Normal erythropoietin response in chronic hepatitis C patients with ribavirin-induced anaemia

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Introduction

The combination of type I interferon (IFN) with ribavirin (RBV) is the current treatment of choice for chronic hepatitis C (CHC) [1]. The administration of RBV is almost invariably associated with the development of haemolytic anaemia, which is characterized by a variable fall in haemoglobin (Hb) concentration [2–4]. Several mechanisms contribute to the pathogenesis of RBV-induced anaemia, such as accumulation of the drug into red blood cells [5,6], extravascular erythrophagocytic destruction [7,8], as well as erythrocyte metabolic impairment [9,10] and membrane oxidative damage [11]. The reduction of Hb appears early during treatment and progresses to a nadir value during the first 4–8 weeks of treatment [3]. The manifestations of RBV-induced anaemia include chronic fatigue, reduced physical performances and shortness of breath on exertion [12–15].

In the normal, healthy subject, haemolytic anaemia elicits an increased production of erythropoietin (EPO) by the renal cortex and, to a lesser extent, the liver [16]. EPO is a cell proliferation- and differentiation-promoting glycoprotein hormone, which physiologically stimulates the maturation of red blood cell precursors [17,18]. Its levels are regulated by blood oxygen content and several other factors interacting in a complex manner [19]. When oxygen levels fall, as in anaemia, and renal hypoxia ensues, EPO blood level increases and stimulates red blood cell volume reconstitution [19–21]. Recent studies, reported in preliminary form, suggest a possible role of EPO administration in hepatitis C virus (HCV)-infected patients treated with RBV in association with IFN [22–25]. CHC patients experiencing RBV-induced anaemia had an increased Hb concentration and a lower frequency of RBV dose reduction [23,24], as well as deriving symptomatic relief [25] when treated with EPO, 40000 U/week sc., compared with controls. However, the effect of RBV...
administration on endogenous EPO production has not been evaluated [22].

Accordingly, the aim of the present study was to further investigate the possible role of EPO in this clinical setting, by evaluating the dynamics of endogenous EPO in response to the RBV-induced anaemia that occurs in CHC patients undergoing antiviral combination treatment.

Materials and methods

Study subjects
Eighteen anti-HCV and HCV RNA-positive patients, treated in our unit from October 2000 to April 2001 with α-2b-IFN, 3 M U three times weekly sc., plus oral RBV, 800–1200 mg/day, were studied under informed consent. All patients were either non-responder or had relapsed after a previous IFN treatment. Subjects with pre-existing anaemia (Hb level <13 or <12 g/dl in males and females, respectively), evidence of kidney disorder or abnormal creatinine values, chronic obstructive pulmonary disease or lung fibrosis, cardiac or vascular disease, and HBV or HIV co-infection were excluded.

Clinical and laboratory evaluation
Physical examination and laboratory exams, including liver and kidney function tests, were performed every 14 days during the first 12 weeks of treatment. Patient weight was recorded at each visit and the total plasma volume was derived by estimated blood volume (8% of body weight) and relevant haematocrit (Hct) level. Creatinine clearance (CC) was calculated as: \((\text{140 - age}) \times \text{weight} / (72 \times \text{creatinine}) \times 0.85 \text{ in females})\). A liver biopsy was performed in all patients within 18 months prior to treatment start. The pathological assessment was made by a single pathologist according to standard criteria [26]. The Hb nadir was defined as the lower value of Hb observed during the first 12 weeks of combination treatment. The two blood counts obtained, respectively, within 30 days before treatment start (baseline) and at the Hb concentration nadir were used for the purpose of this investigation. At the same time-points, serum samples were obtained and stored at –20°C until further use. Serum EPO was measured at baseline and at the Hb concentration nadir with EPO-Trac I125 (DiaSorin, Saluggia VC, Italy), which is a highly specific, competitive binding, disequilibrium radioimmunoassay procedure utilizing recombinant human EPO for both tracer and standards. EPO concentration ([EPO]) is expressed in mU/ml of serum.

Mathematical analysis and modelling
To investigate whether EPO response to the RBV-induced anaemia was adequate or, in contrast, negatively influenced by the presence of chronic liver disease or the antiviral treatment itself, the results obtained in the 18 CHC patients were compared with a reference regression line [27]. The line coefficients were derived from experimental data [28] obtained, also by radioimmunoassay, in subjects with ‘uncomplicated’ [29], non-renal anaemia due to a single aetiology/mechanism and no liver disease [28]. The reference regression line was calculated using the least squares method [27,28] and its equation was: \(\log_{10}[\text{EPO}] = 3.42 - (0.056 \times \text{Hct})\) for Hct <40% and \(1.311 - (0.003 \times \text{Hct})\) for Hct >40% [28]. The predicted \(\log_{10}[\text{EPO}]\) value was estimated by extrapolation from the reference regression equation. The \(\log_{10}\) observed/predicted ratio (O/P) was then calculated and used to estimate the adequacy of EPO response in the CHC subjects [27,28]. The 95% confidence limits for normal EPO O/P were 0.8–1.22 [28].

The adequacy of EPO response was further evaluated by comparing the distribution of the individual values of EPO concentration per Hb in CHC subjects with the 95% confidence interval area [30] of normal EPO concentration per Hb value derived in 50 subjects with ‘uncomplicated’, iron-deficiency and autoimmune haemolytic anaemia (data kindly provided by Dr ME Roque, Universidad Nacional del Sur, Argentina) [31].

To compare the extent of the increase in EPO production after RBV treatment start in our patient group with the dose of rHuEPO previously recommended [22], an ad hoc mathematical model was developed.

The baseline daily EPO production in CHC patients was first quantified. A discrete time model was applied under the following assumptions: mean native EPO half-life is 2 h [32]; before treatment start, [EPO] is at the steady state and equal to the concentration found in each of the CHC patients studied.

Subsequently, the excess amount of EPO produced in response to RBV-induced anaemia was estimated, using a discrete time model and assuming that [EPO] in CHC patients increases steadily from baseline to a level corresponding to its concentration at the Hb nadir. The model describing [EPO] increase was: \(x(k+1) = ax(k) + 0.5 + ax(k), \) where \(x(k)\) is [EPO] at time \(k, \) \(k\) is the time interval equal to 2 h and \(a\) is the [EPO] increasing rate, chosen in individual patients depending on the time to Hb nadir and relevant [EPO].

It was also assumed that the endogenous EPO production in rHuEPO-treated patients is not influenced by rHuEPO administration, rHuEPO has the same structure/function profile of native EPO, and its bioavailability after subcutaneous injection is 20% [33].
Statistics

All values were expressed as mean ±SD, unless otherwise specified. Two-tailed, paired Students' t-test was applied to compare EPO values before and during treatment. The maximal reduction in Hb concentration (referred to as Δ-Hb) and the variation in [EPO] (referred to as Δ-EPO) were compared with simple correlation by the Pearson method. CC values were compared with Δ-Hb and Δ-EPO values with the Spearman rank test. A 5% level was assumed to denote statistical significance. The Primer Statistics 1.0 package was used for the analysis.

Results

The median age of the study population was 59 years (range 24–73); 11 patients (60%) were male (Table 1). Seven patients (40%) showed stage 4 fibrosis on liver biopsy. Hepatic synthetic function was well conserved in all patients.

Anaemia, erythropoietin concentration and liver disease

Mean Hb at baseline was 14.6 ±1.6 g/dl. It decreased to 11.5 ±1.5 g/dl at the concentration nadir. The average Δ-Hb was 3.1 ±1.8 g/dl (Table 1). The mean time to Hb nadir was 40 ±17 days (Table 2). Baseline mean [EPO] was normal and all individual values were within normal limits (Table 1). However, six patients (33%) showed an abnormal baseline log10[EPO] O/P (data not shown). Among these, three had an inappropriately high [EPO] relative to Hb, while in the other three patients [EPO] was lower than predicted from the Hb level. The serum [EPO] significantly increased in CHC patients at the Hb nadir compared with baseline (P<0.0001). Mean [EPO] at the Hb nadir was 55.5 ±30.5 mU/ml. The average Δ-EPO was 37.6 ±29.5 mU/ml. A significant direct correlation was found between Δ-Hb and Δ-EPO (r=0.58; P=0.011) (Figure 1). No significant association was found between EPO concentration and the stage of liver disease (Table 1), as well as the liver synthetic function (data not shown).

In addition, there was no correlation between Δ-Hb and CC (r=–0.22; P=0.37) or between CC and Δ-EPO (r=–0.35; P=0.15) (Table 1). However, the highest Δ-Hb values were observed in the two subjects with the lowest levels of CC (Table 1).

Degree of erythropoietin response to the ribavirin-induced anaemia

The range of EPO response pattern for the degree of anaemia was overlapping in the CHC patients and the control anaemic subjects. In fact, most (16 of 18; 89%) of the individual values representing the relationship between Hb and EPO concentrations in serum of CHC patients, at the Hb nadir during RBV administration, fell within the 95% confidence bounds of the reference regression line obtained in the control group (Figure 2).

Table 1 shows the results of the EPO O/P analysis. The average O/P for log10[EPO] was 1.11 ±0.15, indicating the overall EPO response to RBV-induced
anaemia in CHC patients was adequate. Indeed, the sample mean fell within the 95% confidence limits of reference normal log10\[EPO\] O/P. Three patients (16.6%) showed an over-response relative to their Hb level. In two cases, this inappropriately higher EPO response was minor (O/P, 1.24). One patient, whose baseline [EPO] was already inappropriately low, showed a similarly ‘inadequate’ EPO response to RBV-induced anaemia.

Quantitative estimate of erythropoietin excess production
Average daily EPO production before RBV start was 107.2 ±75.5 mU/ml (Table 2). CHC patients treated with RBV physiologically produced an estimated mean excess of 3915 mU/ml of EPO in the time period between treatment start and Hb nadir. Since our study population had a calculated average total plasma volume of 3.7 l during this time period, the amount of excess EPO produced would represent about 14 500 U of EPO, that is, barely 30% of the effective rHuEPO dose the patients would have received on average if they were treated with 40 000 U/week sc.

Conclusions
RBV is an effective adjunct to the IFN-based treatment in CHC [12–15]. However, a substantial group of patients receiving RBV develop anaemia, which often requires dose reduction and sometimes discontinuation [3,12–15]. At least for HCV genotype 1 infection, which is the most prevalent in Europe and North America, the maintenance of a higher daily RBV dose in CHC patients may be associated with a higher rate of virological response [34,35]. Moreover, the direct antiviral mutation-promoting activity of RBV has been shown in vitro to increase in a dose-dependent manner [36,37]. Therefore, preventing or correcting anaemia in these patients might be important to improve the overall treatment efficacy [38]. With this aim, investigators have recently proposed the administration of rHuEPO to CHC patients treated with the IFN–RBV combination [22–25].

Table 2. Results of the mathematical analysis showing the log10 EPO concentration predicted from the reference regression line and the extent of EPO additional production in response to the RBV-induced anaemia

<table>
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<tr>
<th>Pts</th>
<th>Day of Hb nadir</th>
<th>Observed Hb at Hb nadir</th>
<th>Observed log10 EPO at Hb nadir</th>
<th>Predicted log10 EPO at Hb nadir</th>
<th>Log10 EPO O/P before RBV</th>
<th>Daily EPO produced until Hb nadir</th>
<th>EPO over-production</th>
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M ±SD 40 ±36 55.4 ±30.5 1.68 ±0.26 1.52 ±0.22 1.11 ±0.15 107.2 ±75.5 8372.9 ±7555 3915 ±5162

EPO level is in mU/ml of serum. Rx, treatment; Hb, haemoglobin; O/P, observed/predicted ratio. Abnormal values of log10 EPO O/P are shown in bold. The values of EPO production represent an estimate obtained by mathematical modelling.

Figure 1. Correlation between the variation in haemoglobin concentration (\(\Delta\) Hb, g/l) and the change in erythropoietin serum level (\(\Delta\) EPO, mU/ml) at the haemoglobin nadir compared with baseline.
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The present study shows that unselected CHC patients without anaemia have serum EPO concentrations within the normal range [28]. Moreover, EPO concentration in HCV-positive subjects undergoing antiviral treatment with IFN and RBV increases physiologically and adequately in response to the RBV-induced anaemia, resembling other ‘uncomplicated’ anaemia conditions [28,29]. In fact, the degree of such an increase is comparable to that observed in anaemic subjects with normal hepatic and renal function [28,31]. Overall, these findings suggest that EPO production is not impaired in patients with compensated liver disease due to HCV and that CHC does not affect EPO response to RBV-induced anaemia. The adequacy of EPO response can be maintained even though the anaemia itself is not corrected, as a result of continued RBV intake and haemolysis.

The effective weekly dose of rHuEPO received by CHC patients enrolled in the recent pilot investigation [22–25] appears to be more than three-times greater than the physiological increase in EPO production in response to the RBV-induced anaemia. Since the EPO response is usually sufficient to slow the rate of Hb reduction during RBV treatment and eventually to stabilize Hb levels in most patients [3], the dosing regimen of rHuEPO for RBV-treated CHC patients might be reconsidered in a more patient-tailored fashion. The assessment of log10[EPO] O/P in individual patients could be a useful tool to track CHC patients with an inadequate EPO response relative to their Hb level. In patients with HCV genotype 2 or 3 infection, RBV dose reduction should still be regarded as the first-line intervention for the management of anaemia.

RBV-induced anaemia has a haemolytic pathogenesis and, therefore, is not a recognized indication for rHuEPO treatment [16]. rHuEPO is currently approved for the treatment of anaemia in patients with end-stage renal failure, and in those undergoing anti-tumour chemotherapy or a donation/auto-transfusion programme before major surgery [18–20,39–41]. The drug needs be administered cautiously in subjects with arterial hypertension [42,43], increased cardiovascular risk or thrombophilia [44,45], and hyperkalaemia [46,47]. However, in patients with normal haemoglobin...
and EPO values, rhEPO administration has been shown to be safe [32,48].

Since RBV is excreted mainly through the kidney [4], even minor impairments in renal function could increase the risk of RBV-induced anaemia. Our data, derived from a small sample, show that in general the degree of anaemia or the EPO response in RBV-treated patients is not consistently affected by the actual renal function. It should be noted that the calculated CC was normal in 17 of the 18 patients. However, the highest reduction in Hb levels occurred in the two patients with the lowest CC values. Thus, the hypothesis that minor reductions in renal function could place patients at increased risk of RBV-induced anaemia should be further tested in a larger patient sample.

In conclusion, since the EPO production is normal in CHC patients before and during combination treatment, the rationale of adding exogenous rhEPO to the antiviral regimen appears ‘pathophysiologically’ not straightforward. Nevertheless, the clinical relevance of the issue warrants further study, possibly in the form of a dose-finding investigation coupled with EPO O/P assessment. Subsequently, the clear-cut evidence of a clinical benefit of rhEPO in RBV-induced anaemia will remain to be demonstrated in a randomized, controlled trial.

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