PART V

DIALYSIS: Anaemia

Chairmen: C van Ypersele de Strihou
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THE VARIABLE ROLE OF ERYTHROPOIETIN DEFICIENCY IN THE PATHOGENESIS OF DIALYSIS ANAEMIA

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Summary

To evaluate the role of reduced erythropoietin (Ep) production in the pathogenesis of dialysis anaemia, measurements of serum erythropoietin (SEp) concentration by means of a highly sensitive in vitro bioassay were performed in 88 non-nephrectomised non-transfused regular haemodialysis patients. In 50 patients (group 1) SEp levels were lower than 120 mU/ml. In the remaining 38 patients (group 2) SEp concentrations ranged from 120–369 mU/ml. Group 1 showed a highly significant positive correlation between SEp concentration and haematocrit as did 13 anephric patients investigated for comparison. In contrast, group 2 displayed a highly significant negative correlation between SEp concentration and haematocrit. The results demonstrate the existence of two distinctive groups of similar size in regular haemodialysis patients: those with an absolute (group 1) and those with a relative deficiency of Ep (group 2). In the case of the latter, lack of Ep is probably a secondary factor in the pathogenesis of anaemia, whereas uraemic toxicity and blood loss may play a primary role.

Introduction

Lack of erythropoietin (Ep) is considered to be the primary cause for the development of renal anaemia. This concept is based on the fact that, so far, only rarely have elevated serum erythropoietin (SEp) levels been demonstrated in azotaemic anaemic patients. As in general the degree of anaemia correlates with the severity of azotaemia, it is assumed that decrease of excretory ability of the kidney is accompanied by an equivalent reduction of endocrine renal function.

Even though this explanation appears plausible, its validity has still to be established. Because of the insensitive bioassays used and the small number of patients investigated, actual data on SEp levels in uraemia are scarce.

In the present study SEp concentrations were measured in a large number of
regular haemodialysis patients by means of a highly sensitive in vitro bioassay. In addition healthy controls, anaemic patients without renal disease and non-dialysed uraemic subjects were investigated.

Patients and Method

A total of 88 (63 male, 25 female) non-binephrectomised regular haemodialysis patients were investigated. All of them were treated by 3 x 6–10 hr/wk haemodialysis for at least six months. None required transfusion therapy. All patients were on oral or intravenous iron substitution and 14 received testosterone propionate 100–250 mg/wk intramuscularly. For comparison, 13 bilaterally nephrectomised regular haemodialysis patients (3 of them with intermittent transfusion therapy and 8 receiving 500 mg testosterone propionate/wk intramuscularly), 11 azotaemic patients on the day of first dialysis, 9 anaemic patients without renal disease, and 30 healthy controls were investigated.

Measurement of SEp concentrations was performed using foetal mouse liver cell culture as described by Dunn and Wardle. Haemopoietic livers were harvested from 14 day-old mouse foetuses and suspended in a culture medium consisting of 85% minimal essential medium, 10% foetal calf serum, and 5% bicarbonate buffer in an atmosphere of CO₂. Fixed volume aliquots of serial dilutions of test serum or standard were added to constant volumes of cell suspension and 5 replicates were made of every concentration step. After a pre-incubation period of 20 hours at 37°C radioiron transferrin was added and the culture incubated for another 4 hours for pulse labelling. Subsequently cellular lysis in distilled water was performed and haeme extracted using HCl and Drabkin's solution in butanone. Haeme radioactivity was measured in a β-liquid scintillation counter. Rate of radioiron incorporation into haeme served as a measure of Ep activity.

In order to be able to express SEp concentrations in international units we calibrated our laboratory standard (commercial sheep plasma standard step III, Connaught, Toronto) by use of the International Standard Preparation B, courteously made available through the WHO International Laboratory for Biological Standards, Mill Hill, London. As the foetal mouse liver cell assay is sensitive down to 0.5 mU Ep Standard/ml final dilution, and the test serum portion of culture medium is 10% at the maximum, SEp concentrations down to 5 mU/ml can be measured accurately. The calculation of Ep titre, with 95% confidence limits and testing of significance of regression, linearity and parallelism, were performed by analysis of variance.

Results

Figure 1 shows the results for the 88 regular haemodialysis patients studied. SEp concentration is plotted against the corresponding haematocrit. SEp concentrations range from 23–369 mU/ml with a mean of 130 mU/ml. In patients with haematocrits higher than 40% SEp concentration approximates to 120 mU/ml. This SEp level is almost identical with the mean of 126 ± 50 (SD) mU/ml found in 30 healthy controls. From the data, two distinctive groups of patients
Figure 1. Serum erythropoietin (SEp) concentration and haematocrit (Hct) in regular haemodialysis patients. Mean ± SD of 30 healthy controls is indicated by the dotted line.

become apparent. In the first group decreasing haematocrits are accompanied by decreasing SEp concentrations, whereas in the second group SEp concentrations rise with falling haematocrits. The first group consists of patients with SEp concentrations below 120 mU/ml, whereas in the second group all SEp concentrations are above 120 mU/ml. In Figure 2 the values of the first group are presented separately. The mean haematocrit is 29% and mean SEp concentration 67 mU/ml.
Figure 2. Correlation between serum erythropoietin (SEp) concentration and haematocrit (Hct) in regular haemodialysis patients with SEp concentrations lower than 120 mU/ml

\[ y = 7.38 + 2.04x \]
\[ r = 0.58 \]
\[ p < 0.001 \]
\[ n = 50 \]

Figure 3. Correlation between serum erythropoietin (SEp) concentrations and haematocrit (Hct) in regular haemodialysis patients with SEp concentrations higher than 120 mU/ml

\[ y = 487.2 - 9.3x \]
\[ r = -0.76 \]
\[ p < 0.001 \]
\[ n = 38 \]
There is a highly significant positive correlation between SEp levels and haematocrits ($r=0.58$, $p < 0.001$). In contrast the results in the second group (Figure 3) exhibit a highly significant negative correlation ($r=0.76$, $p < 0.001$). The mean haematocrit is 29%, mean SEp concentration 214 mU/ml.

Discussion

The need for maintenance haemodialysis treatment is evidence of the minimal residual renal excretory function in all patients investigated. In contrast, endocrine renal function varies widely, as judged by SEp concentrations ranging from very low levels to values three times higher than the mean of normal controls. In more than half of the patients SEp concentrations are lower than the mean of normal controls, i.e. absolutely decreased. In these patients Ep deficiency appears to play a primary role in the pathogenesis of anaemia, as can be deduced from the observation that haematocrit and SEp concentrations are positively correlated. Higher SEp levels are accompanied by lesser degrees of anaemia. This is the pattern we also found in anephric patients, where Ep deficiency is known to be extreme$^{14,15}$. In a group of 13 bilaterally nephrectomised subjects with haematocrits ranging from 15–29% SEp concentrations and haematocrits were significantly positively correlated ($r=0.68$, $p < 0.01$). The mean SEp concentration was 114 mU/ml and individual values ranged from 30–215 mU/ml.

In the remaining patients with SEp levels above the mean of normal controls, the kidneys are obviously able to respond to anaemia by producing increased amounts of Ep. However, when compared with 9 severely anaemic patients (mean haematocrit 23%) without renal disease, where SEp levels ranged from 333–996 mU/ml, SEp levels in these patients are relatively low. Nevertheless, one has to conclude that in this group of patients other factors such as uraemic toxicity and blood loss may be the primary causes of anaemia. This concept is supported by the findings of Wallner$^{16}$ and our own results in 11 azotaemic patients studied on the first day of dialysis, when anaemia is most severe. The mean SEp concentration of these patients was $604 \pm 364$ (SD) mU/ml, while the mean haematocrit was 21%. In view of this good compensatory ability of the Ep producing sites it is not surprising that adequate detoxification by regular haemodialysis is often accompanied by a significant improvement in peripheral haemoglobin levels$^{17}$.

References


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Open Discussion

RABIN (Ottawa) First, what is the percentage of serum in the bioassay? Second, did you transfuse those patients who appeared to be dependent on erythropoietin and was the transfusion accompanied by a fall in the serum erythropoietin?

RADTKE In answer to the first question: The maximum test serum proportion in the culture medium was 10% but in most instances we put only 5% test serum into the medium. In answer to the second question: Only three of the bilaterally nephrectomised patients were on transfusion therapy. The other 88 patients were not transfused. I have no idea whether I would find a response to transfusion.

RABIN But don’t you feel that it would be necessary in order to prove the hypothesis that anaemia actually stimulates the release of erythropoietin?

RADTKE You may be right but we have transfused only for clinical indications.

MARRY (Los Angeles) These are fascinating data. Do you see any difference between the two groups in terms of parathyroid hormone? You know that excess parathyroid hormone causes fibrosis of the bone marrow cavity. Three laboratories have shown that after parathyroidectomy you can improve anaemia significantly. I wonder whether those patients who had high erythropoietin and still had anaemia were those who had a greater degree of bone marrow fibrosis secondary to higher levels of parathyroid hormone.

RADTKE We have not checked the parathyroid hormone regularly in our patients so I cannot answer your question. We saw in two out of 20 cases that the haematocrit improved following parathyroidectomy.

POSEN (Ottawa) Did you check the level of residual renal function in each group? Secondly, is there any relationship to the type of renal disease in each group?

RADTKE We are starting to check residual renal function in our patients. So far we see no difference in residual renal function between the two groups.

We could not find any prevalence of one type of renal disease in one or other group.

POSEN Did you study polycystic kidney disease?
RADTKE Yes, and there was no prevalence in any one group.

CHAIRMAN (VAN YPERSELE, Louvain) May I ask you whether the patients had a steady haemoglobin level at the time of measurement? You could have increased levels of erythropoietin because of hidden or obvious blood losses in the previous 7 weeks.

RADTKE All the patients we investigated were on home dialysis on similar treatment. They all had very little blood loss and there was no accidental blood loss at the time of observation.