Low Erythropoietin Plasma Levels during Exacerbations of COPD

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Key Words

Emphysema • Chronic bronchitis • Hemoglobin

Abstract

Background: It is known that pro-inflammatory cytokines suppress in vitro the gene expression and protein production of erythropoietin (Epo). We hypothesized that systemic inflammation in patients with chronic obstructive pulmonary disease (COPD) may influence Epo production, particularly during episodes of exacerbation of the disease (ECOPD) where an inflammatory burst is known to occur. **Objectives:** We compared the plasma levels of Epo and high-sensitivity (hs) C-reactive protein (hsC-RP) in patients hospitalized because of ECOPD ($n = 26$; FEV_1 : $48 \pm 15\%$ predicted), patients with clinically stable COPD ($n = 31$; FEV_1 : $49 \pm 17\%$ predicted), smokers with normal lung function ($n = 9$), and healthy never smokers ($n = 9$). **Methods:** Venous blood samples were taken between 9 and 10 a.m. after an overnight fast into tubes with EDTA (10 ml) or without EDTA (10 ml). Plasma levels of Epo (R&D Systems Inc., Minneapolis, Minn., USA) and hsC-RP (BioSource, Belgium) were determined by ELISA. **Results:** Log-Epo plasma levels were significantly lower (0.46 ± 0.32 mU/ml) in ECOPD than in stable COPD (1.05 ± 0.23 mU/ml), smokers (0.95 ± 0.11 mU/ml) and never smokers with normal lung function (0.92 ± 0.19 mU/ml) ($p < 0.01$,

each). In a subset of 8 COPD patients who could be studied both during ECOPD and clinical stability, log-Epo increased from 0.49 ± 0.42 mU/ml during ECOPD to 0.97 ± 0.19 mU/ml during stability ($p < 0.01$). In patients with COPD log-Epo was significantly related to hsC-RP ($r = -0.55$, $p < 0.0001$) and circulating neutrophils ($r = -0.48$, $p < 0.0001$). **Conclusions:** These results show that the plasma levels of Epo are reduced during ECOPD likely in relation to a burst of systemic inflammation.

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Introduction

The best known function of erythropoietin (Epo) is the regulation of red blood cell production in the bone marrow [1]. However, Epo has other relevant, albeit less well characterized, biological effects. For instance, expression of the Epo receptor and biologic responses to Epo have been described in endothelial, neural, muscle, and cardiac cells [2–5]. Likewise, Epo appears to mobilize endothelial progenitor cells from the bone marrow (EPCs)

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and to increase neovascularization in coronary ischemia [6]. In fact, in an in vitro angiogenesis assay the stimulation of capillary outgrowth by Epo was comparable to that produced by the vascular endothelial growth factor (VEGF) [7, 8]. On the other hand, it is known that the pro-inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) suppress Epo gene expression and Epo protein secretion in vitro, and may be responsible for impaired Epo synthesis in inflammatory diseases in vivo [9].

Chronic obstructive pulmonary disease (COPD) is characterized by pulmonary and systemic inflammation [10, 11–14], both of which flare up during exacerbations of the disease (ECOPD) [15–19]. Epo regulation in relation to systemic inflammation has been seldom studied in COPD. One study reported that Epo levels were increased in patients with stable COPD with respect to controls, only if anemia (defined by a hemoglobin (Hb) concentration lower than 13.5 g/dl) was present [20]. Interestingly, the subgroup of patients with COPD and anemia showed more systemic inflammation than those without it [18]. To our knowledge, no previous data has been reported on Epo regulation during ECOPD, where a burst of systemic inflammation is known to occur [15–19]. We hypothesized that, contrary to what has been described in patients with stable COPD [20], plasma Epo levels will be reduced during ECOPD. To test this hypothesis, we compared the plasma levels of Epo in patients hospitalized because of ECOPD, patients with stable COPD, smokers or ex-smokers with normal lung function and healthy never smokers. To investigate the role of systemic inflammation, we quantified in these individuals the plasma levels of high-sensitivity (hs) C-reactive protein (hsC-RP).

Methods

Subjects and Ethics

We prospectively included in the study 26 patients with ECOPD, 31 patients with clinically stable COPD, 9 smokers or ex-smokers with normal lung function and 9 healthy never smokers, all of them male. All participants signed their informed consent once aware of the nature and purpose of the study. The study protocol had been approved by IRB of the Balearic Islands.

Study Design

Patients with ECOPD ($n = 26$) were consecutively recruited from the emergency room of our hospital. The diagnosis of COPD was established according to the criteria of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [21]. Exclusion criteria included clinical evidence of pneumonia, pulmonary embolism, acute cardiovascular diseases, renal insufficiency, liver

diseases, autoimmune diseases, cancer of any origin, respiratory or metabolic acidosis and signs of gastrointestinal bleeding. According to clinical interview, the onset of ECOPD occurred at least 48 h prior to hospitalization in all patients studied. In these patients, all tests were performed during the first 24 h of hospital admission and only if the patient had received a maximum of one dose of systemic corticosteroids and/or antibiotics. In 8 of these 26 ECOPD patients, measurements could be repeated during clinical stability, three months after hospital discharge. Treatment of ECOPD was standardized in all patients and included: (1) aerosolized bronchodilators (ipratropium bromide 500 μ g and fenoterol 250 μ g four times daily); (2) systemic glucocorticoids (inpatient regimen: methylprednisolone 40 mg three times daily for one day, 40 mg twice daily one day and 40 mg once daily up to hospital discharge. Home regimen: descending doses of oral prednisone for twelve days starting from 30 mg); (3) antibiotics (amoxycillin 875 mg and clavulanic acid 175 mg three times daily for seven days or levofloxacin 500 mg once daily for seven days), and (4) controlled oxygen therapy to maintain a transcutaneous oxygen saturation (SaO_2) above 90%.

Patients with stable COPD ($n = 31$) were consecutively recruited from the outpatient clinic of our institution. The diagnosis of COPD was established according to the GOLD guidelines [21]. Clinical stability was defined by the absence of ECOPD or treatment change during the 8 weeks that precede the inclusion in the study; none of these patients was receiving systemic steroids or domiciliary oxygen therapy at the time of the study and none had neuromuscular, rheumatologic and/or unstable cardiovascular or cerebrovascular disease.

Smokers with normal lung function ($n = 9$) were recruited from the pulmonary function laboratory of our department, whereas healthy volunteers ($n = 9$) were recruited from the general population using a newspaper announcement. All of them were older than 50 years of age, had a sedentary lifestyle, a normal spirometry and wish to participate in the study.

Blood Sampling and Measurements

Venous blood samples were taken between 9 and 10 a.m. after an overnight fast into tubes with EDTA (10 ml) or without EDTA (10 ml) for biochemical determinations. The latter were centrifuged immediately. After centrifugation serum and plasma were separated in aliquots and stored at -80°C until analysis. Measurements were done in duplicate, and mean values were used for analysis. Total, differential leukocytes and red blood cells counts and Hb concentration were determined automatically in whole blood (Sysmex K-4500, Toa Medical Electronics Co. Ltd., Kobe, Japan). The plasma levels of Epo (R&D Systems Inc., Minneapolis, Minn., USA) [22, 23] and hsC-RP (BioSource, Belgium) were determined by ELISA.

Lung Function

Spirometry (Medical Graphics Corporation, St. Paul, Minn., USA) was determined in all participants according to international guidelines [24, 25]. Reference values were those of a Mediterranean population [25]. In patients hospitalized because of ECOPD, spirometry was determined at hospital discharge. Arterial blood gases (IL BG3, Izasa, Spain) were obtained in all ECOPD on admission breathing room air. SaO_2 (Criticare System Inc., Waukesha, Wisc., USA) was obtained in ECOPD and stable patients with COPD also breathing room air.

Table 1. Anthropometric, clinical and functional data of all participants

	ECOPD	Stable COPD	Smokers	Never smokers	p value ANOVA
n	26	31	9	9	
Age, years	67 ± 8	68 ± 7	60 ± 9	61 ± 4	<0.05
Body mass index	26.3 ± 5.5	26.4 ± 4.6	26.8 ± 2.4	25.0 ± 3.4	NS
Active smokers, n (%)	13 (50)	10 (32)	5 (56)	Ø	
FEV ₁ /FVC _{post-bronchodilation} , %	51 ± 14 ^a	51 ± 14 ^a	77 ± 6	92 ± 14	<0.001
FEV ₁ post-bronchodilation, %	48 ± 15 ^a	49 ± 17 ^a	105 ± 8	103 ± 10	<0.001
FVC _{post-bronchodilation} , %	75 ± 8 ^a	76 ± 10 ^a	118 ± 6	108 ± 4	<0.001
FEV ₁ pre-bronchodilation, %	46 ± 15 ^a	50 ± 18 ^a	108 ± 6	109 ± 5	<0.001
GOLD stage, n (%)					
1 or 2	9 (35)	15 (48)			
3 or 4	17 (65)	16 (52)			
Cardiovascular risk factors	16 (46)	18 (58)	6 (60)	Ø	

^a Significantly different from non-COPD controls.

Statistical Analysis

Results are expressed as mean ± SD. A Kolmogorov-Smirnov test was used to assess if variables followed a normal distribution. The distribution of Epo values was significantly different from a normal distribution and fitted better with a log-normal distribution that was used for statistical analyses. One-way analysis of variance (ANOVA) was used to compare differences between groups, followed by post-hoc contrasts (Bonferroni) if appropriate. The statistical significance of differences in the subset of 8 patients studied during ECOPD and clinical stability was investigated by the paired Student's t test, whereas between patients with and without anemia was investigated by the unpaired Student's t test. The Pearson test was used to analyze potential correlations between variables of interest. Statistical significance was set at $p < 0.05$.

Results

Table 1 presents the main clinical, anthropometric and lung function data of all participants. Age and body mass index (BMI) were similar in all groups. The prevalence of systemic hypertension, diabetes mellitus and hypercholesterolemia was also similar in COPD patients and smokers with normal lung function, but higher from that seen in healthy subjects. Both groups of COPD patients showed moderate to severe airway obstruction, whereas spirometry was normal in the other two groups (table 1). At the emergency room, patients with ECOPD showed respiratory failure (pH 7.40 ± 0.04 , PaO₂ 59.2 ± 6.4 mm Hg, PaCO₂ 43.2 ± 8.6 mm Hg, SaO₂ $90.5 \pm 3.7\%$). SaO₂ determined at rest in patients with stable

COPD was $94.6 \pm 1.8\%$. Mean Hb levels were similar in the 4 groups studied (table 2). If anemia is defined by hemoglobin levels lower than 13.0 mg/dl [22], 4 of the 31 patients with stable COPD (13%) and 7 of the 26 patients with ECOPD (27%) had anemia.

Table 2 shows the individual and mean values (bars) of log-Epo plasma levels in the four groups of individuals studied. These were significantly lower (0.46 ± 0.32 mU/ml) in patients with ECOPD than in those clinically stable (1.05 ± 0.23 mU/ml), smokers with normal lung function (0.95 ± 0.11 mU/ml) and never smokers (0.92 ± 0.19 mU/ml) ($p < 0.01$). log-Epo plasma levels in patients with stable COPD were not significantly different from those of smokers with normal lung function and never smokers (table 2). Likewise, they were not different between GOLD stages of disease severity (table 3). In the subset of 8 COPD patients studied under both conditions, log-Epo plasma levels increased ($p < 0.01$) from 0.49 ± 0.42 mU/ml during ECOPD to 0.97 ± 0.19 mU/ml at clinical stability (table 4). Neither the presence of CV risk factors (systemic hypertension, diabetes mellitus and hypercholesterolemia) nor active smoking influence Epo plasma levels during ECOPD or in stable conditions (data not shown).

During ECOPD, there was evidence of a flare-up of systemic inflammation, as shown by the higher leukocyte and neutrophil counts (table 2), as well as higher plasma hsC-RP levels ($p < 0.01$) in patients with ECOPD (49.5 ± 42.6 mg/l) than in those with clinically stable disease (4.5 ± 4.7 mg/l), smokers with normal lung

Table 2. Mean ($\pm$ SD) values of the blood variables determined in the study

	ECOPD	Stable COPD	Smokers	Never smokers	p value (ANOVA)
n	26	31	9	9	
Epo, mU/ml	3.7 $\pm$ 2.7 ^{a, b}	12.9 $\pm$ 7.9	9.1 $\pm$ 2.5	9.1 $\pm$ 4.4	<0.01
log-Epo, mU/ml	0.46 $\pm$ 0.32 ^{a, b}	1.05 $\pm$ 0.23	0.95 $\pm$ 0.11	0.92 $\pm$ 0.19	<0.01
Hemoglobin, g/dl	14.2 $\pm$ 1.6	14.8 $\pm$ 1.6	15.0 $\pm$ 1.1	14.9 $\pm$ 1.2	NS
hsC-RP, mg/l	49.5 $\pm$ 42.6 ^{a, b}	4.5 $\pm$ 4.7	2.9 $\pm$ 2.3	1.3 $\pm$ 1.1	<0.01
Leukocytes, $\times 10^3$ /ml	10.8 $\pm$ 2.9 ^{a, c}	7.4 $\pm$ 1.9 ^c	8.7 $\pm$ 3.0 ^c	5.3 $\pm$ 1.2	<0.01
Neutrophils, $\times 10^3$ /ml	9.3 $\pm$ 2.6 ^{a, b}	4.8 $\pm$ 1.6 ^c	5.4 $\pm$ 2.5 ^c	3.1 $\pm$ 1.1	<0.01

^a Significantly different from stable COPD; ^b significantly different from non-COPD controls; ^c significantly different from never smokers.

Table 3. Erythropoietin plasma levels, hemoglobin and systemic inflammation in ECOPD and stable COPD patients according to GOLD stages

	ECOPD GOLD 1–2	ECOPD GOLD 3–4	Stable COPD GOLD 1–2	Stable COPD GOLD 3–4
N	9	17	15	16
Epo, mU/ml	2.9 $\pm$ 1.6 ^a	4.0 $\pm$ 3.0 ^b	11.7 $\pm$ 6.2	14.2 $\pm$ 9.3
log-Epo, mU/ml	0.42 $\pm$ 0.23 ^a	0.50 $\pm$ 0.31 ^b	1.02 $\pm$ 0.19	1.07 $\pm$ 0.27
Hb, g/dl	15.1 $\pm$ 1.2	14.1 $\pm$ 1.8 ^b	15.0 $\pm$ 1.2	14.6 $\pm$ 1.9
hsC-RP, mg/l	59.3 $\pm$ 41.5 ^a	50.1 $\pm$ 43.6 ^b	3.8 $\pm$ 3.5	5.2 $\pm$ 5.6
Leukocytes, $\times 10^3$ /ml	10.8 $\pm$ 2.7 ^a	10.9 $\pm$ 3.3 ^b	7.5 $\pm$ 1.9	7.3 $\pm$ 1.9
Neutrophils, $\times 10^3$ /ml	9.4 $\pm$ 2.6 ^a	9.5 $\pm$ 2.8 ^b	4.9 $\pm$ 1.5	4.7 $\pm$ 1.7

^a $p < 0.01$: significantly different from stable COPD GOLD stage 1–2; ^b $p < 0.01$: significantly different from stable COPD GOLD stage 3–4.

function (2.9 ± 2.3 mg/l) and never smokers (1.3 ± 1.1 mg/l). The latter was confirmed in the subset of 8 COPD patients studied both during ECOPD (10.1 ± 4.1 mg/ml) and at stability (4.6 ± 4.0 mg/ml, $p < 0.01$) (table 4). In patients with COPD, Epo levels were related to both hsC-RP ($r = -0.55$, $p < 0.0001$; fig. 1a) and circulating neutrophils ($r = -0.48$, $p < 0.0001$; fig. 1b), but not to Hb levels ($r = -0.05$, $p = 0.7$) or arterial PO_2 ($r = 0.04$, $p = 0.8$), albeit it should be noted that we measured arterial PO_2 only in patients with ECOPD. HsC-RP levels were similar in different GOLD stages of disease severity (table 3).

When patients were divided according to the presence or absence of anemia (hemoglobin levels lower than 13.0 mg/dl [22]), we did not observe any difference

Table 4. Mean ($\pm$ SD) values of erythropoietin, systemic inflammation and hemoglobin levels in 8 COPD patients studied during an exacerbation and in stability

	ECOPD	COPD in stability
Epo, mU/ml	4.6 $\pm$ 4.0*	10.1 $\pm$ 4.1
log-Epo, mU/ml	0.49 $\pm$ 0.42*	0.97 $\pm$ 0.19
hsC-RP, mg/l	66.1 $\pm$ 35.9*	7.6 $\pm$ 8.3
Leukocytes, $\times 10^3$ /ml	11.5 $\pm$ 2.4*	8.9 $\pm$ 2.8
Neutrophils, $\times 10^3$ /ml	10.2 $\pm$ 2.1*	5.5 $\pm$ 2.6
Hb, g/dl	13.5 $\pm$ 1.7	13.8 $\pm$ 0.9

* $p < 0.05$: significantly different from stable COPD.

Fig. 1. Significant negative relationship between log-Epo and both hsC-RP plasma levels ($r = -0.55$, $p < 0.0001$) (a) and neutrophils ($r = -0.48$, $p < 0.0001$) (b). See text for further details. Stable COPD, $n = 31$; ECOPD, $n = 26$.

Table 5. Erythropoietin plasma levels and systemic inflammation in ECOPD and stable COPD patients with and without anemia

	ECOPD without anemia	ECOPD with anemia	Stable COPD without anemia	Stable COPD with anemia
n	19	7	27	4
Hb, g/dl	14.9 ± 1.2^a	12.4 ± 0.6	15.1 ± 1.3^b	12.5 ± 0.9
log-Epo, mU/ml	0.44 ± 0.23^c	0.51 ± 0.53^d	1.02 ± 0.21	1.26 ± 0.27
hsC-RP, mg/l	56.8 ± 43.9^c	29.2 ± 33.0^d	3.9 ± 3.2	8.5 ± 10.2
Leukocytes, $\times 10^3/\text{ml}$	10.5 ± 3.1	11.6 ± 2.1	7.5 ± 2.0	6.6 ± 1.2
Neutrophils, $\times 10^3/\text{ml}$	9.1 ± 2.7^c	10.1 ± 2.6^d	4.8 ± 1.6	4.5 ± 1.2

^a $p < 0.05$: significantly different from ECOPD with anemia; ^b $p < 0.05$: significantly different from stable COPD with anemia; ^c $p < 0.05$: significantly different from stable COPD without anemia; ^d $p < 0.05$: significantly different from stable COPD with anemia.

in log-Epo plasma levels in both stable patients and patients with ECOPD (table 5). Also, hsC-RP plasma levels as well as leukocyte and neutrophil counts were similar in patients with and without anemia, both during ECOPD and clinical stability (table 5). Finally, ECOPD patients, with and without anemia, had lower log-Epo plasma levels and higher systemic inflammation ($p < 0.05$, both) than stable COPD patients, with and without anemia (table 4).

Discussion

The main and novel finding of this study is that Epo plasma levels are reduced during ECOPD likely in relation to a burst of systemic inflammation.

Previous Studies

Several previous studies have investigated Epo regulation in patients with stable COPD [20, 27–30]. In general, these studies showed that Epo concentration in COPD

was similar to that seen in control subjects, except in those patients with chronic respiratory failure, severe nocturnal desaturation or anemia, in whom Epo levels were increased [20, 27–29]. The influence of systemic inflammation on Epo regulation in COPD is less well understood. A previous study in patients with stable COPD and mild arterial hypoxemia failed to find any relationship between the systemic inflammation and Epo levels [20]. All in all, these observations are in keeping with the results of our study since we also found that patients with stable COPD with mild hypoxemia had similar Epo plasma levels than controls and that, in the subgroup of patients with anemia Epo levels were increased (table 5), although they do not reach statistical significance probably due to a type II error since only 4 patients with stable COPD had anemia.

Interpretation of Findings

We found that Epo plasma levels are significantly reduced during ECOPD (tables 1, 4). In keeping with this observation, a previous paper in patients with a number of causes of acute respiratory failure, including COPD, pneumonia and heart failure, also reported reduced Epo plasma levels [31]. We propose here that Epo down-regulation during ECOPD can be explained by the burst of inflammation that occurs during these clinical circumstances [32], as shown here by a significant increase in leukocyte and neutrophil counts, as well as by the raised concentration of hsC-RP during ECOPD (table 5). Several pieces of evidence support this proposal. First, in vitro experiments have demonstrated that IL-1 and TNF- α suppress Epo expression and secretion by activating the transcription factors GATA-2 and NF- κ B [9]. Second, clinical investigations indicate that TNF- α suppresses Epo secretion in patients with advanced solid tumors and chronic infection [33, 34]. Third, Epo plasma levels in anemic patients who suffer concomitantly of other inflammatory process are often lower than expected in relation to their hemoglobin concentration [35]. Fourth and finally, the results of our study also support this proposal by identifying a significant negative association between Epo plasma levels and both hsC-RP and neutrophils in the patients studied here (fig. 1). Interestingly, this inflammatory mechanism appears to overcome the regulation of EPO by oxygen, given that the hypoxemia that occurs during exacerbations of COPD would be a strong stimulus for Epo production [36, 37].

The prevalence of anemia in different studies of patients with clinically stable COPD ranges between 13 and

17% [20, 38]. Our results in stable patients (13%) are in keeping with these estimates. Interestingly, the percentage of patients with anemia during ECOPD appeared to be much higher (27%). The fact that ECOPD patients with anemia showed lower log-Epo plasma levels (0.51 ± 0.53 vs. 1.26 ± 0.27 mU/ml, $p < 0.05$) and higher both hsC-RP concentrations (29.2 ± 33.0 vs. 8.5 ± 10.2 mg/l, $p < 0.05$) and neutrophil cell counts (10.1 ± 2.6 vs. $4.5 \pm 1.2 \times 10^3$ /ml, $p < 0.05$) than those with stable COPD and anemia (table 5), is in keeping with our interpretation that Epo is downregulated during ECOPD regardless of the existence of anemia and likely in relation to systemic inflammation.

Clinical Implications

We did not explore the potential clinical implications of the observed Epo downregulation during ECOPD. However, we can speculate that these reduced Epo levels can contribute to: (1) the increased prevalence of anemia reported in COPD [38], because Epo is a key regulator of red blood cell production in the bone marrow [1]; (2) cardiovascular morbidity and mortality during ECOPD [39–40] since Epo has been implicated in the neovascularization process that occurs during acute coronary artery events [6], and, perhaps (3) the pathogenesis of emphysema given that Epo is known to interfere with the VEGF pathway [41], and blockade of VEGF-receptor 2 causes emphysema in experimental animals [42]. Of course, all these possibilities require testing in future studies.

Potential Limitations

Several potential limitations of our study deserve comment. First, we studied four independent groups of subjects, but results were basically reproduced in the admittedly small subgroup of patients that could be studied both during ECOPD and clinical stability. Second, it has been suggested that the physiological circadian rhythm of Epo is lost in COPD patients [43] and we did not study it, but, as indicated above, all samples were taken at the same time (between 9 and 10 a.m.). Third, we did not measure arterial PO₂ in patients with stable COPD and, therefore, we were not able to fully investigate the role of blood oxygenation in these patients. Fourth, we did not study the influence of iron metabolism or red cell life span on Epo levels in our patients. Finally, steroids can certainly reduce inflammation [44] and have the potential to influence Epo gene expression directly in experimental animals [45], but their effects upon Epo regulation in vivo are less clear [46]. In our study, stable COPD

patients did not receive systemic steroids, and ECOPD patients had received one dose of systemic glucocorticoids before sampling at most.

Conclusions

Our study shows that Epo plasma levels are reduced during ECOPD and suggest that this is likely related to the burst of systemic inflammation that occurs under

these circumstances. The clinical implications of this observation will have to be investigated in future studies.

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