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Dialysate related cytokine induction and response to recombinant human erythropoietin in haemodialysis patients

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Abstract

Background. Chronic inflammatory disorders or infections represent a major cause of hyporesponsiveness to recombinant human erythropoietin (rHuEpo). To test the hypothesis that dialysate-related cytokine induction alters the response to rHuEpo, we conducted a prospective

study with matched pairs of chronic haemodialysis patients. We compared the effect of two dialysis fluids, differing in their microbiological quality, on the rHuEpo therapy.

Methods. Thirty male patients with end-stage renal disease maintained on regular haemodialysis were assigned either to a group treated with conventional (potentially microbiologically contaminated) dialysate (group I) or to a group treated with online-produced ultrapure dialysate (group II). Randomization was stratified according to the maintenance dose of rHuEpo necessary to maintain a target haemoglobin level of 10–10.5 g/dl. Patients were followed for 12 months. Kt/V was calculated by the formula of Daugirdas. Haemoglobin levels were measured weekly and serum ferritin concentrations were determined at 6-week intervals. C-reactive protein (CRP) and interleukin-6 (IL-6) was measured by an ELISA at the start of the study and after 3, 6 and 12 months.

Results. In group I, continuous use of bicarbonate dialysate did not change the rHuEpo dosage given to achieve the target haemoglobin level and was associated with elevated surrogate markers (CRP, IL-6) of cytokine-induced inflammation. The switch from conventional to online-produced ultrapure dialysate in group II resulted in a lower bacterial contamination with a significant decrease of CRP and IL-6 blood levels. It was accompanied by a significant and sustained reduction of the rHuEpo dosage, which was required to correct the anaemia. Using multiple regression analysis, IL-6 levels are shown to have a strong predictive value for rHuEpo dosage in both groups.

Conclusions. Our data demonstrate that dialysate-related factors such as low bacterial contamination can induce the activation of monocytes, resulting in elevated serum levels of IL-6. Dialysate-related cytokine induction might diminish erythropoiesis. The use of pyrogen free ultrapure dialysate resulted in a better response to rHuEpo. Not only would it save money, but it would also help to maintain an optimal haemoglobin level without further increase in rHuEpo dosage.

Keywords: biocompatibility; erythropoietin; interleukin-6; renal anaemia; ultrapure dialysate



Introduction

The cause of anaemia in patients with end-stage renal disease treated by haemodialysis is complex and multifactorial. The main reason for the development of renal anaemia is insufficient erythropoietin production by the damaged kidneys. In addition to this other important factors contributing to anaemia, such as iron deficiency, uraemia, infection, and the patients' co-morbidity should be considered [1,2].

Concerning recombinant human erythropoietin (rHuEpo) therapy, infection, and/or inflammation, which are often not clinically apparent are, besides iron deficiency, frequent causes for a poor response to the treatment with rHuEpo. C-reactive protein (CRP) is known to

be a useful marker to assess the severity of an inflammation in dialysis patients. Recently, Barany *et al.* [3] have reported that increased levels of CRP are a predictor of resistance to erythropoietin. Erythropoietin dosage and serum CRP were both inversely correlated with serum albumin and serum iron. These data indicate that inflammatory disorders may inhibit the response to rHuEpo by the release of cytokines inhibiting erythropoiesis and by an altered iron metabolism (i.e. functional iron deficiency).

Uraemia can lead to inflammatory reactions, but dialysis can significantly contribute to the systemic inflammatory syndrome that characterizes haemodialysis patients. Activation of monocytes with the subsequent enhanced release of inflammatory cytokines can be caused by membrane-induced complement activation, by direct cell–membrane interaction, and by dialysis fluids containing endotoxins. Whether the chronic inflammatory state caused by dialysis-related haemoincompatibility might influence the responsiveness to rHuEpo remains to be seen.

The aim of our prospective study was to compare the effect of two dialysis fluids, differing in their microbiological quality, on the rHuEpo therapy. We determined the rHuEpo dose being necessary to maintain a defined target haemoglobin level. To assess the state of a chronic inflammation we measured the markers CRP and interleukin-6 (IL-6).

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▶ **Subjects and methods**

Study population

Thirty male patients with end-stage renal disease maintained on regular haemodialysis were selected from a large outpatient dialysis centre. Their original diagnoses, based on history, laboratory tests, radiological signs, and the histology of biopsies were chronic glomerulonephritis (in six patients of group I and eight patients of group II), chronic tubulointerstitial nephritis (in four patients of group I and four patients of group II), diabetic nephropathy (in three patients of group I and two patients of group II), and hypertensive nephroangiosclerosis (in two patients of group I and one patient of group II). In all of them normocytic normochromic anaemia was diagnosed; other forms of anaemia were excluded by appropriate tests. None of the patients was suffering from chronic infection, an inflammatory disorder, malignancy, excess hyperparathyroidism, chronic gastrointestinal loss of blood, or haemolysis. Patients treated with

immunosuppressive drugs, angiotensin-converting enzyme inhibitors, or angiotensin-receptor antagonists were also excluded from the study.

All of the patients had received haemodialysis for at least 6 months. Dialysis was carried out for 4–5 h three times a week by using synthetic biocompatible high-flux membranes (polysulphone 6000, F60, Fresenius, Germany) and commercial bicarbonate dialysates. Dialysers were not reused. Erythropoietin (Epoetin beta: NeoRecormon[®], Boehringer Mannheim, Germany) was administered intravenously after haemodialysis. It was given in varying dosages to achieve and maintain a predialytic haemoglobin concentration of 10–10.5 g/dl. After detailed information about the study, all patients had given their written consent and the study protocol was approved by the local Ethical Committee.

Study design

The patients were assigned either to a group treated with commercial (potentially microbiologically contaminated) dialysate or to a group treated with online-produced ultrapure dialysate. All patients were treated with the same dialysis machines MTS 4008 (Fresenius, Germany). Randomization was stratified according to the maintenance dose of rHuEpo necessary to maintain the defined haemoglobin level. Patients were followed for 12 months. Iron (iron gluconate) was given i.v. to keep ferritin levels above 400 ng/ml.

Study parameters

The demographic data from the patients were obtained from the medical records. At the time of study recruitment intact parathyroid hormone (iPTH) was determined by radio immunoassay and serum aluminium by atomic spectroscopy; Kt/V was calculated by the formula of Daugirdas [5]. Haemoglobin levels were measured weekly and serum ferritin concentrations were determined in 6-week intervals. CRP was measured using an enzyme linked immunosorbent assay (ELISA) (normal value: <0.5 ng/ml). The concentration of plasma IL-6 was measured by an ELISA (normal value: <3.5 pg/ml) (R&D System, Abingdon, Oxon, UK). Measurements were done at the commencement of the study and after 3, 6 and 12 months. Microbiological evaluation of the dialysate was carried out every month by standard agar tests (colony-forming units (c.f.u.)/ml dialysate). The individual rHuEpo dose was calculated by the dosage per week divided by the body weight.

Statistics

The mean±standard deviation was calculated for each group. The significance of effects was tested using analysis of variance (ANOVA). A *P* value less than 0.05 was considered significant. In addition, simple and multiple regression analyses were used to investigate the relationship between rHuEpo dose (dependent variable) and different independent variables.



Results

As shown in Table 1*, between the two groups of patients there were no significant differences

in demographic characteristics and laboratory data such as iPTH, aluminium, ferritin, and Kt/V_{urea} at the time of study recruitment. The mean levels of CRP and IL-6 were significantly elevated compared to healthy controls. The weekly rHuEpo dose required to maintain the haemoglobin concentration between 10.0 and 10.5 g/dl was identical for both groups. During the observation period of 12 months, none of the patients suffered from bacterial infection or any serious illness affecting erythropoiesis or resulting in blood loss.

View this table: [\[in this window\]](#) **Table 1.** Demographic characteristics and laboratory data at time of study recruitment
[\[in a new window\]](#)

Table 2 \blacklozenge shows the effect of ultrapure dialysate on changes in inflammatory markers and rHuEpo dosage in comparison to conventional dialysate. In group I, continuous use of bicarbonate dialysate did not change the rHuEpo dosage given to achieve the target haemoglobin level. In addition, the elevated serum concentrations of CRP and IL-6 were not affected and microbiological evaluation of the dialysate by standard agar test revealed bacterial contamination as shown by the number of c.f.u. per ml dialysate (Table 2 \blacklozenge).

View this table: [\[in this window\]](#) **Table 2.** Comparison of haemoglobin, rHuEpo dose and inflammatory markers during the study period using two different dialysates
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In contrast, after changing from conventional to ultrapure dialysate, bacterial growth could not be detected in the dialysate (0 c.f.u./ml dialysate) in group II. Accordingly, a significant decrease of CRP and IL-6 levels occurred. Mean concentrations of iPTH, aluminium, and ferritin were not affected. The lower levels of IL-6 and CRP under treatment with ultrapure dialysate were accompanied by significant and sustained decrease of the rHuEpo dosage required to correct the anaemia.

Using a simple regression analysis we found after 3 months a strong linear relationship between the serum levels of IL-6, CRP or the number of c.f.u., and rHuEpo dosage in both groups (Table 3 \blacklozenge). A strong regression coefficient was also confirmed after 12 months observation period. In addition, the number of c.f.u. showed a significant relationship with the concentrations of IL-6 and CRP (Table 3 \blacklozenge). In a model of multiple regression analysis using rHuEpo dosage as

dependent variable and c.f.u., IL-6, and CRP as independent parameters, IL-6 is shown to have the strongest predictive value for rHuEpo dosage in both groups (Table 4⁺). The linear relationship between IL-6 and rHuEpo is shown in Figure 1⁺.

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Table 3a. Simple regression analysis after 3 and 12 months observation period (rHuEpo vs c.f.u., IL-6, or CRP)

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Table 3b. Simple regression analysis after 3 and 12 months observation period (c.f.u. vs IL-6, or CRP)

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Table 4. Multiple regression analysis after 3 and 12 months observation period: rHuEpo vs three independents (CRP, c.f.u., and IL-6)

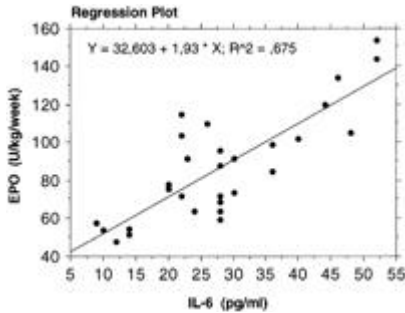


Fig. 1. A linear relationship is shown between IL-6 levels and rHuEpo dose in patients of both groups ($n=30$) by regression analysis.

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Discussion

In this prospective study, two dialysate solutions differing in their microbiological quality were compared. At first it was demonstrated that dialysate-related factors such as low bacterial contamination can induce the activation of monocytes resulting in elevated serum levels of IL-6. Consequently the enhanced cytokine release may aggravate the systemic inflammatory syndrome, which is frequently seen in dialysis patients. Secondly, a strong relationship between the inflammatory syndrome and the resistance to rHuEpo therapy was detected.

Our data are in accordance with clinical observations describing acute-phase proteins as the best indicators for insufficient responsiveness to rHuEpo therapy in patients suffering from chronic inflammation [3,4]. Up to now only uncontrolled studies have been published, indicating that lower rHuEpo dosages were needed when ultrapure dialysis fluids were used: Kleophas *et al.* [6] reported their long term experience with batch-type dialysis machines using ultrapure dialysis fluids and noted that low doses of rHuEpo (37 ± 28 units/kg body weight/week) were needed by 41 patients. Maduell *et al.* [7] found that the change from conventional haemodiafiltration (1–3 l/h) to online pyrogen-free haemodiafiltration (6–12 l/h) resulted in a better response to rHuEpo. However, patients receiving online haemodiafiltration had higher dialytic doses for both small and middle molecular substances, with a decrease in uraemic toxicity, which affects also erythropoiesis. These findings are consistent with previous observations by Eiselt *et al.* [8]. These authors reported that during acetate-free biofiltration the rHuEpo dosage needed declined in comparison to haemodialysis. Finally, preliminary data from Masakane *et al.* [9] suggest that the beneficial effect of ultrapure dialysate may be counteracted by cellulose membranes.

The mechanisms by which inflammatory processes may affect erythropoiesis are complex. There is growing evidence that cytokines can influence erythropoiesis by interaction with erythropoietin at the cellular level. Macdougall *et al.* [10] reported that serum IL-6 concentrations might be a useful marker to control the suppressed erythropoiesis during inflammation in dialysis patients receiving rHuEpo. This assumption is supported by our results

of a multiple regression analysis, which showed that compared with numbers of c.f.u. and CRP levels, IL-6 levels are the strongest predictors of rHuEpo dosage. Goicoechea *et al.* [11] found a relationship between the IL-6 concentrations and tumour necrosis factor alpha (TNF- α) and the rHuEpo doses needed by dialysis patients treated with either polysulphone or cuprophane membranes. Therefore it is reasonable to suggest that inflammatory cytokines such as IL-6 and TNF- α may interfere with erythropoiesis by enhancing the synthesis of interferon- β and - γ , which inhibit the development of erythroblasts [12]. The reduced iron availability may represent an additional mechanism that can cause a poor responsiveness to rHuEpo dosage.

The introduction of rHuEpo into clinical treatment is considered one of the most important advances in the management of end-stage renal disease. However, a major 'side-effect' of rHuEpo therapy is the high cost. Optimizing the response to rHuEpo by the use of ultrapure dialysate and biocompatible membranes would not only save money, but would also help to maintain an optimal haemoglobin level without further increase in rHuEpo dosage.




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