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Impaired erythrocyte fluidity during treatment of renal anaemia with erythropoietin

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Abstract. Linde T, Sandhagen B, Danielson BG, Wikström B (Department of Internal Medicine and Department of Clinical Physiology, University Hospital, Uppsala, Sweden). Impaired erythrocyte fluidity during treatment of renal anaemia with erythropoietin. *Journal of Internal Medicine* 1992; 231: 601–606.

Seventeen haemodialysis patients with renal anaemia were treated with recombinant human erythropoietin (rhEPO) and observed for 30 weeks. The viscosity of whole blood and plasma, the erythrocyte aggregation tendency, and the erythrocyte deformability, measured as fluidity, were analysed every second week. All patients responded with increasing haematocrit and whole-blood viscosity. The plasma viscosity and the erythrocyte aggregation tendency were already increased before the start of treatment, and remained unchanged during treatment. The basal erythrocyte fluidity tended to be impaired, although not significantly so. During treatment, significant impairment of fluidity was observed at the beginning of the treatment period. After 24 weeks the fluidity started to increase, and it later reached values observed before the start of treatment. Hence, the quality of the erythrocytes formed during the corrective phase of rhEPO treatment differs in some respects from that of cells formed at a normal production rate. The impaired fluidity might have important implications for the flow resistance in small vessels, and contribute to the development or aggravation of hypertension that is often seen during rhEPO treatment.

Keywords: blood viscosity, erythrocyte deformability, erythropoietin, hypertension, renal anaemia, uraemia.

Introduction

Recombinant human erythropoietin (rhEPO) has been shown to be effective in reversing anaemia in haemodialysis patients [1, 2]. However, treatment with rhEPO is associated with side-effects. Approximately one-third of haemodialysed patients treated with rhEPO will experience either an aggravation of pre-existing hypertension or develop *de novo* hypertension [3]. An increase in the apparent whole-blood viscosity, caused by an increased haematocrit [4], has been suggested as one possible reason for the elevation of blood pressure [5, 6].

In vitro, the whole-blood viscosity is strongly correlated with the haematocrit. However, measurements of whole-blood viscosity only reflect the situation in large vessels. In the microcirculation many other properties of the blood are important for flow resistance. The whole-blood viscosity decreases with increasing shear rates, i.e. the *in-vivo* viscosity is lowest in the microcirculation, where the shear rate is highest. Other factors working in concert are the Fåhraeus-Lindquist effect [7], and the fact that the haematocrit in the capillaries is much lower than that in the larger vessels [8], thereby substantially reducing the effect of the haematocrit on the microcirculation. The ability of the erythrocytes to deform in the passage of the narrowest capillaries is of vital importance for the flow resistance. Also, the plasma viscosity and to some extent the erythrocyte aggregation tendency are important for the microcirculation.

Abbreviations: rhEPO = recombinant human erythropoietin, Hb = haemoglobin concentration, MCV = mean corpuscular volume, MCHC = mean corpuscular haemoglobin concentration.

In uraemia, the erythrocyte deformability, measured as filterability, has been shown to be decreased [9, 10], and the erythrocyte aggregation tendency to be increased [11, 12]. The aim of this study was to examine some haemorheological variables in uraemia in the basal state, and during treatment with rhEPO.

Study population

Seventeen haemodialysis patients (nine men and eight women) with renal anaemia (haemoglobin concentration (Hb) 73.2 ± 13.1 g l^{-1}) were studied during 30 weeks of rhEPO treatment. By coincidence, the proportion of patients on antihypertensive drugs was high (14/17), but all patients had a well-controlled blood pressure before the start of treatment ($161 \pm 28/87 \pm 13$ mmHg). Their mean age was 54 (range 25–72) years, and they had been treated with maintenance haemodialysis for a mean period of 21 (1–131) months. Original kidney diseases were chronic glomerulonephritis in eight cases, chronic pyelonephritis in three cases, diabetic nephropathy in three cases, and other renal diseases in three cases.

Regular haemodialysis was performed three times weekly. The starting dose of rhEPO (Cilag, Sollentuna, Sweden) was 50 U kg^{-1} body weight administered after each dialysis. After 4 weeks the dose was adjusted individually to achieve a haematocrit in the range 30–40%, which resulted in a mean dose in the range 100–140 U kg^{-1} after each dialysis. After 17 weeks the target haematocrit was reached and the doses were reduced. Initially, 14 patients were given the drug intravenously. In seven of these patients the treatment was changed to subcutaneous injections when the target haematocrit was achieved. The remaining three patients received the drug subcutaneously from the start. Oral, and in some cases intravenous, iron preparations were given as needed.

The results of the haemorheological analyses were compared with reference values obtained earlier [13] from 83 healthy individuals (35 men and 48 women), of mean age 37 (range 19–65) years.

Methods

All blood samples were collected before the start of the dialysis procedure. Haemoglobin, reticulocyte count, serum iron, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were analysed, using standard laboratory techniques, every second week. Blood pressure was measured before dialysis with a sphygmomanometer cuff.

Haemorheological variables were analysed every second week. Haematocrit was analysed by micro-haematocrit centrifugation at 11 000 *g* for 5 min without correction for trapped plasma. All other rheological variables were assessed at 37 °C in a Low Shear 30 rotational viscometer (Contraves AG, Zürich, Switzerland). Plasma viscosity was analysed at a shear rate of 38 s^{-1} and apparent whole blood viscosity at 100 s^{-1} at native haematocrit.

Erythrocyte aggregation tendency was analysed as whole-blood viscosity at a shear rate of 1 s^{-1}, and corrected for plasma viscosity and haematocrit [13].

Erythrocyte fluidity, a measure of erythrocyte deformability, was analysed by bulk viscometry at a low shear rate (0.95 s^{-1}) as the reciprocal apparent viscosity. The erythrocytes were washed and resuspended to a haematocrit of 55% in isotonic phosphate-buffered saline at pH 7.4 supplemented with 5 mmol l^{-1} glucose [13].

Data are expressed as mean values $\pm$ SD in the text and as mean values $\pm$ SEM in Fig. 1. Differences in mean values between patients and reference values were analysed by Student's unpaired *t*-test. Changes in variables during treatment with rhEPO were analysed by a parameterized-modified Winkel and Jörgensen's method for calculation of age-dependent (here used as time-dependent) reference intervals [14]. The strength of the correlation between fluidity and other variables was assessed by least-squares correlation analysis. The level of statistical significance was set at $P < 0.05$.

Results

The results are shown in Fig. 1. All patients responded with increasing Hb, reticulocyte count and haematocrit. MCV remained unchanged up to week 28, but was significantly increased in week 30. MCHC decreased, and was significantly lowered during weeks 4–10 and 16–22. Serum iron remained unchanged up to week 18, but was significantly increased during the remaining weeks.

The whole-blood viscosity (reference value 4.49 ± 0.44 mPa) increased from 2.82 to a maximum of 4.17 mPa after 18 weeks. The mean haematocrit, Hb and whole-blood viscosity varied in a parallel manner.

The plasma viscosity was significantly elevated

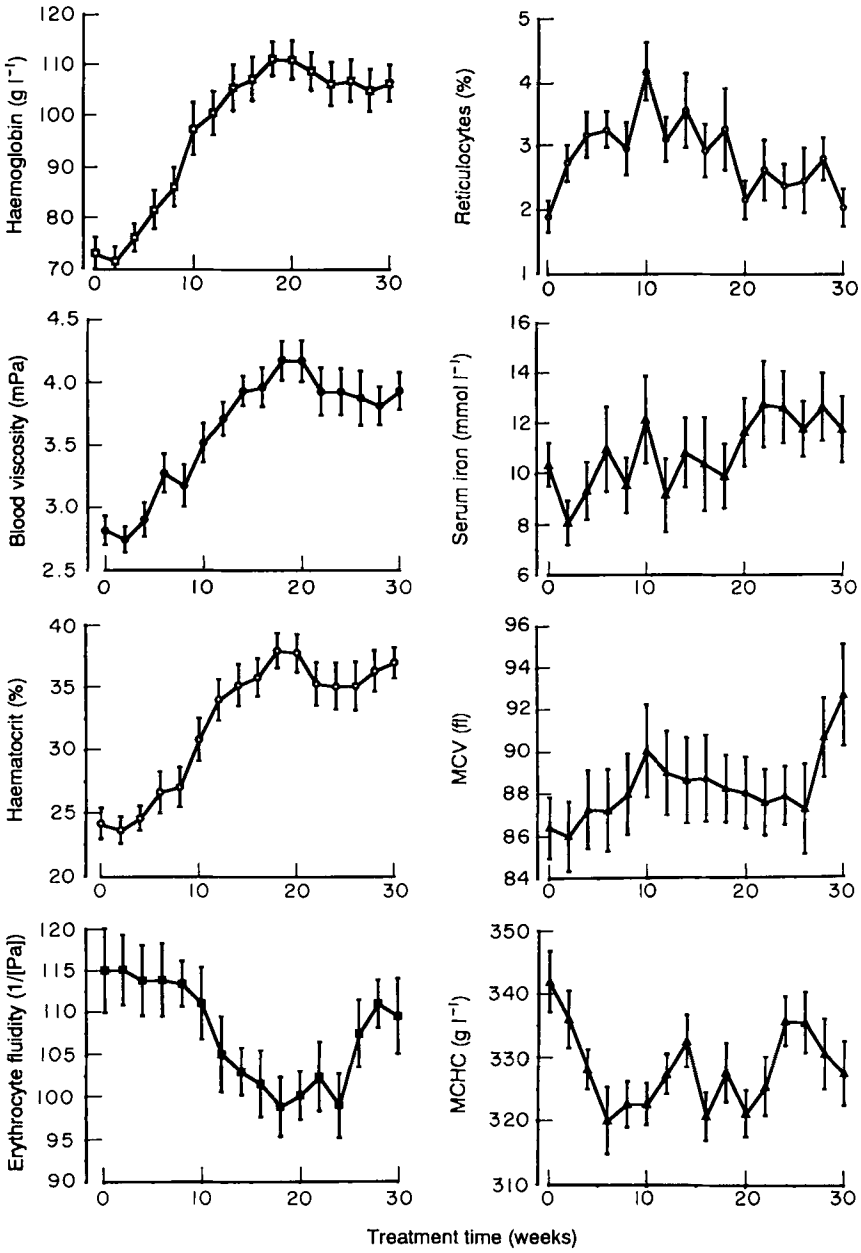

Fig. 1. Mean and standard error of those haematological and haemorheological variables for which significant changes occurred during rhEPO treatment.

before rhEPO treatment (1.42 ± 0.10 mPa) compared to that of the reference group (1.31 ± 0.07 mPa). Furthermore, the erythrocyte aggregation tendency was significantly elevated (1.15 ± 0.19) compared to that of the reference group (1.00 ± 0.10). No changes in either the plasma viscosity or the aggregation tendency were observed during treatment.

The erythrocyte fluidity (reference value 122 ± 10 Pa) was reduced (115 ± 21 Pa), although not significantly so at the start, but decreased further during treatment to a level significantly lower than that of the reference group from the fourth week. Compared to the pre-treatment patient value, the fluidity was significantly lowered between weeks 10 and 24. However, after 24 weeks, the fluidity started to increase.

No patients were excluded from the study during the 30-week period. Owing to increasing blood

pressure in nine of the 17 patients, antihypertensive drugs had to be introduced, or existing medication had to be increased. The changes were mainly performed after the 15th week of rhEPO treatment. The drugs that were introduced or administered in increasing doses were calcium antagonists in four cases, ACE-inhibitors in five cases, beta-blockers in two cases and alpha-blockers in three cases. After these corrections, no increase in mean systolic or diastolic blood pressure was observed.

One patient with diabetes mellitus developed grand mal seizure after 15 and 28 weeks of treatment. Apart from grand mal seizure and an increased requirement for antihypertensive drugs, no serious side-effects were noticed.

Discussion

The basal haemorheological values in our patients with chronic renal failure confirm previous reports on increased plasma viscosity [9–11] and erythrocyte aggregation tendency [11, 12]. It has been suggested that the higher levels of these variables are due to the increased fibrinogen concentration observed in uraemia [5, 9, 11]. Kopperstein *et al.* noted an increase in red cell aggregation during rhEPO treatment [15]. This increase was probably due to the significantly increased haematocrit, since whole blood was used in the assessment and no correction was made for the haematocrit.

Earlier reports [9, 10] have described a decreased basal erythrocyte deformability, measured as filterability, in uraemia. Our patients displayed a tendency (although not significant) towards an impaired erythrocyte fluidity. The conflicting results might be explained by the fact that the three major constituents of erythrocyte deformability (intracellular viscosity, cell membrane viscosity and the quotient between cell membrane area and cell volume) affect the results differently in the two methods.

During the corrective phase of treatment, when high doses of rhEPO were given, there was a significant decrease in erythrocyte fluidity. This change was reversed during the steady-state phase. The deterioration of the erythrocyte deformability appears to occur in parallel with the increasing fraction of rapidly produced erythrocytes during the corrective phase. Our study thus implies that these erythrocytes differ from normal ones.

The reason for the impaired erythrocyte fluidity is unknown. Towards the end of the erythrocyte life-span, MCHC and the membrane viscosity are known to increase, causing impaired erythrocyte deformability [16]. Probably due to decreased mean cell age, MCHC was decreased during the period of impaired fluidity in our study (Fig. 1), which should enhance the erythrocyte fluidity. Thus changes in MCHC or cell age cannot explain the deterioration of fluidity in this study, nor was there any correlation between fluidity and MCV or serum iron levels.

Changes in the antihypertensive therapy could have affected the erythrocyte fluidity. The calcium antagonist isradipine has been shown to improve erythrocyte deformability [17]. The introduction of calcium antagonists could thus contribute to the improvement in fluidity observed at the end of the study. Antihypertensive drugs were mainly introduced after the 15th week of treatment. The significant reduction in erythrocyte fluidity from as early as week 10 thus cannot be explained by changes in the antihypertensive treatment.

Patients with untreated essential hypertension have been shown to have impaired fluidity [18]. One proposed explanation is an enlarged metabolic pool of free calcium ions in erythrocytes from hypertensive patients. Decreased red-cell deformability associated with increased calcium content has also been found in haemodialysis patients [19]. Erythropoietin has been shown to increase free calcium concentration in human erythroid precursors [20] and platelets [21]. Altered erythrocyte content of calcium could thus explain the impaired fluidity observed during rhEPO treatment.

Recent studies on uraemic children [22, 23] have not shown any deterioration of erythrocyte deformability during rhEPO treatment, but only an improvement, even from the start of treatment. In these studies, however, only the membrane elastic shear modulus was measured, and this variable reflects only part of the total deformability of the red cell.

Experimental findings have shown that erythrocytes produced in rats and rabbits during erythropoietin stimulation induced by anaemia or low barometric pressure have a much shorter life-span than normal [24, 25]. In addition, human foetal erythrocytes that are produced during the last 2 months of gestation at a relative rate of 3– to 5-fold that of normal adults have been shown to have a shorter life-span (45–70 d) than normal adult erythrocytes [26]. These findings imply that the quality of rapidly produced erythrocytes differs from that of erythrocytes formed under normal conditions.

Cotes *et al.* [27] found a normal red cell survival time during rhEPO treatment. However, these measurements were made during a period with stable haematocrit. The red cell survival time during the corrective phase of rhEPO treatment, when we have found a decreased fluidity, is unknown and probably difficult to measure.

The haemodynamics of hypertension in erythropoietin-treated patients have been examined in several studies and summarized by Raine [28]. There is a fall in cardiac output, but a proportionately greater increase in vascular resistance. This increase in vascular resistance has been proposed to be due to an increase in blood viscosity and a loss of vasodilation stimulated by hypoxia. In addition to an increase in whole-blood viscosity, we have found an impaired erythrocyte fluidity, which could contribute to the increase in vascular resistance.

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