Nocturnal desaturation and serum erythropoietin: a study in patients with chronic obstructive pulmonary disease and in normal subjects

Michael F. FITZPATRICK, Tom MACKAY, Kenneth F. WHYTE, Martin ALLEN, Robert C. TAM*, Caroline J. DORE†, Michael HENLEY†, P. Mary COTES* and Neil J. DOUGLAS
Respiratory Medicine Unit, Department of Medicine (RIE), City Hospital, Edinburgh, Scotland, U.K., and *Haemostasis Research Group and †Section of Medical Statistics, Clinical Research Centre, Harrow, Middlesex, U.K.

(Received 30 March/24 September 1992; accepted 20 November 1992)

1. To clarify the relationship between nocturnal oxygen desaturation and erythropoietin production in patients with chronic obstructive pulmonary disease, we determined arterial oxygen saturation and serum immunoreactive erythropoietin levels over 24 h in eight patients with chronic obstructive pulmonary disease and in nine normal subjects.

2. In the normal subjects, there was a significant circadian variation in serum erythropoietin levels with the highest mean deviation from the geometric mean at 22.00 hours and the nadir at 05.00 hours.

3. The three patients with chronic obstructive pulmonary disease with the most marked nocturnal desaturation (lowest arterial oxygen saturation <57%) and most marked daytime hypoxaemia (daytime arterial partial pressure of oxygen <6 kPa) had raised nocturnal serum erythropoietin levels. In two of these patients, the serum erythropoietin level was raised throughout the 24 h and erythrocyte mass was also raised. In the other patient, the serum erythropoietin level was not raised in five daytime samples and erythrocyte mass was normal.

4. The other five patients with chronic obstructive pulmonary disease with less severe nocturnal hypoxaemia (lowest arterial oxygen saturation range 78-86%) had serum erythropoietin levels (range 14-36 m-i.u./ml) which were indistinguishable from normal (range 12-44 m-i.u./ml) and showed circadian changes which were not significantly different (P=0.35) from those in the normal subjects.

5. Thus, mild nocturnal oxygen desaturation is not associated with elevation of serum erythropoietin levels, whereas daytime hypoxaemia with associated severe nocturnal desaturation is associated with increased serum erythropoietin levels both by day and by night.

INTRODUCTION

The relationship between erythrocyte mass and awake oxygen saturation is linear in healthy human subjects [1], but is highly variable in patients with chronic obstructive pulmonary disease (COPD) [2-5]. Some of this variability may relate to chronic infection or the anaemia from chronic disease [5, 6] or to varying nocturnal hypoxaemia in patients with COPD, with such nocturnal hypoxaemia contributing to the development of polycythaemia [7, 8]. Although there is no objective evidence in support of this contention, when sleep hypoxaemia in COPD was modelled in rats, intermittent hypoxaemia for as little as 2 h/day for 28 days significantly raised erythrocyte mass [9]. Recently, it has been reported that patients with COPD who desaturated at night to at least 85% saturation with more than 5 min spent below 90% saturation had significantly higher erythrocyte masses (ml/kg body weight) than patients with COPD who did not desaturate to this extent [10], suggesting that nocturnal hypoxaemia could be important.

Thus, to investigate the role of nocturnal desaturation in stimulating erythropoiesis, we have determined the concentration of erythropoietin (EPO) in serum and the arterial oxygen saturation (SaO₂) throughout a 24 h period in nine normal subjects and in eight patients with COPD.

METHODS

Subjects and protocol

We studied eight hypoxic patients with COPD, mean age 67 (SD 8) years, with previously documented daytime arterial partial pressure of oxygen (Pao₂) of 8.5 kPa or lower, who had been clinically stable for at least 6 weeks and were not on domiciliary oxygen therapy, and nine normal subjects without evidence of respiratory or renal disease, mean age 65 (SD 7) years (see Table 1). Eight of the nine normal subjects were lifelong non-smokers; the other had been an intermittent light smoker but had

Key words: circadian rhythm, chronic bronchitis, emphysema, erythropoietin, hypoxaemia, polycythaemia.

Abbreviations: COPD, chronic obstructive pulmonary disease; EPO, erythropoietin; Pao₂, arterial partial pressure of oxygen; SaO₂, arterial oxygen saturation.

Correspondence: Dr N. J. Douglas, Respiratory Medicine Unit, City Hospital, Greenbank Drive, Edinburgh EH10 5SB, Scotland, U.K.
not smoked for 2 years and had no respiratory symptoms and normal respiratory function. The mean body mass index of the COPD patients was 22 (SD 4) kg/m² and of the normal subjects was 24 (SD 3) kg/m². In each subject, a venous cannula was inserted into the forearm and flushed with 5 ml of saline (150 mmol/l NaCl) containing 2 units of heparin/ml. A 5 ml blood sample was taken via the cannula every 2 h during the day (10.00-22.00 hours) and hourly at night (23.00-09.00 hours), after which the cannula was flushed with heparinized saline. The 5 ml blood samples were allowed to clot at room temperature for 30 min before being centrifuged at 2000 g for 10 min. One millilitre of each supernatant serum was then transferred to a plain polypropylene tube and stored at -70°C until the EPO assay. Total blood loss from sampling during each 24 h study was less than 150 ml per subject. \( \text{SaO}_2 \) was measured for 30 min at each sample time during the day, and continuously at night, with an earlobe oximeter (Ohmeda Biox 3700). An arterial blood gas measurement was made on each subject during the daytime, when patients and normal subjects had been seated erect for at least 30 min.

During each individual’s study, overnight polysomnography was carried out, using our standard electrode placement [11]. This included electroencephalography, electrooculography, electromyography, inductance plethysmography (Respirotrac) and measurement of airflow with oronasal thermocouples. Overnight sleep studies began at 23.30 hours and ended at 06.30 hours.

EPO was estimated by radioimmunoassay using ‘method a’ described by Egrie et al. [12] and the Second International Reference Preparation of EPO as standard [13]. The mean interassay coefficient of variation of estimates was 7%. Serum samples from each subject were assayed as a batch in two independent assays and EPO concentrations are reported as the geometric mean of these two estimates. As far as possible, each assay included samples from both patients with COPD and control subjects. Samples were coded so that the analyst knew neither the time of collection of samples nor whether they came from the COPD group or the control group.

Erythrocyte mass and carboxyhaemoglobin were measured on all eight patients at least 1 month after the date of study.

Lung function tests (forced expiratory volume in 1 s and forced vital capacity) were performed on all patients and normal subjects during the early afternoon of the day of study.

All subjects gave their written informed consent to the study, which had the approval of the local Ethical Advisory Committee.

Statistical analysis

Analysis of variance was performed on the log-transformed serum EPO levels with the factors group and time using the program Genstat [14]. Estimates for the missing values (six in patients with COPD and eight in control subjects) were obtained using an iterative approach where the residuals corresponding to missing observations are set to zero [15]. The effect of group was assessed relative to the variation between individuals, whereas the effect of time and the interaction between group and time were assessed relative to the variation within individuals. It was originally intended to analyse the patients with COPD as a single group, but estimates of serum EPO levels in these subjects (Fig. 1), in contrast with control subjects, showed marked heterogeneity both between and within individuals. Similar heterogeneity was apparent in terms of oxygenation. The bronchitic patients were, therefore, divided on the basis of oxygenation into a severely hypoxic group (daytime \( \text{PaO}_2 \leq 6 \) kPa, nocturnal \( \text{SaO}_2 < 70\% \)) and a more normoxic group (daytime \( \text{PaO}_2 > 7.7 \) kPa, nocturnal \( \text{SaO}_2 > 70\% \)). There were no patients with a daytime \( \text{PaO}_2 \) between 6.1 and 7.6 kPa. An unpaired Student’s \( t \)-test or Wilcoxon rank test was used to compare data between patients and normal subjects.

RESULTS

\( \text{SaO}_2 \)

Mean daytime (10.00-23.00 hours) \( \text{SaO}_2 \) in the patient group, 83 (SEM 5)% was lower than in the normal subjects, 95 (SEM 0.3)% (\( P < 0.02 \)). The maximum overnight fall in \( \text{SaO}_2 \) was larger in the patients with COPD [18 (SEM 4)%] than in the normal subjects [5(SEM 1)%; \( P < 0.01 \)]. The minimum overnight \( \text{SaO}_2 \) in the patients with COPD ranged from 18 to 86%, and all patients with COPD spent at least 135 min with an \( \text{SaO}_2 \) below 90% and in four patients the lowest \( \text{SaO}_2 \) was <80%. The minimum overnight \( \text{SaO}_2 \) in the normal subjects ranged from 84 to 93%, and in seven of the nine subjects the lowest \( \text{SaO}_2 \) was >90%, whereas in the other two subjects there were brief episodes in which \( \text{SaO}_2 \) fell below 90% (Table 1).

Sleep results

All patients with COPD and normal subjects slept for at least 4 h during the study [patients with COPD, mean 5.7 (SEM 0.4) h, normal subjects, 6.0 (SEM 0.3) h; \( P = 0.52 \)] and passed through all sleep stages including rapid eye movement sleep. Polysomnographic analysis revealed no evidence of the sleep apnoea/hypopnoea syndrome in any patient with COPD or normal subject.

Serum EPO level

Estimates of serum EPO levels in individual patients and control subjects are shown in Fig. 1. In the three most hypoxaemic of the eight patients
Nocturnal desaturation and erythropoietin

Table 1. Data on the patients with COPD and normal subjects studied. The symbols indicate the identity of the patients and subjects in Fig. 1. Abbreviations: FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; Paco₂, arterial partial pressure of carbon dioxide.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Symbol in Fig. 1</th>
<th>Sex</th>
<th>Lowest Ŝao₂ at night (%)</th>
<th>Lung function test (% of predicted)</th>
<th>Daytime arterial blood gas partial pressures (kPa)</th>
<th>Erythrocyte mass* (I/kg)</th>
<th>Plasma volume† (I/kg)</th>
<th>Carboxyhaemoglobin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>▲</td>
<td>M</td>
<td>80</td>
<td>41</td>
<td>8.2</td>
<td>5.3</td>
<td>0.031</td>
<td>0.044</td>
</tr>
<tr>
<td>2</td>
<td>△</td>
<td>F</td>
<td>82</td>
<td>62</td>
<td>8.5</td>
<td>5.6</td>
<td>0.023</td>
<td>0.032</td>
</tr>
<tr>
<td>3</td>
<td>□</td>
<td>F</td>
<td>78</td>
<td>98</td>
<td>7.7</td>
<td>5.7</td>
<td>0.027</td>
<td>0.049</td>
</tr>
<tr>
<td>4</td>
<td>○</td>
<td>M</td>
<td>85</td>
<td>57</td>
<td>8.2</td>
<td>5.6</td>
<td>0.035</td>
<td>0.045</td>
</tr>
<tr>
<td>5</td>
<td>●</td>
<td>M</td>
<td>86</td>
<td>37</td>
<td>8.1</td>
<td>5.4</td>
<td>0.026</td>
<td>0.036</td>
</tr>
<tr>
<td>6</td>
<td>△</td>
<td>M</td>
<td>57</td>
<td>86</td>
<td>5.7</td>
<td>9.3</td>
<td>0.030</td>
<td>0.034</td>
</tr>
<tr>
<td>7</td>
<td>□</td>
<td>M</td>
<td>18</td>
<td>40</td>
<td>6.0</td>
<td>9.5</td>
<td>0.061</td>
<td>0.049</td>
</tr>
<tr>
<td>8</td>
<td>●</td>
<td>M</td>
<td>30</td>
<td>20</td>
<td>6.0</td>
<td>9.5</td>
<td>0.042</td>
<td>0.050</td>
</tr>
</tbody>
</table>

Control subjects

| 9        | ▼                | M   | 93                       | 96                                | 10.9                                         | 4.7                      |                      |                       |                       |
| 10       | ◆                | M   | 92                       | 115                               | 10.4                                         | 5.9                      |                      |                       |                       |
| 11       | ▲                | F   | 92                       | 114                               | 10.0                                         | 5.7                      |                      |                       |                       |
| 12       | ○                | M   | 90                       | 116                               | 11.5                                         | 4.8                      |                      |                       |                       |
| 13       | □                | M   | 84                       | 104                               | 9.3                                          | 5.1                      |                      |                       |                       |
| 14       | △                | M   | 91                       | 108                               | 10.6                                         | 4.4                      |                      |                       |                       |
| 15       | □                | M   | 80                       | 104                               | 11.3                                         | 5.4                      |                      |                       |                       |
| 16       | ●                | M   | 92                       | 103                               | 10.7                                         | 5.5                      |                      |                       |                       |
| 17       | ◆                | F   | 93                       | 105                               | 10.5                                         | 5.9                      |                      |                       |                       |

*Normal values: 0.027-0.035 I/kg (male), 0.023-0.029 I/kg (female).
†Normal value: 0.04-0.05 I/kg.

with COPD (nos. 6, 7 and 8) all estimates on all samples (except for five of the 15 samples from patient no. 6) were greater than the range of estimates (12-44 m-i.u./ml) seen in the control subjects. These three patients who had the most severe nocturnal hypoxaemia all showed rises in the serum EPO level between 03.00 and 08.00 hours, unlike the normal subjects (Fig. 2). In the remaining five patients with COPD, estimates of serum EPO levels were indistinguishable from those in the control subjects (Fig. 3) who had a marked circadian pattern of change in serum EPO levels with the highest levels at 22.00 hours and a nadir at 05.00 hours. The relatively normoxic bronchitic patients showed a nadir in serum EPO level at 02.00 hours, but the circadian pattern of change was not clear in this group (Fig. 3), although, from the analysis of variance, the pattern did not differ significantly (P = 0.35) between the non-desaturating bronchitic patients and the control subjects (Fig. 3). For these two groups combined, there were highly significant (P < 0.001) circadian changes.

Erythrocytosis

Erythrocyte mass was above normal in two patients only, nos. 7 and 8 (Table 1), the two patients in whom the serum EPO level was continuously raised. These were among those (patients nos. 6–8) who had the most severe daytime hypoxaemia (Pao₂ < 6 kPa) and had the lowest Ŝao₂ values at night (18 and 30%, Table 1). The pattern of nocturnal desaturation in patient no. 6, who did
not develop erythrocytosis, was considerably less severe than in patient nos. 7 and 8 with erythrocytosis (Fig. 2).

**DISCUSSION**

This study shows that, in patients with COPD, the serum EPO concentration was increased only in those with marked hypoxaemia throughout the 24 h period. Specifically, we found no evidence that short-term nocturnal desaturation resulted in elevation of the serum EPO concentration.

The three patients with the most severe nocturnal hypoxaemia all had increases in serum EPO levels later in the night. As the time lag between the onset of a hypoxic stimulus and the demonstration of an elevation of serum EPO level in man is 2–4 h [16, 17], this early morning elevation of serum EPO level could be related to nocturnal hypoxaemia.

Previous studies have failed to demonstrate any clear relationship between daytime serum EPO concentrations and either the degree of hypoxia or the presence or absence of polycythaemia in patients with COPD [18, 19]. Possible explanations include: (1) the transient nature of the serum EPO response to hypoxaemia [20, 21]; (2) the presence of concomitant respiratory or metabolic acidosis impairing the EPO response to hypoxic stimuli [22]; and (3) the possibility that drug treatment of associated cardiac failure may impair the EPO response to hypoxia [23]. None of the patients in the present study was acidotic or was taking an angiotensin-converting enzyme inhibitor. The three patients in the current study with elevated daytime serum EPO levels had a resting PaO₂ of 4–6 kPa. Only three of 20 patients with COPD studied by Wedzicha et al. [18] had a PaO₂ less than 6.6 kPa and in that study EPO was not estimated in serum samples collected at night. Our results demonstrate marked variability in serum EPO levels during the day in the three patients with elevated daytime serum EPO levels and in one patient the concentration remained within the normal range between 12.00 and 18.00 hours. Guidet et al. [19] found that serum EPO levels were similar in 12 severely hypoxic (mean PaO₂ 6.2 kPa) COPD patients with polycythaemia and 12 non-polycythaemic COPD patients matched for PaO₂. However, the latter finding was based on a single serum sample taken at 10.00 hours, and EPO was measured by an *in vitro* bioassay, which the authors state was not specific for EPO.
Miller et al. [24] demonstrated, on a single morning sample, an increase in serum EPO level in six out of 28 COPD patients with mild hypoxaemia or normoxia (Pao₂ > 6.8 kPa). However, on retesting after 3–6 months, the serum EPO level was normal in four of the five patients in whom it was initially elevated. Miller et al. [24] also measured serum EPO levels in 18 patients with COPD over 24 or 48 h and noted a diurnal variation in the group, with the highest serum EPO levels occurring at 24.00 hours rather than in the morning hours. The pattern of diurnal change in serum EPO concentrations in the patients studied by Miller et al. [24] was similar to that seen in the normal subjects in our study as well as by Cotes and Brozovic [25] in a normal subject and by Wide et al. [26] in hospital patients. Wedzicha et al. [18] noted that their polycythaemic COPD patients had a greater nocturnal desaturation than non-polycythæmic COPD patients with a similar daytime Pao₂, but found no significant difference between serum EPO levels at 07.00 hours and in the afternoon in the polycythaemic group.

Both the severity and the duration of hypoxia appear to be important in determining the EPO response. In rats, EPO secretion may not increase until the Sao₂ falls below 80% [27], and the minimum necessary duration of hypoxaemia is 30 min [28]. All three patients in the present study who had elevated serum EPO levels at night had daytime Sao₂ values below 85% and all spent most of their sleep time with an Sao₂ below 80% (Fig. 2). However, one of the less hypoxic patients (patient no. 3) had a drop in overnight Sao₂ to below 80% for 15–30 min without any rise in serum EPO level. Similarly, the serum EPO level has been found to be normal during the night and early morning in patients with obstructive sleep apnoea, despite recorded intermittent drops in Sao₂ to below 50% [29, 30]. This apparent anomaly seems likely to be explained by a difference in the duration of the hypoxic stimulus between patients with severe COPD and those with sleep apnoea: sleep apnoea is usually associated with very brief episodes of desaturation, whereas in severe COPD prolonged desaturation at night is superimposed on pre-existing hypoxaemia. Thus, the more prolonged nocturnal hypoxic stimulus in patients with COPD might accentuate the response of the renal EPO-producing cells to a greater extent than the recurring brief episodes of hypoxia seen in the sleep apnoea/hypopnoea syndrome.

The lack of change in serum EPO level after desaturation to 80% or below strongly suggests that the difference in erythrocyte mass between ‘desaturators’ and ‘non-desaturators’ reported by Fletcher et al. [10] did not result solely from differences in nocturnal oxygenation. Indeed, the ‘desaturators’ in that study were significantly more hypoxaemic when awake than the ‘non-desaturators’. However, further studies are required to delineate the threshold for EPO release in terms both of magnitude and duration of hypoxaemia in human subjects and the increased EPO production needed to induce erythrocytosis.

We have thus documented significant circadian variation in serum EPO concentrations in normal subjects (Fig. 3). The study showed that the serum EPO level was higher during the day and night in the three most severely hypoxic COPD patients as compared with normal subjects. In these three patients, there was marked nocturnal desaturation and a nocturnal increase in the serum EPO level from about 03.00 hours to 07.00–08.00 hours. There was no evidence of elevated serum EPO concentrations in the other five patients, despite the fact that all spent more than 2 h at night with an Sao₂ below 90%.

ACKNOWLEDGMENTS

We thank Dr J. C. Egrie of Amgen for antisera used in the radioimmunoassay, and the World Health Organization for the International Reference Preparation of EPO.

REFERENCES