Erythropoietin Response to Acute Hypoxemia in Patients with Chronic Pulmonary Disease*

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Chronic hypoxemia is associated with development of secondary polycythemia. To evaluate effects of transient hypoxemia on serum EPO activity in patients with chronic lung disease, we studied six oxygen-dependent patients who underwent either a 4-h oxygen withdrawal or their routine therapy, in a randomized, blinded fashion, on two separate days. Serum EPO did not differ at baseline between study days. Erythropoietin levels did not change significantly over time during normoxic conditions. Under hypoxic conditions, serum EPO levels rose over 4 h with the change from baseline first becoming significant at 2 h. The log of serum EPO response showed an inverse correlation with the level of arterial oxygen saturation. We conclude that patients with chronic lung disease are able to produce EPO in response to acute hypoxic stress. Transient episodes of hypoxemia, such as occur during sleep or exercise, may result in increased red blood cell production stimulated by this EPO response. (Chest 1992; 102:482-85)

Severe chronic obstructive pulmonary disease frequently is complicated by hypoxemia and its sequelae. In such patients, the administration of continuous low flow oxygen reduces morbidity and prolongs survival. Both the British Medical Research Council trial and the multicenter Nocturnal Oxygen Therapy Trial demonstrated these benefits in selected patients who had continuous hypoxemia during wakefulness at rest. However, continuous and noninvasive means of monitoring oxyhemoglobin saturation have shown that recurrent, transient hypoxemia occurs often during sleep or vigorous exercise despite adequate arterial oxygen tensions or oxygen saturation measured at other times. The physiologic and clinical importance of such hypoxemic episodes are unknown. To assess the significance of these events, we measured one physiologic consequence of transient hypoxemia: EPO production.

In healthy volunteers, exposure to high altitude stimulates EPO production and subsequently, polycythemia. Gradual ascent has made it impossible to determine the rate of EPO response to hypoxemia in this setting. Although it has long been known that EPO levels are above normal between 12 and 39 h following ascent to high altitude, more precise definition of EPO production rates had not been possible. Eckardt and colleagues circumvented this difficulty by making healthy volunteers hypoxic abruptly in a hypobaric chamber. They demonstrated that serum EPO levels were significantly above baseline values at 84 min following exposure to simulated altitudes of 4000 m. Such a time course suggests that EPO level measurement might be feasible when evaluating hypoxic events in COPD patients.

Previous studies have sought and failed to find a clear association between degree of hypoxemia, EPO level and polycythemia in patients with chronic lung disease. It is possible, therefore, that chronically hypoxic patients are unable to augment EPO production in response to an acute hypoxic stimulus. Alternatively, such patients may have a heightened threshold for response or in some other way exhibit adaptation to the chronic stimulus. Therefore, we undertook the present study to determine whether patients with chronic hypoxic lung disease produce EPO in response to a short-term hypoxic event. By studying patients previously stabilized on long-term supplemental oxygen, we were able to mark precisely the onset of hypoxemia from the time of oxygen withdrawal and thereby determine the degree and time course of EPO level changes following oxygen withdrawal.

**Methods**

We studied six patients (four men, two women) whose clinical and biochemical characteristics are described in Table 1. All patients suffered from chronic pulmonary disease and required continuous supplemental oxygen to maintain normoxemia. No patient had significant concurrent hematologic or renal disease and all were nonsmokers at the time of the study. None of the patients was

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polycytemic. Informed consent was obtained from all subjects according to the Guidelines on the Use of Human Subjects of the University of Toronto.

Each patient served as his or her own control by participating in each of two study days separated by at least 24 h but by no more than five days. Because of the known diurnal variation in immunoreactive EPO levels in patients with chronic lung disease, studies took place at the same time of day for each subject and, as much as possible, took place in the morning hours. Each patient was assigned to receive in random sequence either his or her usual oxygen prescription by nasal prongs for 4 h (normoxic condition) or the same flow rate of compressed air by nasal prongs (hypoxic condition).

Oxygen saturation was measured at baseline and whenever blood was withdrawn on both experimental days, using a transcutaneous pulse oximeter (Biox IIA: B.T. Inc., Boulder, Colo). An indwelling venous cannula was inserted in the forearm at least 60 min before manipulation of the supplemental gas mixture and blood was drawn at baseline and 15, 30, 60, 120, 180 and 240 min following this adjustment. The serum was separated by low-speed centrifugation and was frozen for later assay of EPO.

The EPO levels were measured in patient serum samples, as described in more detail previously. The assay measures tritiated thymidine incorporation into splenic erythroblasts isolated from phenylhydrazine-treated mice. Strain C57DL/6J x C3H/HEJ mice (Jackson Laboratories, Bar Harbor, Me) were injected with 1.2 mg phenylhydrazine hydrochloride (J.T. Baker Chemical Co., Phillipsburg, NJ), intraperitoneally, on two consecutive days. Splenectomy was performed two days later and nucleated erythroblasts (accounting for >95 percent of spleen cells) were obtained. Cells were incubated in MEM alpha medium plus 20 percent vol/vol 1X serum substitute at 4 x 10^6 ml in the presence of known amounts of EPO (Connaught Laboratories, Canada) or test samples, for 22 h, 37°C, 5 percent CO₂ in humidified air. Tritiated thymidine, 2.0 Ci/mmol (Dupont-NEN, Boston, Mass), was added at 2 μCi/ml in PBS. Following a 2-h incubation, cells were harvested onto glass filter paper (PHD cell harvester, Cambridge, Mass), and radioactivity was measured in a Beckman LS1800 liquid scintillation counter. Normal human serum contains less than 25 μEPO/ml by this method.

All results are expressed as mean ± SEM. Repeated measures analysis of variance over time for each experimental condition was performed to look for significant changes in EPO levels over time. If significant time trends were detected, mean peak EPO levels at each interval were compared to the baseline value using the Wilcoxon sign rank test. A relationship between log serum EPO level and oxygen saturation was sought using least squares linear regression. For this analysis, all individual values for EPO at each time point were plotted against the corresponding arterial oxygen saturation. Results were considered significant at the p<0.05 level.

RESULTS

While the patients were receiving the usual prescribed dose of supplemental oxygen the baseline arterial oxygen saturation did not differ significantly between the two experimental days. In contrast, while they were receiving compressed air the arterial oxygen saturation fell from a mean (SEM) of 95.2 percent (1 percent) to 85.3 percent (1.9 percent), which yielded p<0.01. At 30 min, the earliest recorded measurement following the switch to compressed air, oxygen saturation was significantly different (p<0.02) from that observed at the same time period on the normoxic day, a trend that persisted for the duration of the experiment (Fig 1).

Serum EPO levels did not differ significantly between normoxic and hypoxic conditions at baseline. During normoxic conditions, serum EPO levels did not vary significantly over time (Fig 2) and the mean (SEM) peak serum EPO level at 4 h was not significantly higher than that at baseline: 57 (44) mU/ml at O h compared with 92 (79) mU/ml at 4 h. By contrast, when patients were made hypoxemic, serum EPO levels rose gradually over time, peaking at 4 h after oxygen withdrawal (Fig 2). As early as 2 h after the onset of hypoxemia, EPO levels increased above baseline (p<0.05). Increases in EPO levels over time were observed in serum samples from each patient studied, although the magnitude of each varied from serum sample to serum sample, as reflected in the

### Table 1—Characteristics of Study Subjects

<table>
<thead>
<tr>
<th>Patient/Sex/Age (yr)</th>
<th>Diagnosis</th>
<th>Hgb (g/L)</th>
<th>Hct (%)</th>
<th>Cr (μmol/L)</th>
<th>BUN (mmol/L)</th>
</tr>
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<tbody>
<tr>
<td>1/M/77</td>
<td>COPD</td>
<td>147</td>
<td>42.6</td>
<td>125</td>
<td>7.7</td>
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<tr>
<td>2/M/67</td>
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<td>132</td>
<td>39.0</td>
<td>80</td>
<td>5.9</td>
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<tr>
<td>3/M/74</td>
<td>COPD</td>
<td>141</td>
<td>40.8</td>
<td>104</td>
<td>5.2</td>
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<tr>
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<td>COPD</td>
<td>136</td>
<td>39.0</td>
<td>49</td>
<td>4.3</td>
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<tr>
<td>5/F/76</td>
<td>Sarcoidosis</td>
<td>120</td>
<td>35.9</td>
<td>84</td>
<td>7.4</td>
</tr>
<tr>
<td>6/M/57</td>
<td>COPD</td>
<td>118</td>
<td>36.6</td>
<td>93</td>
<td>6.3</td>
</tr>
</tbody>
</table>

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21652/ on 06/26/2017)
wide error bars at later time points (Fig 2). Serum EPO rose from a mean (SEM) of 230 (148) mU/ml at 0 h to 1,259 (676) mU/ml at 4 h (p<0.05).

The log serum EPO increase was inversely correlated with arterial oxygen saturation (r = -0.36, p<0.05).

**DISCUSSION**

Here we have monitored the time course of EPO release under conditions approximating the clinical setting, hypoxia induced by transient removal from supplemental oxygen in patients with chronic pulmonary disease. In contrast, previous studies of patients with chronic lung disease used erythropoiesis as the stimulus for EPO release, measured clinical changes in EPO without correlating these to changes in oxygen saturation, or measured serum EPO at a single point in time. Other investigators have monitored the time course of EPO release in normal subjects either taken to high altitudes or subjected to decompression chambers in order to simulate the hypoxia occurring naturally in the present group of patients. None of our patients had detectable underlying renal or hematologic disease that might have affected serum EPO levels. We found that patients with chronic lung disease are able to produce a rise in serum EPO as early as 2 h following the onset of an acute hypoxic stress. Moreover, the magnitude of the EPO response correlated with the degree of induced hypoxemia.

The time course of the rise in serum EPO in our study is similar to that recently described in normal subjects exposed to simulated altitudes of 4,000 m in a decompression chamber. The lag time of 1 1/2 to 2 h between the onset of hypoxemia and the first detectable rise in serum EPO levels also is consistent with the time course of renal EPO messenger RNA production in rodents. Messenger RNA for EPO can be demonstrated in the kidneys of rats after 1 h of hypoxemia and the serum EPO levels rise approximately 30 min later.

The linear relationship between hypoxemia and red blood cell production in normal man is well known. Yet the recognized association of chronic hypoxemia with polycythemia has shown no consistent relationship between red blood cell mass, arterial oxygen content and serum EPO in those individuals. The reasons for this apparent discrepancy are not clear but several hypotheses have been put forward. Earlier reports suggested that the impaired erythropoietic response to hypoxemia in subjects with chronic lung disease may be due to chronic inflammation in the bronchial tree, analogous to the situation in the anemia of other chronic inflammatory diseases. The finding of normal serum iron, iron binding capacity and transferrin saturation in patients with chronic lung disease argues against this mechanism.

Erythropoiesis can be impaired not only by defective EPO production but also by abnormalities in the EPO-responsive cell (ie, the erythroid progenitor cells). This might explain why serum EPO levels in patients with chronic lung disease have been reported as being either normal or high independent of the presence of polycythemia. However, more recent work has shown that the proliferative capacity of erythroid progenitors from both polycythemic and nonpolycythemic patients with chronic hypoxic lung disease is normal in vitro when exogenous EPO is added to culture. These data suggest that another mechanism is responsible for impaired erythropoiesis in chronic hypoxic lung disease.

In patients with hypoxic lung disease, correlations have been found between the red cell mass in smokers and carboxyhemoglobin levels and between serum EPO and carboxyhemoglobin. This is not surprising since increased carbon monoxide levels would lead to decreased oxygen content at the tissue level which should lead to a heightened EPO response. To eliminate this variable from the present study, the subjects in our study were not current smokers and, therefore, the EPO responses were unlikely modified by the generation of carboxyhemoglobinemia.

In rats, intermittent hypoxemia induces a larger increase in red cell mass than does continuous hypoxemia. In addition, mice made polycythemic by blood transfusion have a greater EPO response to hypoxemia if they have had previous hypoxic exposures. Polycythemic patients with chronic hypoxic lung disease had more episodes of nocturnal oxygen desaturation to < 80 percent than did their nonpolycythemic counterparts. The results of these studies along with the time course of EPO rise

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**Figure 2.** Change in serum EPO levels over time under conditions of normoxia (open squares) or hypoxia (solid triangles). Mean ± SEM levels for all samples are shown. *p < 0.05 as compared to time 0.
demonstrated in the present study suggest that transient episodes of hypoxemia, such as occur during sleep or exercise may lead to increased red blood cell production stimulated by EPO. This might explain the poor correlation between red blood cell mass, degree of hypoxemia and serum EPO levels reported in the past. That is, intermittent brief episodes of hypoxemia might transiently stimulate EPO production. Although, the daytime serum EPO levels would return to normal, the subsequent polycythemia would be sustained.

We have found that serum EPO rises within 2 h after the onset of hypoxemia. We continued our hypoxic challenge for 4 h and found that serum EPO continued to rise throughout the study period. It would be of interest to determine whether shorter periods of hypoxemia also lead to a rise in serum EPO. We now plan to study this question and to examine the reoxygenation recovery period to determine the duration of the EPO response in this group of patients.

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