VASCULAR ENDOTHELIAL GROWTH FACTOR AND BASIC FIBROBLAST GROWTH FACTOR IN CHILDREN WITH CYANOTIC CONGENITAL HEART DISEASE

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Objective: **Vascular endothelial growth factor and basic fibroblast growth factor are potent stimulators of angiogenesis. Children with cyanotic congenital heart disease often experience the development of widespread formation of collateral blood vessels, which may represent a form of abnormal angiogenesis. We undertook the present study to determine whether children with cyanotic congenital heart disease have elevated serum levels of vascular endothelial growth factor and basic fibroblast growth factor.** ***Methods:*** **Serum was obtained from 22 children with cyanotic congenital heart disease and 19 children with acyanotic heart disease during cardiac catheterization. Samples were taken from the superior vena cava, inferior vena cava, and a systemic artery. Vascular endothelial growth factor and basic fibroblast growth factor levels were measured in the serum from each of these sites by enzyme–linked immunosorbent assay.** ***Results:*** **Vascular endothelial growth factor was significantly elevated in the superior vena cava (*P* = .04) and systemic artery (*P* = .02) but not in the inferior vena cava (*P* = .2) of children with cyanotic congenital heart disease compared to children with acyanotic heart disease. The mean vascular endothelial growth factor level, determined by averaging the means of all 3 sites, was also significantly elevated (*P* = .03). Basic fibroblast growth factor was only significantly elevated in the systemic artery (*P* = .02).** ***Conclusion:*** **Children with cyanotic congenital heart disease have elevated systemic levels of vascular endothelial growth factor. These findings suggest that the widespread formation of collateral vessels in these children may be mediated by vascular endothelial growth factor. (J Thorac Cardiovasc Surg 2000;119:534-9)**

Abnormal angiogenesis occurs in association with numerous conditions, including tumor metastasis, diabetic retinopathy, rheumatoid arthritis, and gastric ulceration.[1] Children with cyanotic congenital heart disease often experience the development of abnormal blood vessel proliferation, which may also represent a form of abnormal angiogenesis. Vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are two potent stimulators of angiogenesis that are elevated in the serum of patients with many types of cancer.[2,3] The production of both factors is known to be upregulated in response to hypoxia.[4,5] We hypothesized that one or both of these angiogenic factors may mediate the abnormal angiogenesis demonstrated by children with cyanotic congenital heart disease. We compared VEGF and bFGF in the serum of children with and without cyanotic congenital heart disease to determine whether systemic levels of these angiogenic factors were elevated in the presence of cyanosis.

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Methods

Blood was collected during elective cardiac catheterization from 22 children with cyanotic congenital heart disease and 19 children with acyanotic congenital heart disease. Patients were excluded if they had undergone operative procedures that converted previously cyanotic conditions to an acyanotic

Table I. *Diagnosis and most recent procedure for patients with cyanotic congenital heart disease*

Diagnosis	*Most recent procedure*	*Patients (n)*
Tetralogy of Fallot	None	4
Tetralogy of Fallot	Blalock-Taussig shunt	1
Hypoplastic left heart syndrome	Norwood procedure	3
Hypoplastic left heart syndrome	Bidirectional Glenn shunt	2
Pulmonary atresia, intact ventricular septum	Blalock-Taussig shunt	2
Pulmonary atresia, intact ventricular septum	Bidirectional Glenn shunt	1
Pulmonary atresia, transposition of the great arteries, ventricular septal defect	Bidirectional Glenn shunt	1
Heterotaxy, pulmonary stenosis	Ventricular septal defect repair, right ventricular outflow patch	1
Heterotaxy, pulmonary atresia	Blalock-Taussig shunt	1
Heterotaxy, pulmonary atresia	Bidirectional Glenn shunt	1
Unbalanced arteriovenous canal	Pulmonary artery band	1
Tricuspid atresia	Blalock-Taussig shunt	2
Tricuspid atresia	Bidirectional Glenn shunt	1
Tricuspid atresia	Waterston shunt	1
Total patients		22

state (Fontan procedure). Patients were also excluded if they had a history of malignancy or inflammatory states that might lead to increased levels of VEGF or bFGF independent of their oxygen saturation. The study was approved by the Institutional Review Board of the Children's Hospital and Regional Medical Center, Seattle, Washington, and all procedures were performed in accordance with their guidelines after obtaining informed consent.

Blood was collected from the superior vena cava (SVC), inferior vena cava (IVC), and a systemic artery by way of indwelling catheters at the time of cardiac catheterization. Samples were allowed to clot for 30 minutes and were then centrifuged at 3000*g* for 30 minutes at 4°C. Serum was removed and stored at –70°C. VEGF and bFGF levels were measured with the Quantikine VEGF and HS bFGF ELISA kits (R&D Systems, Minneapolis, Minn) according to the manufacturer's instructions. In brief, serum samples were added to wells precoated with VEGF or bFGF antibody and incubated at room temperature for 2 or 3 hours, respectively. VEGF antibody conjugated to horseradish peroxidase was added followed by substrate (hydrogen peroxide) and chromogen (tetramethylbenzidine). For the bFGF assay, bFGF antibody conjugated to alkaline phosphatase was added followed by substrate (NADPH) and chromogen (INT-Violet). After stop solution (2N sulfuric acid) was added, optical density was determined within 30 minutes. Wells were read at a wavelength of 450 nm with a correction at 545 nm (VEGF) or 450 nm with a correction at 630 nm (bFGF). Each sample was run 4 times, and the results were averaged, yielding a single value for VEGF and bFGF for each site sampled. The limit of detection was 9 pg/mL for VEGF and 0.50 pg/mL for bFGF. Values of VEGF and bFGF less than the detection limits of the assay were assigned values of 4.5 pg/mL and 0.25 pg/mL, respectively.

Statistical analysis was carried out with SAS statistical software, version 6.12 (SAS Institute Inc, Cary, NC). VEGF and bFGF levels were determined for each site and are expressed as median (25th, 75th percentile). VEGF and bFGF levels for each patient were also averaged across sampling sites. Wilcoxon rank sum was used to assess differences in VEGF and bFGF levels between patients with cyanotic heart disease and with acyanotic heart disease. Repeated measures analysis of variance was used to determine whether the sampling site was associated with VEGF or bFGF levels.

Table II. *Diagnosis for patients with acyanotic congenital heart disease*

Diagnosis	*Patients (n)*
Recurrent tachycardia	5
Aortic stenosis	4
Patent ductus arteriosus	3
Pulmonary stenosis	2
Aortic coarctation	1
Aortic coarctation, aortic stenosis	1
Aortic coarctation, mitral stenosis	1
Aortic coarctation, aortic stenosis, ventricular septal defect	1
Mitral stenosis, aortic insufficiency	1
Total patients	19

Results

The median age was 1.6 years (25%, 0.7; 75%, 3.7) for children with cyanotic congenital heart disease and 12.2 years (25%, 6.9; 75%, 16.1) for children with acyanotic congenital heart disease. Mean systemic room air oxygen saturation was 79.8% ± 7.7% for children with cyanotic congenital heart disease and 99.5% ± 1.0% for children with acyanotic congenital heart disease. There were 11 girls and 11 boys in the cyanotic group and 9 girls and 10 boys in the acyanotic group.

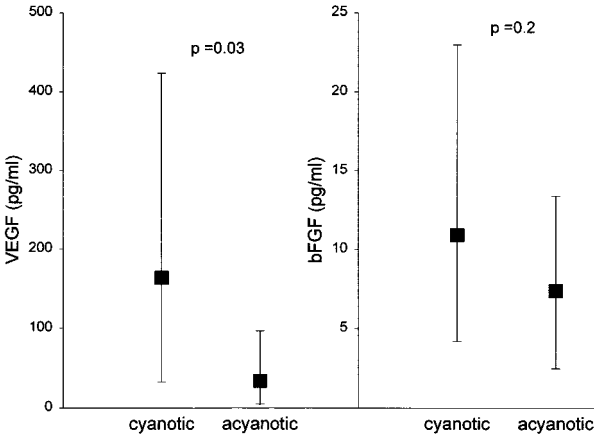

Fig 1. Average VEGF and bFGF levels for the three sites sampled (SVC, IVC, and systemic artery). Results are expressed as median values; the error bars represent the 25th and 75th percentiles.

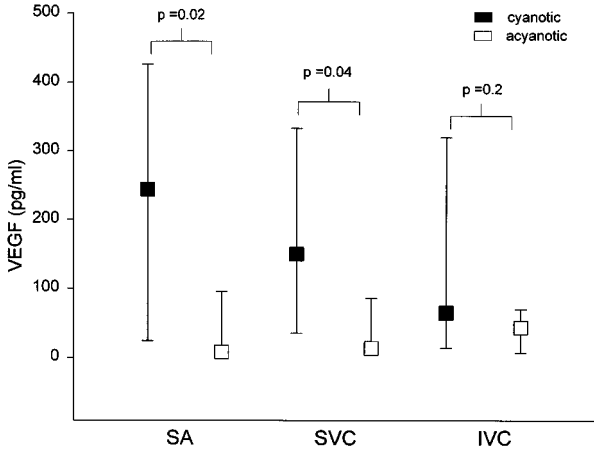

Fig 2. Median (25th, 75th percentile) VEGF levels in systemic artery, SVC, and IVC in patients with cyanotic congenital heart disease and acyanotic children.

The clinical diagnosis and past surgical procedures for children with cyanotic congenital heart disease and the clinical diagnosis for children with acyanotic congenital heart disease are listed in Tables I and II, respectively.

Twenty-one of the patients with cyanotic congenital heart disease and all of the patients with acyanotic congenital heart disease had serum VEGF measured. One patient with cyanotic congenital heart disease did not have VEGF measured because of lack of sufficient sample. Not all children had samples available from all three sites. Among patients with cyanotic congenital heart disease (n = 21 patients), the VEGF level averaged across all three sampling sites (SVC, IVC, and systemic artery) was 129.6 pg/mL greater than in patients with acyanotic congenital heart disease (n = 19 patients; P = .03; Fig 1). Among patients with cyanotic congenital heart disease (n = 22 patients), the bFGF level averaged across all three sampling sites was 3.5 pg/mL greater than in patients with acyanotic congenital heart disease (n = 19 patients) but this did not reach statistical significance (P = .2; Fig 1).

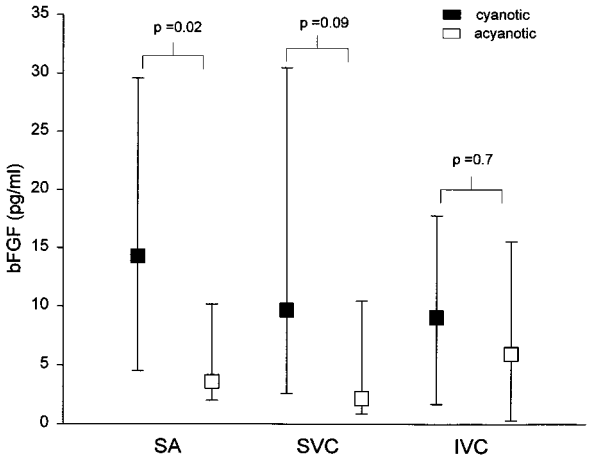

Fig 3. Median (25th, 75th percentile) bFGF levels in systemic artery, SVC, and IVC in patients with cyanotic congenital heart disease and acyanotic children.

The sites from which blood samples were obtained did not significantly affect VEGF levels. Patients with cyanotic congenital heart disease (n = 12 patients) had a median arterial VEGF level 235.5 pg/mL greater than patients with acyanotic congenital heart disease (n = 14 patients; P = .02; Fig 2). Samples obtained from the SVC showed a similar pattern, with levels among patients with cyanotic congenital heart disease (n = 11 patients) 136.0 pg/mL greater than patients with acyanotic congenital heart disease (n = 13 patients; P = .04). For samples obtained from the IVC, VEGF levels tended to be higher among the patients with cyanotic congenital heart disease (n = 17 patients) than among the patients with acyanotic congenital heart disease (n = 16 patients), but this was not statistically significant (P = .2).

Median arterial bFGF levels in cyanotic children (n = 14) were 10.7 pg/mL greater than in patients with acyanotic congenital heart disease (n = 15 patients; P = .02; Fig 3). Samples obtained from the SVC had bFGF levels in patients with cyanotic congenital heart disease (n = 13 patients) that were 7.5 pg/mL greater than among patients with acyanotic congenital heart disease (n = 14 patients; P = .09). For samples obtained from

the IVC, bFGF levels were 3.0 pg/mL greater among the patients with cyanotic congenital heart disease (n = 18 patients) than among the patients with acyanotic congenital heart disease (n = 16 patients; $P = .7$).

Discussion

Children with cyanotic congenital heart disease may experience the development of abnormal vascular channels that become the source of significant morbidity. Abnormal blood vessel proliferation in these children may take several forms, including systemic venous collaterals, pulmonary arteriovenous malformations, or aortopulmonary collateral arteries.[6-8] Systemic venous collateral vessels may develop extensively in any child with cyanotic congenital heart disease. Pulmonary arteriovenous malformations developing after cavopulmonary anastomosis in children with single ventricle physiology cause progressive cyanosis from right to left shunting. Aortopulmonary collateral arteries associated with pulmonary atresia may cause a number of problems, including significant left to right shunting, progressive obliteration after unifocalization procedures, and pulmonary "steal" from systemic blood flow during cardiopulmonary bypass. The management of children with cyanotic congenital heart disease may be complicated by the development of these vascular lesions and may require interventional cardiac catheterization or surgical treatment.

The proliferative nature of these vascular channels and their widespread occurrence suggest that these lesions form in response to a systemic angiogenic stimulus. Our interest in this area comes from previous work that examined pulmonary arteriovenous malformations that develop in children after cavopulmonary anastomosis. The development of these pulmonary arteriovenous malformations may represent a more localized form of abnormal angiogenesis that is under hepatic control. Conditioned media derived from cultured hepatocytes demonstrates an inhibitory effect on endothelial proliferation, and this inhibitory activity can be partially purified by serial column chromatography.[9] Presumably, the absence of this factor in the pulmonary circulation after cavopulmonary anastomosis might lead to unchecked vascular proliferation, resulting in clinically apparent pulmonary arteriovenous malformations.

The present study was undertaken to determine whether systemic levels of stimulators of angiogenesis were elevated in the serum of children with cyanotic congenital heart disease. The role of VEGF and bFGF as angiogenic stimulators is well established, and therefore these growth factors represent likely candidates for mediating the abnormal proliferation of blood vessels that occur in these children. Both of these factors have been shown to be mitogenic for endothelial cells[10,11] and act synergistically to induce angiogenesis.[12] Elevated systemic levels of these factors have been found in the sera of patients with cancer of many cell types including lung, ovarian, uterine, colorectal, and renal.[2,3] Significant elevations of these factors in patients with cancer correlate with a more aggressive course, presumably because of an increased angiogenic state of the tumor that leads to early metastasis.

Hypoxia is a strong stimulus for angiogenesis and leads to an upregulation of both VEGF and bFGF during hypoxic conditions in cell culture.[4,5] Glioma cells exposed to hypoxia demonstrate upregulation of VEGF mRNA that is reversed when cells are returned to normal oxygen levels.[4] Upregulation of VEGF has also been demonstrated in vivo where VEGF messenger RNA is increased 2- to 3–fold in the lungs of animals exposed to chronic hypoxia.[13] Recent reports have documented increased serum levels of VEGF in systemic hypoxic conditions in humans. Asano and colleagues[14] reported increased serum VEGF levels in athletes who were trained at high altitude and then returned to normal levels 1 month after the athletes returned to sea level. There is also evidence that bFGF is upregulated by hypoxia. The production of bFGF mRNA has been found to increase in the brains of animals exposed to hypoxia.[5,15]

The present study demonstrates that VEGF is elevated in the serum of children with cyanotic congenital heart disease. This finding was consistent for serum obtained from the SVC and the systemic artery and for the average value obtained from all three sites. bFGF was significantly elevated in the systemic artery of children with cyanotic congenital heart disease, and there was a trend toward increased bFGF levels in serum obtained from the SVC and the average value from the three sites. The lack of significant elevations of bFGF may relate to the small sample size of this study. However, based on the known biologic features of these two angiogenic factors, it seems reasonable that VEGF is more likely to be systemically elevated in patients with cyanotic congenital heart disease. bFGF lacks a signal sequence necessary for secretion, and it is not known how bFGF is exported from cells.[16] bFGF has been shown to be stored in the extracellular matrix and may be released from these extracellular storage sites with cell injury or death.[17] bFGF may not be released into the circulation in these children with chronic hypoxic without the cellular injury that occurs in tumor proliferation or inflammatory states in which

this factor is elevated. VEGF, on the other hand, does contain a signal sequence and has been shown to be secreted from cells.[18]

We sampled several vascular beds (SVC, IVC, and systemic artery) in an attempt to identify the source of production of these factors in these children. This strategy might be predicted to be unsuccessful because of the rapid equilibration and dilution of factors produced by a given organ (the liver for example) in high-volume, high-flow vessels such as the great veins and the arterial circulation. We were unable to definitively pinpoint the site of origin or metabolic breakdown of either factor in these children. We are currently looking at tissue protein and mRNA levels of VEGF and bFGF in children with cyanotic congenital heart disease using immunocytochemistry and in situ hybridization.

A weakness of this study is that the levels of VEGF that were observed in children with cyanotic congenital heart disease in this study were not appreciably different from normal values that have been previously obtained in some reports.[19,20] Recent studies have demonstrated that serum values of VEGF increase with the time that blood is allowed to clot before being centrifuged, possibly related to VEGF release by activated platelets.[21] Samples obtained for the present study were kept on ice and centrifuged within 30 minutes of collection. It is possible that the VEGF values obtained in this study were generally lower than those previously reported because we had minimal platelet activation in our samples because of prompt processing. In addition, other studies have demonstrated VEGF levels for normal control patients similar to our results for patients with acyanotic congenital heart disease.[22] We believe the validity of the conclusions reached in this study is supported by the concurrent performance of the enzyme-linked immunosorbent assay on serum obtained from children with acyanotic congenital heart disease, with identical handling of the samples from each group.

Another weakness occurs in that, despite attempts to balance the study groups for age, children with acyanotic congenital heart disease were significantly older than children with cyanotic congenital heart disease. This was largely attributable to the fact that most of the samples obtained in children with cyanotic congenital heart disease were obtained during cardiac catheterizations before palliative operations performed in infancy. The acyanotic group demonstrated conditions that often did not require intervention in infancy such as arrhythmias or progressive aortic valvular disease. Several authors have looked at the influence of age on bFGF and VEGF levels in patients with cancer, children with inflammatory bowel disease, and children with diabetes and have found no correlation.[19,23,24] Rivard and colleagues[25] reported an age–dependent impaired angiogenic response to ischemia that was probably due to a decrease in VEGF transcription. However, this study compared tissue VEGF levels in young versus elderly animals. There have been no studies to our knowledge that demonstrate a correlation of serum VEGF levels with age. Lassus and colleagues[26] measured plasma VEGF levels in neonates and found levels similar to those reported in normal adults using the same methods used in the present study.

Summary

The present study represents a preliminary attempt to identify factors that may have an impact on manifestations of cyanotic congenital heart disease. VEGF appears to be systemically elevated in patients with chronic cyanosis and may contribute to the formation of extensive collateral vessels that sometimes develop in these children. Issues related to the exact origin of these factors are not specifically answered by this study. However, these findings may have broader implications regarding the pathophysiologic features of cyanotic heart disease, while further study of affected children may aid in understanding the control mechanisms of angiogenesis.

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