Serum Immunoreactive Erythropoietin in Children With Cyanotic and Acyanotic Congenital Heart Disease

By Per Hågå, P. Mary Cotes, Janice A. Till, Barbara D. Minty, and Elliot A. Shinebourne

Serum immunoreactive erythropoietin (siEp) was measured in 27 cyanotic and 21 acyanotic children with congenital heart disease, age 4 months to 10 years. The geometric mean value was 9 mlU/mL for each group with 95% range from 3 to 26 mlU/mL and 4 to 22 mlU/mL for the cyanotic and acyanotic subjects, respectively. The levels are similar to those found in normal adults using the same assay system. Three cyanotic subjects showed increased siEp values. One was anemic relative to his hypoxemia, and the other two showed signs of increasing hypoxia. There was a significant negative correlation between siEp and arterial oxygen content. However, siEp did not correlate significantly with hemoglobin, hematocrit, PaO₂, or SaO₂. Despite normal siEp levels, the cyanotic children showed compensatory erythropoiesis with significantly elevated hemoglobin and hematocrit levels, which did correlate inversely with PaO₂ and SaO₂. Arterial oxygen content was also significantly higher in the cyanotic subjects (p < 0.02). The cyanotic children seemed to display the same pattern as observed in man and animals exposed to prolonged hypobaric hypoxia, where after an initial rise in erythropoietin values the levels fall to normal, while increased erythropoiesis is sustained.

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MATERIALS AND METHODS

The subjects of the study were patients undergoing cardiac catheterization or about to have cardiac surgery. The catheterized patients were under general anesthesia with a FiO₂ of 33%, in accordance with hospital routine. Blood was sampled from the catheter at the start of the procedure. In the patients about to be operated on, blood was obtained from an arterial line after the patient was stabilized under general anesthesia.

Twenty-seven cyanotic children and twenty-one acyanotic children having congenital heart disease were studied. Their age ranged from 4 months to 10 years. Cyanosis or arterial desaturation was defined as a systemic arterial oxygen saturation (SaO₂) of less than 95%. Clinical findings are shown in Table 1. Growth was assessed on the standard height and weight charts of Tanner & Whitehouse.

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correlation was tested by linear regression. SiEp shows a log normal distribution and was therefore tested after logarithmic transformation; means were expressed as geometric means. Some of the acyanotic patients displayed hyperoxemia on being artificially ventilated at a FiO2 of 33% and PO2 values above 15 kPa were excluded from analysis. Two cyanotic patients (subjects II & III in Table 3) had siEp values 6.0 and 10.8 standard deviations, respectively, above the rest of the group. For this reason they were excluded from the statistical analysis. The study was approved by the Brompton Hospital Ethical Committee.

RESULTS

The estimates of siEp are shown in Fig 1 and Table 2. The geometric mean was 9 mU/mL for each group, with a 95% range† from 3 to 26 mU/mL for cyanotic and 4 to 22 mU/mL for acyanotic subjects. The difference is not statistically significant. The levels are similar to those of 21 normal adults using the same assay system (geometric mean 9 mIU/mL for acyanotic subjects. The difference is not statistically significant. The levels are similar to those of 21 normal adults using the same assay system (geometric mean 8 mIU/mL, 95% range from 4 to 16 mIU/mL).

Three cyanotic patients (subjects I, II, III; Table 3) had siEp values 3.8, 7.6, and 13.5 standard deviations, respectively, higher than their counterparts (Fig 1). Subject I, with arterial oxygen saturation 80.9% and hemoglobin concentration 12.7 g/dL, may be considered anemic in relation to his hypoxemia. This relative anemia was most likely due to iron deficiency (serum ferritin 8 μg/L). This may explain the

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raised siEp concentration. Subjects II and III had both shown symptoms and signs of increasing cyanosis and hypoxia during the months leading up to hospital admission. In addition, subject III suffered from iron deficiency anemia, as judged from red cell indices and serum ferritin (Table 3).

Hemoglobin concentration and hematocrit were significantly higher in the cyanotic than the acyanotic subjects (p < 0.001, n = 46). Arterial oxygen content (1.36 x hb x SaO2) was also significantly higher in the cyanotic group (p < 0.02, n = 44) (Table 2).

The siEp concentrations did not show any significant correlation with hemoglobin concentration or hematocrit, or with systemic arterial oxygen tension (PaO2) or SaO2. This was so both for the cyanotic and acyanotic patients as well as for the two groups combined. However, there was a significant negative correlation between siEp and arterial oxygen content in the cyanotic group (r = -0.56, P < 0.01, n = 25) (Fig 2). The inverse correlation was also significant for all patients combined, although to a lesser extent (r = -0.43, P < 0.01, n = 44).

Hemocrit was inversely correlated with arterial PO2 and oxygen saturation for both the cyanotic group and all subjects combined (p < 0.05 in all instances). Hemoglobin concentration showed the same pattern, but the relation did not attain significance in the cyanotic group. The best correlation was obtained between hematocrit and SaO2 in the total sample (r = -0.74, P < 0.001, n = 44).

**Table 2. Hematologic and Physiologic Data of the Study Groups**

<table>
<thead>
<tr>
<th></th>
<th>n Cyanotics</th>
<th>n Acyanotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoietin (mIU/mL)</td>
<td>25</td>
<td>9 (3-26)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>25</td>
<td>15.1 ± 1.8*</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>25</td>
<td>0.46 ± 0.05*</td>
</tr>
<tr>
<td>PO2 (kPa)</td>
<td>25</td>
<td>7.2 ± 1.2</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>25</td>
<td>84.0 ± 7.8</td>
</tr>
<tr>
<td>Arterial O2 content (mL/dL)</td>
<td>25</td>
<td>17.1 ± 2.2*</td>
</tr>
<tr>
<td>P50 (kPa)</td>
<td>9</td>
<td>3.9 ± 0.44</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>25</td>
<td>24.5</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>25</td>
<td>0.41</td>
</tr>
<tr>
<td>Ferritin (g/L)</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>PO2 (kPa)</td>
<td>25</td>
<td>7.5</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>25</td>
<td>80.9</td>
</tr>
</tbody>
</table>

Values are means ± SD, except for erythropoietin values, which are geometric means (% range).

*P < 0.001 when compared with the values of acyanotics (two-tailed unpaired t-test, degrees of freedom = 44).

† 1 kPa = 7.5 mm Hg; 1 mm Hg = 0.133 kPa.

‡ P < 0.02 when compared with the values of acyanotics (two-tailed unpaired t-test, degrees of freedom = 42).

**DISCUSSION**

The low siEp concentrations found in the cyanotic group were unexpected. Our findings are not in agreement with either the general notion of raised Ep levels in secondary
polycythemia nor with the raised levels reported in adults with CCHD in other radioimmunoassay studies. In fact, Koeffler and Goldwasser found the patients with cyanotic heart disease to have the highest average concentration among the secondary polycythemia subjects.7 Our findings cannot be explained by insufficient severity of hypoxemia, since the PaO2 ranged from 5.0 to 8.9 kPa, with an even spread of values.

A FiO2 of 33% is used in general anesthesia to counteract the negative effects of artificial ventilation on oxygenation. Some acyanotic subjects displayed hyperoxemia when ventilated in this way, and there is a possibility that the SaO2 of some of the cyanotic patients with a high pulmonary blood flow was also increased by artificial ventilation.

Oxygen delivery to the tissues depends on many factors, in particular on the total number of circulating erythrocytes, PaO2, SaO2, the position of the oxygen-hemoglobin dissociation curve, cardiac output, and regional blood flow.

A possible reason for these low siEp values in children with CCHD is that their hypoxemia was totally compensated, and consequently no tissue hypoxia was experienced. Compensatory mechanisms to counteract tissue hypoxia clearly operated in the patients of our study group. We did not measure red cell mass, but hemoglobin levels and hematocrit were well above normal in our cyanotic patients and, moreover, were inversely correlated with PO2 and SaO2, indicating increasing production according to increasing needs. A rightward shift of the oxygen-hemoglobin dissociation curve is considered advantageous for oxygen delivery when lowered PaO2 is caused by right to left shunting of blood,20 which is in line with experimental findings.21 We found an increase in standard P50 in the cyanotic patients tested in our study (Table 2). Furthermore, for all subjects tested, P50 levels correlated significantly with hemoglobin concentrations and hematocrit. Most importantly, the arterial oxygen content was found to be significantly higher in the cyanotic than in the acyanotic subjects (Table 2). However, although we have shown compensatory adjustments at work, whether total compensation of hypoxemia occurs is not answered by this investigation. Total compensation for any deficiency state in a child would entail "normal" growth and development. Our patients showed reduced growth, both in height and weight, as is a common finding in CCHD. However, the growth impairment was not significantly more severe in the cyanotic than the acyanotic group. Hypoxia seems to be only one of several factors, albeit an important one, responsible for impaired growth in congenital heart disease.22 A recent study found an inverse association between cognitive function and time of corrective surgery (6 months to 6 years) in children with simple transposition, suggesting that the length of hypoxic exposure has an adverse effect and that the hypoxemia is not totally compensated.23 On balance, we believe that total correction of tissue hypoxia most likely is not achieved and that the low Ep values cannot be fully explained through such a mechanism. One may argue that through reduction in growth, oxygen consumption, and physical exercise, oxygen needs are reduced to a lower level whereby equilibrium is reestablished through lower demands. However, such a concept seems unlikely in infants and children because of the major emphasis during this period on growth and development.

When exposed to hypobaric hypoxia, both experimental animals and humans show an initial rise in erythropoietin concentration that falls gradually to normal levels in the ensuing days or weeks. Even though the Ep levels return to normal, increased erythropoiesis is sustained, with a continuous rise in hemoglobin concentrations and hematocrit levels.24,25 Looking at other subjects chronically exposed to hypoxia, we find little data on erythropoietin secretion in persons living permanently at high altitude. However, in one study, urinary Ep was increased in only two out of six subjects.26 In patients with severe hypoxic lung disease with an associated polycythemia, siEp was above normal in only 5 out of 10 patients.10 Thus, from both experimental work in animals and humans as well as clinical studies, the axiom of a direct relationship between the degree of hypoxemia and serum or urinary erythropoietin concentrations is not borne out under long-term hypoxic conditions. We believe the cyanotic patients in the present study fit the pattern outlined above and that their low siEp levels may be explained in the context of chronic hypobaric hypoxia.

The raised siEp concentrations of the three patients in Table 3 must be explained differently. Subject 1 was relative to his cyanotic heart disease anemic (SaO2 80.9%) at the time of study, most likely due to iron deficiency, and this may be the reason for his elevated siEp value. The other two subjects had both shown severe signs and symptoms of increasing cyanosis and hypoxia during the preceding months. They were thus not in a "stable chronic state," and both in humans and animals exposed to prolonged hypoxic conditions, it has been shown that increasing the hypoxic stimulus causes a renewed rise in Ep levels,24,26,27 Our finding of a significant negative correlation between siEp levels and arterial oxygen content in CCHD (Fig 2) indicates that oxygen supply is a factor in the regulation of erythropoietin production even when the Ep concentrations are in the "normal" range.

Single determinations of concentrations of erythropoietin both as a measure of the production rate of the hormone and as a reflection of the serum levels over time may be seen as wanting. Diurnal variations in siEp values in humans,28,29 as well as fluctuating levels in a polycythemic adult, have been observed.11 Fig 3 shows that multiple samples from the same individual showed fairly stable levels of siEp. This indicates that the single measurements of the patients in our study can reasonably be expected to give good estimates of the steady state siEp levels.

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