluded from the lungs, with its concomitant increased risk of developing PAVMs.

Secondly, these preliminary results could make a case for leaving an intact ventricular–PA continuity or other alternative sources of pulmonary blood flow, and hence hepatic venous return to the lungs. The possible advantages [11,13,14] and disadvantages [15] of accessory pulmonary flow with a CPA have been debated, although no consensus exists as to the usefulness or deleterious effect of such a circulation. In some reports, leaving alternative sources of

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HLHS, hypolastic left heart syndrome; TA, tricuspid atresia; TGA, transposition of the great arteries; DILV, double inlet left ventricle; DORV, double outlet right ventricle; DKS, Damus–Kaye–Stansel; IAA, interrupted aortic arch; CPA, cavopulmonary anastomosis; VEGF1, pre-CPA levels; VEGF2 = post CPA levels = pre-TCPC levels; VEGF3, post TCPC levels; sats., saturations.
pulmonary blood flow during CPA has resulted in less development of aortopulmonary collaterals and PAVMs [13,14]. However, these findings are not unanimous. Despite the uncertainty, be it for the sole purpose of potentially avoiding PAVMs, one may argue that accessory pulmonary flow from the hepatic veins is routinely justified potentially avoiding PAVMs, one may argue that accessory pulmonary flow from the hepatic veins is routinely justified. In conclusion, this small series suggests that systemic VEGF levels measured in peripheral venous blood fluctuate in an almost predictable manner during the single ventricle surgical pathway. Although the etiology and function remain obscure, using serial plasma VEGF levels as a biochemical marker for enhanced angiogenesis may allow to identify those patients at risk of developing PAVMs, notably after CPA.

5. Study limitations

Ideally, in any given patient, the entire pathway including all surgical stages of single ventricle physiology, from birth to after completion of Fontan, would give a complete curve of plasma VEGF levels for each individual. This was not available for all of our patients at the time of this report, as some patients are still awaiting completion of TCPC.

None of our patients had angiographic evidence of PAVMs. However, the most sensitive way to detect subclinical PAVMs is bubble-contrast echocardiography. This was not performed in our patients, and it would have been interesting to see if VEGF levels correlated with the degree of eventual PAVM development, as measured by this diagnostic modality.

These preliminary results need to be further validated in larger studies, and ideally include patients with and without documented PAVMs, to assess the potential specificity of plasma VEGF levels.

Acknowledgements

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References