Effects of dialyser and dialysate on the acute phase reaction in clinical bicarbonate dialysis

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Abstract

Background. In chronic haemodialysis (HD), morbidity may result from repetitive induction of the acute phase response, caused by a bioincompatible dialysis membrane and/or contaminated dialysate. In the present study, cytokine release (interleukin-6, IL-6) and subsequent production of acute phase proteins (C-reactive protein, CRP and secretory phospholipase A₂, sPLA₂) were assessed to investigate whether the HD-induced acute phase reaction depends mainly on the type of membrane or on the sterility of the dialysate.

Methods. In 11 patients, IL-6, CRP and sPLA₂ levels of accumulated solutes and excess fluid from HD were assessed in blood samples drawn before (t₀), at the end (t₁₈₀) and 24 h after the start of HD (t₁₄₄₀). All patients were dialysed on Cuprammonium (CU) and Polysulphon (PS) dialysers and seven patients underwent an additional HD session on CU plus a dialysate filter (CUf).

Results. IL-6 levels were increased significantly at t₁₈₀ compared with t₀ (P<0.02) with both CU and CUf. At t₁₄₄₀, IL-6 levels had returned to baseline. In contrast, marked fluctuations did not occur during HD with PS. At t₁₈₀, IL-6 was significantly greater with CU and CUf devices, than with PS (P<0.02). Following HD with CU and CUf, a significant increase in CRP was observed at t₁₄₄₀, compared with post-dialysis values (P≤0.05). In addition, sPLA₂ levels were markedly increased at t₁₄₄₀, compared with t₁₈₀, but only significant in the case of CU (P=0.01). IL-6 levels at t₁₈₀ were significantly correlated with CRP (r=0.50, P<0.01) and sPLA₂ (r=0.47, P=0.01) values at t₁₄₄₀. During HD with PS membranes, neither CRP nor sPLA₂ values were markedly changed.

Conclusions. In contrast to PS, both CU and CUf resulted in elevated IL-6 plasma levels at the end of HD, compared with t₀, which correlated with increased CRP and sPLA₂ values 24 h later. Therefore, the type of membrane, rather than the bacterial quality of the dialysate, seems to be responsible for the induction of the acute phase response during clinical bicarbonate HD.

Keywords: acute phase response; bioincompatibility; C-reactive protein; haemodialysis; interleukin-6; secretory phospholipase A₂

Introduction

The main goal of haemodialysis (HD) is the removal of accumulated solutes and excess fluid from HD patients. Depending on the type of membrane used, both acute and long-term side-effects have been described [1]. The sum of specific interactions between the blood of HD patients and the artificial material of the extra-corporeal circuit has been termed ‘bioincompatibility’ [2]. Because the material is foreign to the body, this process is best described as an inflammatory response. The repetitive induction of the acute phase response induced by repeated exposure to the dialysis circuit [2], has been related to a number of HD-associated complications, such as an increased incidence of infection [3], malnutrition and catabolism [4], dialysis-related amyloidosis [5] and mortality [5,6]. Hence, the type and magnitude of the acute phase response may be important determinants of bioincompatibility in chronic haemodialysis (CHD) patients [7].

The acute phase response is part of the body’s reaction to inflammation and tissue damage, and is regulated by an integrated network of mediators, including pro-inflammatory cytokines, such as interleukin-1β (IL-1β), tumour necrosis factor α (TNFα) and interleukin-6 (IL-6). In human peripheral blood, mononuclear cells (PBMC) are the main source of IL-6. This cytokine is the major inducer of acute phase protein production, including C-reactive protein (CRP) [8] and secretory phospholipase A₂ (sPLA₂) [9,10]. In clinical disease, CRP is most frequently used to monitor the course and magnitude of the acute phase response. Approximately 8–10 h after an inflammatory
stimulus, plasma levels of CRP start to increase up to several hundred-fold normal physiological concentrations [11]. Plasma levels of sPLA₂ usually increase 6–8 h after the inflammatory stimulus [11].

Early HD-related symptoms of the acute phase response, such as fever and hypotension, have been related to the release of pro-inflammatory cytokines from activated PBMC as described in the interleukin hypothesis [12]. In HD patients, PBMC activation may result from a number of stimuli, such as complement activation [13] and direct contact with the dialyser membrane [14] and acetic dialysate [15]. In addition, evidence supports that bacterial substances in the dialysate compartment can induce monocyte activation in the blood phase in vitro [16]. However, whether the presence of cytokine-inducing substances provokes the induction of the acute phase response in clinical HD remains highly questionable [17,18].

The present study was designed to investigate both the cause and magnitude of the acute phase reaction in CHD patients. Pro-inflammatory cytokines are mainly released during dialysis [13] and, therefore, IL-6 was measured before and at the end of HD. Plasma levels of CRP and sPLA₂ increase between dialysis sessions [11] and these substances were assessed after 24 h. The effect of the type of membrane was studied by comparing a markedly bioincompatible material (Cuprammonium, CU) with a relatively biocompatible membrane (Polysulphon, PS). The influence of dialysate contamination was assessed by comparing conventional bicarbonate dialysate with ultrapure dialysate during HD with CU devices only.

Subjects and methods

Patients

Eleven patients, four males and seven females, undergoing intermittent HD for at least 18 months (median 40 months; range 19–166) participated in the study. Written informed consent was obtained from each patient. The median age was 72 years (range 41–77). The aetiology of renal insufficiency was hypertensive nephrosclerosis in seven patients, adult polycystic kidney disease in two, chronic pyelonephritis in one and one patient had analgesic nephropathy. Exclusion criteria were known hypersensitivity to the membranes used, signs of intercurrent infection, malignancy, liver disease, autoimmune disease and treatment with drugs known to interfere with the immune system.

Study design

The present analysis consisted of two parts. The first included a randomized cross-over study comparing high complement-activating CU membranes with low complement-activating PS devices. In order to establish constant blood-membrane exposure times all patients were dialysed on each membrane for a period of 3 weeks, three times a week, for 3 h. To exclude carry-over effects, blood samples were collected during a single dialysis session in every third study week. Blood samples were drawn from the afferent line before HD (t₀) and from the efferent line at the end of HD (t₁₄₄₀).

Twenty-four hours after the start of HD (t₁₄₄₀), blood samples were obtained by venous puncture. At tₐ, a dialysate sample was taken aseptically. In the second part of the study, seven patients underwent an additional HD session with CU dialysers and ultrapure dialysate, according to the above-mentioned protocol.

Dialysis procedure

All patients were dialysed with first-use CU (AM-UP-75, Asahi, Tokyo, Japan) and PS (F60S, Fresenius, Bad Homburg, Germany) dialysers (Table 1). Prior to HD, all dialysers were rinsed with 1000 ml 0.9% NaCl containing 5000 IU heparin. According to the individual needs of the patients, blood flow rates varied between 200 and 300 ml/min. Ultrafiltration (UF) rates were constant for each individual patient (300–1000 ml/h). Anti-coagulation was achieved using heparin, starting with an initial dose of 1500–4000 IU, followed by a continuous infusion of 250–1250 IU/h. The dialysate was prepared using tap water, purified by reversed osmosis, for dilution of a concentrated bicarbonate solution. In order to obtain ultrapure dialysate an endotoxin filter (PS SPS 600) was used [19].

Analytical methods

IL-6. IL-6 was determined in EDTA plasma, using an enzyme immunoassay (ELIA; Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) [20]. After centrifugation (10 min, 1500 g), the samples were stored at −20°C until required for testing. Samples were analysed in duplicate, according to the manufacturer’s instructions. The lower limit of detection was 0.5 pg/ml.

CRP. Serum CRP concentrations were determined by nephelometry (B.N. II, Behring Diagnostics Benelux, N.V., Rijswijk, The Netherlands). The lower limit of detection of CRP was 2 mg/l, the inter-assay coefficient was 5.9%. For statistical analysis, results below the detection limit were assigned a value of 1 mg/l.

sPLA₂. Secretory phospholipase A₂ was determined in EDTA plasma using an enzyme immunoassay as described by Wolbink et al. [21] (lower limit of detection 0.1 ng/ml, inter-assay coefficient <15%). Levels in healthy volunteers are below 5 ng/ml.

Correction for haematocrit (Ht). Values of IL-6, CRP and sPLA₂ at t₁₈₀ and t₁₄₄₀ were corrected for changes in Ht: corrected value t₁₈₀ = (Ht t₀/Ht t₁₈₀) × value t₁₈₀ (idem t₁₄₄₀).

Endotoxin assay. The dialysate samples were collected aseptically in sterile tubes. The samples were stored at −20°C until required for testing. Endotoxin activity was quantified by mixture of a dialysate sample with the Limulus ameboocyte lysate (LAL)/substrate reagent. The mixture was placed in a Kinetic-QCL Reader (Boehringer Ingelheim Bioproducts).

Table 1. Dialyser characteristics

<table>
<thead>
<tr>
<th>Dialyser</th>
<th>AM-UP-75</th>
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<tbody>
<tr>
<td>Material</td>
<td>Cellulose</td>
<td>Polysulfon</td>
</tr>
<tr>
<td>Surface area (m²)</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>Sterilization method</td>
<td>Gamma</td>
<td>Steam</td>
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<tr>
<td>Structure</td>
<td>Capillary</td>
<td>Capillary</td>
</tr>
<tr>
<td>Ultrafiltration factor (ml/mmHg/h)</td>
<td>20</td>
<td>40</td>
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Verviers, Belgium) and monitored automatically over time for the appearance of a yellow colour. The time required before the appearance of a yellow colour is inversely proportional to the amount of endotoxin present. The concentration of endotoxin in unknown samples can be calculated from a standard curve. All determinations were performed in duplicate. Dialysate samples were heated for 10 min at 75°C. Standard series of purified Escherichia coli 055:B5 endotoxin (Boehringer Ingelheim Bioproducts) were made in endotoxin-free water (LAL-reagent water). All standard series showed linearity from 0.01 to 0.25 EU of endotoxin per ml. Inhibition and interference testing was performed on each sample using an endotoxin spike equal to 0.05 EU of endotoxin per ml. Recoveries of spikes between 50 and 150% were accepted.

Microbiological evaluation of the dialysate. Dialysate samples were collected in sterile tubes. Total plate counts were performed on glucose yeast agar (Merck, Darmstadt, Germany; Oxoid, Basingstoke, UK) and on Columbia agar (Oxoid) with sheep red blood cells after 48 h of incubation at 37°C.

Data analysis

Data are expressed as mean ± SD, or median and range when appropriate. Statistical analysis was performed with the SPSS/PC+ software system using paired Student’s t-tests. A Kolmogorov-Smirnov test was performed for the dialysate data. Correlations were estimated using Pearson’s product moment correlation. P-values < 0.05 were considered significant.

Results

IL-6

Predialysis values for the three modalities (PS, CU and CUf) were not statistically different. Marked fluctuations in IL-6 concentrations were not observed during HD with PS membranes. In contrast, IL-6 levels had increased significantly after HD with both CU and CUf, compared with predialysis values (CU: 3.6 ± 2.6 pg/ml at t0 to 10.9 ± 7.9 pg/ml at t180, \( t = -3.00, \text{df} = 10, P = 0.01; \text{CUf}: 2.7 ± 2.2 pg/ml at t0 to 8.6 ± 4.1 pg/ml at t180, \( t = -3.17, \text{df} = 6, P < 0.02 \)). At the end of HD, a statistically significant difference was observed between PS and both CU and CUf (\( P < 0.02 \)). Differences were not observed between CU and CUf. Twenty-four hours after the start of HD, IL-6 concentrations had returned to baseline for both CU and CUf (Figure 1).

CRP and sPLA2

For the three modalities studied, predialysis values of CRP and sPLA2 were not statistically different. During HD with PS membranes, neither CRP nor sPLA2 showed marked variations. In contrast, following HD with CU membranes, a significant increase in the concentrations of both CRP (Figure 2) and sPLA2 (Figure 3) was observed at t1440, compared with post-dialysis values (CRP: 5.9 ± 6.1 mg/l at t180 to 8.6 ± 7.7 mg/l at t1440, \( t = -2.73, \text{df} = 10, P = 0.02; \text{sPLA2}: 8.5 ± 4.7 mg/ml at t180 to 12.5 ± 7.5 mg/ml at t1440, \( t = -3.16, \text{df} = 10, P = 0.01 \)). As for CUf, both CRP and sPLA2 concentrations were notably increased at t1440 compared with t180; however, only the difference for CRP was significant (CRP: 7.7 ± 5.9 mg/l at t180 to 10.8 ± 6.4 mg/l at t1440, \( P = 0.05; \text{sPLA2}: 7.9 ± 2.4 mg/ml at t180 to 12.8 ± 5.9 mg/ml at t1440, \( P = 0.09 \)). CRP values at t1440 were substantially higher with CU and CUf compared with PS, but the difference was only significant in the case of CUf (CU: \( t = 2.00, \text{df} = 9, P = 0.08; \text{CUf}: \ t = 4.32, \text{df} = 6, P < 0.01 \)). In addition, sPLA2 concentrations at t1440 were...
W. E. M. Schouten et al.

Fig. 3. Plasma sPLA\textsubscript{2} values in ng/ml (mean ± SD) during and after dialysis with Polysulphon (PS), Cuprammonium (CU) and Cuprammonium membranes plus endotoxin filter (CUf). Predialysis values of the three modalities were not statistically different. With CU, sPLA\textsubscript{2} was increased significantly at t\textsubscript{1440}, compared with t\textsubscript{180} (* P = 0.01). When comparing both CU and CUf with PS at t\textsubscript{1440}, sPLA\textsubscript{2} levels were significantly higher (** P < 0.05).

Endotoxin content of the dialysate

Ultrapure dialysate, prepared by the placement of an endotoxin filter in the dialysate compartment, contained significantly fewer endotoxins compared with standard dialysate (filtered: median 0.06 (0.05–0.19) EU/ml; standard: median 0.18 (0.05–1.13) EU/ml; P = 0.002).

Dialysate cultures

Bacterial growth was detected in minimal amounts in only one sample of ultrapure dialysate. All the other cultures of filtered dialysate were negative. Furthermore, the median bacterial content of unfiltered dialysate was significantly higher compared with filtered dialysate (standard: median 45 (0–480) c.f.u./ml; filtered: median 0 (0–10) c.f.u./ml; P = 0.007).

Correlations between IL-6, and CRP and sPLA\textsubscript{2}

Cumulative data obtained with all three membranes (PS, CU and CUf) showed a positive correlation between the levels of IL-6 at t\textsubscript{180} and both CRP (r = 0.50, P < 0.01; Figure 4a) and sPLA\textsubscript{2} (r = 0.47, P = 0.01; Figure 4b) at t\textsubscript{1440}.

Correlations between endotoxins, and IL-6, CRP and sPLA\textsubscript{2}

As for dialysate endotoxin levels, correlations could not be demonstrated for IL-6 at t\textsubscript{180}, or both acute phase proteins at t\textsubscript{1440} (IL-6: r = −0.24 n.s.; CRP: r = −0.20 n.s.; sPLA\textsubscript{2}: r = −0.16 n.s.).

Discussion

The present study was designed to assess whether HD-induced bioincompatibility, as measured by the generation of acute phase proteins, is determined mainly by membrane characteristics or by the bacterial quality of the dialysate. Two types of dialyser were compared: Cuprammonium (CU, cellulosic material, medium-flux, high complement activating) and Polysulphon (PS, synthetic material, high-flux, low complement activating). Thereafter, CU dialysis with ultrapure dialysate was performed in the majority of the patients. The dialysate contained a bicarbonate buffer in all the experiments.

With PS membrane dialysis, neither IL-6, CRP nor sPLA\textsubscript{2} showed marked variations at any time, confirming the relative biocompatibility of this material. In contrast, in the case of both CU and CUf, plasma levels of IL-6 showed a significant increase at t\textsubscript{180} and both CRP and sPLA\textsubscript{2} increased markedly after 24 h.
Differences were not observed between CU dialysis with standard and ultrapure dialysate. Moreover, the combined data of all experiments did not show any correlation between the endotoxin content of the dialysate and the levels of IL-6 at $t_{180}$ and the two acute phase proteins after 24 h.

Our study showed a significant correlation between the plasma levels of IL-6 at the end of HD and the CRP levels at $t_{1440}$. Comparable data were described in patients with severe burns [8], but not in HD patients. However, CRP was only measured during dialysis, whereas, CRP levels usually do not increase until 8–10 h after an inflammatory stimulus [11].

Elevated plasma levels of CRP have been associated with low serum albumin [22], advanced atherosclerosis and increased cardiovascular risk, even within conventional reference values [23,24]. Higher levels have been described in CHD patients, particularly with CU dialysis, compared with healthy controls and continuous ambulatory peritoneal dialysis (CAPD) patients [25]. Furthermore, elevated CRP levels have been associated with dialysis-related arthropathy [26]. To the best of our knowledge, this is the first study demonstrating an intra-dialysis increase in IL-6 associated with an increase in acute phase proteins after 24 h, which was, by and large, within the normal reference range. Moreover, acute phase proteins have not been assessed previously in the interval between dialysis sessions comparing standard with ultrapure dialysate.

The changes in sPLA$_2$ matched those of CRP. Recently, it has been suggested that the combined effects of CRP and sPLA$_2$ promote phagocytosis of injured cells, thereby enhancing inflammation and tissue damage [10]. It is tempting to speculate that this pathophysiological process underlies some of the aforementioned clinical sequelae of long-term HD.

The transfer of cytokine-inducing substances from the dialysate across the HD membrane to the blood compartment has been studied extensively and is still a subject of debate. Several recirculation experiments have indicated that bacterial contamination of the dialysate compartment is associated with PBMC activation and cytokine generation on the blood side. Our clinical observations contrast with a large amount of in vitro data. However, as outlined extensively elsewhere [18], these data have been obtained under extremely unphysiological conditions, including lipo-polysaccharide (LPS) concentrations $10^3–10^4$ higher than usually found in the clinical situation. If, however, more relevant concentrations of LPS were used and acetate dialysate was replaced by bicarbonate, cytokine production could not be demonstrated [17,27].

To date, only one in vivo analysis has shown PBMC activation, indicated by an increased intracellular content of IL-1 receptor antagonist (IL-1Ra), in HD with standard bicarbonate dialysate compared with ultrapure dialysate [28]. In contrast, however, a previous report from our centre [18] demonstrated no evidence of cytokine (IL-1β, IL-6, TNF α) and cytokine antagonist (IL-1Ra, soluble TNF receptor [sTNFR]) p55 and p75 generation during clinical high-flux HD with standard, as well as ultrapure, bicarbonate dialysate. With respect to these data, it is questionable whether IL-1Ra is a reliable parameter for the systemic induction of the acute phase reaction in CHD patients. Therefore, in the present study, early IL-6 release and the subsequent production of acute phase proteins were assessed as suitable markers of the acute phase response in the clinical situation. In line with earlier data from our centre [18], the present results do not support the view that the bacterial quality of the dialysate, as quantified by endotoxin content and bacterial growth of the dialysate, contributes to either the production of pro-inflammatory cytokines or acute phase proteins in clinical bicarbonate dialysis. Although the use of a bacterial filter does not guarantee endotoxin-free dialysate, it is unlikely that dialysate contamination, at least at levels found in our centre, will have major clinical consequences. As for high-flux PS, neither intra-dialytic cytokine release nor inter-dialytic acute phase protein production could be demonstrated. In the case of medium-flux CU dialysis, however, an intra-dialysis increase in IL-6 and a small, but significant, acute phase reaction was noted after 24 h, apparently independent of the bacterial contamination of the dialysate. Therefore, other factors, such as complement activation, direct contact with the dialysate membrane and/or release of membrane-derived material may play an important role as far as the acute phase reaction in CU dialysis is concerned [7,13,14,29,30].

To summarize, HD with CU dialysers induced elevated IL-6 levels at $t_{180}$, which correlated with increased CRP and sPLA$_2$ values after 24 h, irrespective of the bacterial quality of the dialysate. These findings were not observed when HD was performed with PS dialysers. According to these results, membrane characteristics, rather than the bacterial quality of the dialysate, seem to be involved in the generation of the HD-induced acute phase reaction. Further investigations on the long-term adverse effects of the repetitive induction of the acute phase response during HD are required to permit definitive conclusions.

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