Angiogenic Growth Factors in Patients with Cyanotic Congenital Heart Disease and in Normal Children

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Summary: Previous studies have demonstrated that the expression of angiogenic growth factors is induced in hypoxic models. However, little is known about these factors in patients with cyanotic heart disease. The purpose of this study was to examine the relationship between the plasma level of angiogenic growth factors and the severity of cyanosis. The study included 85 patients with cyanotic heart disease and age matched 81 controls. Median age was 4.2 years in the cyanotic group and 4.8 years in the control group. Mean systemic oxygen saturation was 80.6±7.3% in the cyanotic group and 98.1±0.5% in the control group. In the control group, vascular endothelial growth factor (VEGF) in the neonatal period was significantly elevated, then rapidly decreased within 3 months after birth. After 3 months of age, VEGF levels remained at a plateau. In contrast, this age dependency did not occur in hepatocyte growth factor (HGF) levels. Although VEGF and HGF levels were not different between the cyanotic and control groups within 3 months after birth, the VEGF level in the cyanotic group after 3 months of age was significantly elevated compared to the levels measured in the control group (149.2±105.6 vs. 66.3±22.5 pg/ml, p<0.0001). Moreover, the VEGF level was negatively correlated with oxygen saturation (y=440.6-3.53x, R=0.47, p<0.0001) in cases more than 3 months old. In contrast, no correlation was found between HGF level and oxygen saturation. Although physiologically increased VEGF in the neonatal period was rapidly decreased under normal oxygen saturation, a higher VEGF level persisted if systemic hypoxia was present. Persistently higher VEGF level may be related to the development of systemic to pulmonary collateral arteries in patients with cyanotic heart disease.

Key words vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), angiogenesis, cyanotic congenital heart disease, hypoxia

INTRODUCTION

Angiogenesis is defined as the phenomenon of new capillary vessel formations from the preexisting vasculature, mainly thin vessels [1-3]. It has been demonstrated that angiogenesis is related to physiological phenomenon, such as the formation of circulatory organs in the fetal period [4], changes in uterine mucous membrane in adult females or tissue repair [5] etc. In these regards, vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are considered to induce an angiogenic response via their direct effect on endothelial cells [6,7]. Similarly, recent studies revealed that hepatocyte growth factor (HGF) strongly affects angiogenesis [8]. Administration of angiogenic growth factors as in recombinant protein therapy or gene transfer may be augmented in animal models of myocardial and limb ischemia [9].

Recent studies have revealed that VEGF expression is induced by hypoxia [10-15]. Children with cyanotic congenital heart disease often experience the development of aortopulmonary collateral vessels, enlarged bronchial arteries, or major aortopulmonary collateral arteries: (MAPCA) [16-19]. Sandra and colleagues recently reported that children with
cyanotic congenital heart disease have elevated systemic levels of VEGF [20]. We hypothesized that one or both of these angiogenic factors, VEGF and HGF, was related to the abnormal angiogenesis demonstrated by children with cyanotic congenital heart disease. In this study we evaluated plasma levels of VEGF and HGF in patients with cyanotic heart disease and compared them with the plasma levels in healthy subjects to determine whether these angiogenic factors were elevated in the presence of cyanosis.

MATERIALS AND METHODS

This study involved 85 patients with cyanotic heart disease and age matched 81 control subjects. The cyanotic group consisted of 39 boys and 46 girls, and the control group consisted of 43 boys and 38 girls. Median age was 4.2 years (range, 0 days-40 years) in patients with cyanotic heart disease, and 4.8 years (range, 5 days-31 years) in healthy control subjects. Mean systematic oxygen saturation was 80.6±7.3% for the cyanotic group, and 98.1±0.49% in the control group. Clinical diagnosis in the cyanotic group are listed in Table 1. The control group consisted of healthy subjects without heart disease (patients after Kawasaki disease without coronary abnormality, subjects with an innocent murmur, and normal neonates).

Blood samples were collected from the peripheral vein or the inferior vena cava at the time of cardiac catheterization prior to heparin infusion. In 10 cases in the cyanotic group, blood samples were collected from variable sites, such as the inferior vena cava, peripheral vein, the superior vena cava, a hepatic vein, and a systemic artery in order to evaluate the dispersion of these factors. Blood was collected as plasma, immediately separated by centrifugation, and was then kept frozen at −80 °C until assayed. The analysis was performed by enzyme immunoassays, using commercially available kits (human VEGF Quantikine, R&D Systems, Minneapolis, USA, and a rat HGF EIA kit, Institute of Immunology, Tokyo, Japan). The degree of aortopulmonary collateral arteries development was evaluated by descending aortography and graded on a 4-point scale according to Wernovsky et al. [16].

Statistical analysis

All results were expressed as mean value ± standard deviation. Statistically significant differences among the sampling sites were evaluated by ANOVA. A Mann-Whitney test was used for the comparison between the two groups. Standard linear or polynomial regression analysis was performed to identify correlation among the variables. A value of p<0.05 was interpreted to denote statistical significance.

RESULTS

Dispersion of VEGF and HGF levels

Plasma concentration of VEGF and HGF at each site is shown in Fig. 1. There was no statistical significance among the sampling sites.

VEGF and HGF levels in the control group

Plasma concentrations of VEGF and HGF in the
**Fig. 2.** VEGF and HGF levels in the control group.

**Fig. 3.** Comparison between the cyanotic group and control group.

**Fig. 4.** Correlation between angiogenic growth factors and oxygen saturation.
Comparison between the cyanotic group and control group

In subjects over 3 months old from both the cyanotic and control groups, VEGF and HGF levels did not change with age. The VEGF level of the cyanotic group was significantly increased over that of the control group (149.2±105.6 pg/mL vs. 66.3±22.5 pg/mL, p<0.0001) (Fig. 3). In contrast, the HGF level in the cyanotic group was not significantly different compared to that of the control group (0.28±0.96 ng/mL vs. 0.56±1.25 ng/mL, p=0.19). In analyzing all cases, significant negative correlation was observed between VEGF levels and systemic oxygen saturation, while there was no significant correlation between HGF levels and systemic oxygen saturation (Fig. 4). There were no statistically significant relationships between the degree of aortopulmonary collateral arteries development and plasma VEGF or HGF levels.

DISCUSSION

Patients with cyanotic congenital heart disease often develop aortopulmonary collateral arteries [16-19]. It seems that the development of these aortopulmonary collateral vessels is associated with the severity of the cyanosis. These aortopulmonary collateral vessels may be the major cause of remaining left to right shunts and place a hemodynamic burden on the systemic ventricle in patients having Fontan procedure [19]. Therefore, these vessels are usually closed by transcatheter coil occlusion before or after the operation [17]. The mechanism of development of aortopulmonary collateral vessels is not clear. We hypothesized that persistent systemic hypoxia may induce angiogenesis in patients with cyanotic congenital heart disease, and, as a result, these aortopulmonary collateral arteries may develop.

In this study, we evaluated the plasma concentrations of VEGF and HGF in patients with cyanotic congenital heart disease and healthy control subjects to determine whether the presence of cyanosis influenced these angiogenic factors.

In our study, the plasma concentration of VEGF in normal infants was clearly elevated in the neonatal period, gradually decreasing to the normal range within a few months after birth. Ariadne and colleagues reported that the plasma level of VEGF rose significantly in early neonatal life compared with the levels in umbilical cord blood [21]. Increase of VEGF in the early neonatal period may reflect the physiological development of vessels in the neonatal period [22]. Otherwise, it may reflect transient hypoxia at delivery or sudden hemodynamic changes soon after birth, such as the increase of pulmonary blood flow, etc [13,14,23]. In this study, we demonstrated that elevated plasma concentrations of VEGF in the early neonatal period rapidly decreased to the adult range within a few months after birth. This change may be caused by the stabilization of hemodynamics or by the end of advanced VEGF expression in the neonatal period. However, plasma HGF did not show such age dependency.

In our study, VEGF levels in the cyanotic group were significantly higher than that in the acyanotic control group over 3 months after birth. Plasma concentrations of VEGF may be elevated regardless of the presence of cyanosis in the neonatal period, and immediately decrease to the adult range in the normal acyanotic group. Elevated VEGF levels in the neonatal period in patients with cyanosis may be maintained beyond 3 months. Hypoxia could be considered a strong stimulus for angiogenesis, and leads to an increase of angiogenic factors under hypoxic conditions in the experimental model [15,24]. Recent reports demonstrated that increased serum VEGF levels in athletes who were trained in high altitude conditions [12]. The role of VEGF as an angiogenic stimulator is well established, and therefore VEGF is a candidate for mediating the abnormal proliferation of blood vessels (i.e. aortopulmonary collateral arteries) in patients with hypoxic conditions.

However, HGF levels did not differ between cyanotic and acyanotic groups, and also did not show any correlation with oxygen saturation. These results suggested that VEGF and HGF were probably induced by different mechanisms. If VEGF expressions are linked with abnormal collateral formations in patients with cyanotic heart disease, gene therapy using VEGF inhibitors would be possible in the future.

Several limitations were present in this study. The sample sites, either the peripheral vein or the inferior vena cava, were not fixed in either the cyanotic or control group. However, no significant differ-
ences between VEGF levels of the peripheral vein and the IVC were found in our study. Another limitation in this study was the variation of circulatory dynamics of patients in the cyanotic group. The cyanotic group consisted of patients with various congenital heart diseases, such as Tetralogy of Fallot, decreased pulmonary blood flow, or single ventricle with increased pulmonary blood flow dependent on its hemodynamic condition. Pulmonary artery pressure and pulmonary vascular resistance varied by case. The presence of VEGF may depend not only on systemic oxygen saturation, but other factors. More detailed studies should be performed in this area. Immunohistological examination of aortopulmonary collateral arteries would clarify causes for the presence of angiogenic growth factors.

CONCLUSIONS

Although these limitations were present, our study did clarify that the physiologically increased VEGF in the neonatal period rapidly decreased under normal oxygen saturation, and a higher VEGF level persisted if systemic hypoxia was present. This may be the reason for the development of systemic-to-pulmonary collateral arteries in patients with cyanotic heart disease.

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