Use of ultrafiltered dialysate is associated with improvements in haemodialysis-associated morbidity in patients treated with reused dialysers

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Abstract

Background. Morbidity in haemodialysis patients is associated with chronic inflammation. Microbiological contaminants derived from dialysate are thought to be one inflammatory stimulus and previous studies found that highly purified dialysate reduces inflammation and morbidity. These studies were performed in the absence of practices, such as dialyser reuse, that are potentially inflammatory. We tested the hypothesis that highly purified dialysate reduces inflammation and morbidity even in the presence of other inflammatory stimuli.

Methods. This was a prospective observational study. After obtaining baseline data on inflammation, oxidant stress, nutrition and anaemia correction with standard dialysate, 105 patients were switched to dialysate that was ultrafiltered at the point of use and follow-up data were collected at 3-month intervals for 12 months.

Results. Introduction of ultrafiltered dialysate did not significantly reduce inflammation, as assessed by plasma concentrations of C-reactive protein and interleukin-6 or oxidant stress, as assessed by plasma concentrations of protein carbonyls and protein-free sulphydryls. Neither did it improve anaemia correction, as assessed by plasma haemoglobin and erythropoietin dose. However, introduction of ultrafiltered dialysate was associated with a significant reduction in plasma β2-microglobulin concentration and a significant improvement in nutritional status, assessed by plasma albumin concentration and creatinine generation rate as a marker of muscle mass.

Conclusion. Use of ultrafiltered dialysate was associated with improvements in some measures of morbidity, such as plasma β2-microglobulin and nutrition. These changes occurred in spite of the presence of inflammatory stimuli, such as dialyser reuse, and with no measurable reduction in inflammation and oxidant stress.

Keywords: anaemia; β2-microglobulin; haemodialysis; inflammation; nutritional status; ultrapure dialysate

Introduction

Conventional haemodialysis is associated with chronic inflammation and oxidant stress which may contribute to long-term morbidity and mortality in haemodialysis patients [1]. The two most common markers of inflammation used in clinical studies with haemodialysis have been C-reactive protein (CRP) and interleukin-6 (IL-6). Plasma levels of CRP and IL-6 predict both hospitalization and mortality [2]. In addition, plasma levels of CRP and IL-6 are inversely associated with measures of nutritional status, including thigh muscle area standardized for dry weight and serum albumin [3]. Chronic inflammation has also been shown to correlate directly with hyporesponsiveness to erythropoietin therapy [4].

Microbiologic contaminants derived from dialysate are believed to be one important cause of inflammation in haemodialysis patients. In the United States, current recommendations for dialysate quality are 200 CFU/ml or less for bacteria and 2 EU/ml or less for endotoxin [5]. In recent years there has been considerable debate about the adequacy of this standard for the microbiological quality of dialysate. Small-molecular-weight pyrogens derived from bacterial contamination of the dialysate are able to penetrate both low- and high-flux dialysis membranes [6] and during high-flux haemodialysis backfiltration rates may approach 30 ml/min, which would result in transfer of 5–10 l of contaminated dialysate into the blood compartment of the dialyser. In clinical studies, use of ultrapure dialysate (defined as having levels of bacteria and endotoxin of <0.1 CFU/ml and <0.03 EU/ml, respectively) has
been associated with a decrease in the plasma concentrations of markers of inflammation [7–10], and oxidant stress [10,11], improved nutritional status as measured by serum albumin, clinically estimated dry body weight, mid-arm muscle circumference and protein catabolic rate [7], and an increased responsiveness to erythropoietin as measured by an increase in haemoglobin with similar or decreased doses of erythropoietin [8,12].

Except for one study in the United States, where the endotoxin concentration in the dialysate was maintained at <0.06 EU/ml [13], studies with ultrapure dialysate have been performed in Europe and Asia. The results of these studies may not be applicable to haemodialysis patients in the United States because of marked differences in the practice of haemodialysis. For example, dialyser reuse is common in the United States, with a majority of dialysis centres using reprocessed dialysers, while it is practiced in <10% of the centres in Europe and prohibited in Japan. Also, fistula use in the United States is much lower than in Europe and Japan. Both dialyser reuse and the use of synthetic grafts and catheters for blood access may contribute to an inflammatory response in haemodialysis patients.

Ultrapure dialysate can be prepared by passing dialysate meeting the current quality standards through an endotoxin-retentive ultrafilter immediately prior to its entry into the dialyser. To examine whether or not the reported benefits of ultrapure dialysate can be realized in the presence of other haemodialysis-associated inflammatory stimuli, we investigated the impact of introducing ultrafiltered dialysate on markers of inflammation, oxidant stress, nutrition and anaemia correction in a free-standing haemodialysis unit in a prospective observational study.

Methods

Study design

This was a prospective observational study over 15 months. Patients who consented to participate in the study had baseline laboratory and other data collected 3 months and 1 month prior to the introduction of ultrafiltered dialysate (baseline period). These observations were repeated at 3-month intervals for 12 months following the introduction of ultrafiltered dialysate. The study protocol was reviewed by the Institutional Review Board at the University of Louisville.

Haemodialysis methods

During the baseline period the patients were dialysed with Baxter SPS550 dialysate delivery systems (Baxter Healthcare Corporation, Deerfield, IL). Ultrafiltered dialysate was introduced by switching the patients to dialysis with Phoenix® Dialysis Systems (Gambro Renal Products, Lakewood, CO) incorporating dry powder cartridges for on-line generation of bicarbonate concentrate (BiCart® Cartridge) and ultrafilters for filtration of the final dialysate immediately before it entered the dialyser (Diaclear® Ultrafilter). Throughout both phases of the study all patients were treated with Polyflux® 21R high-flux dialysers (Gambro). Dialysers were processed for reuse with a Renatron® II automated dialysate reprocessing system (Minntech Corporation, Minneapolis, MN) using purified water that was ultrafiltered immediately before it entered the Renatron® II system (Fibero, Minntech Corporation). Renalin® (Minntech Corporation) was used as the cleaning agent and germicide. Bleach was not used as a cleaning agent. Each dialysate was used up to a maximum of 40×, provided its total cell volume exceeded 80% of the initial value, it passed a pressure test for membrane and housing integrity and it was aesthetically acceptable to the patient and the patient-care staff. There were no changes in reprocessing methodology over the course of the study. Each individual patient’s haemodialysis prescription was determined by their primary nephrologist. The delivered dose of dialysis was determined as $K_t/V_{\text{UREA}}$.

Laboratory methods

Serum urea and creatinine concentrations were determined by routine clinical laboratory methods. Serum albumin was determined by the bromocresol green method. Plasma concentrations of high sensitivity CRP (normal range $<10$ mg/l), IL-6 (normal range $<9.7$ pg/ml) and β₂-microglobulin (normal range $1.0–1.7$ mg/l) were determined by immunometric assays (Immulite, Diagnostic Products Corporation, Los Angeles, CA). Plasma protein free sulphhydril groups were determined by a modification of the method of Ellman as previously described [14]. Plasma protein carbonyl groups were determined using a commercially available enzyme-linked immunosorbent assay (Zentech Protein Carbonyl Kit, Zentech Technology Corporation, Dunedin, New Zealand). The bacterial content of the dialysate was determined by applying 1 ml of dialysate to a pour plate containing tryptic soy agar, incubating at 35°C for 48 h, and enumerating the number of colonies under magnification according to applicable regulatory requirements [5]. The endotoxin content of the dialysate was determined using the Limulus amebocyte lysate assay (Associates of Cape Cod, East Falmouth, MA).

Calculations

Normalized protein catabolic rate (nPCR, g/kg/day) was determined from the pre- and post-dialysis blood urea nitrogen (BUN) concentrations and $K_t/V_{\text{UREA}}$ using the method of Depner and Daugirdas [15]:

$$n\text{PCR} = \frac{C_0}{25.8 + 1.15(K_t/V) + 56.8/(K_t/V)} + 0.168$$

where $C_0$ is the mid-week pre-dialysis serum BUN concentration (mg/dl) and $K_t/V = \text{single pool } K_t/V_{\text{UREA}}$. Creatinine generation rate ($G_{Cr}$) was determined from pre- and post-dialysis creatinine concentrations using the method of Shinzato et al. [16]:

$$G_{Cr} = C_0 \left(\frac{7056}{A} + \frac{\Delta BW}{IBW} \times \frac{240}{48-t_a}\right)$$
where $\Delta BW$ is the pre- to post-dialysis change in body weight (kg), $IBW = 0.9 \times (H - 100)$ (kg), $H$ is the height (cm) and

$$A = 3334 + (1.9 \times t_d + 737) \ln \left( \frac{C_R}{C_0} \right) - 1.7 \times t_d$$

$$- \frac{0.0256 \times t_d + 0.997}{(0.00231 \times t_d - 0.0028)} \ln(C_R/C_0)$$

where $t_d =$ dialysis time (h), $C_0$ is the pre-dialysis serum creatinine concentration (mg/dl) and $C_R$ is the post-dialysis serum creatinine concentration corrected for rebound (mg/dl).

$$C_R = \left[ \frac{81.622}{t_d} \times \ln \left( \frac{C_0}{C_1} \right) + 0.942 \right] \times C_1$$

where $C_1 = \text{post-dialysis creatinine (mg/dl)}$.

Data management and statistical analysis

Changes in a variable over time were assessed using a general linear model for repeated measures (SPSS 14.0 for Windows, SPSS Inc, Chicago, IL) using age, gender, the presence or absence of diabetes and dialysis vintage as covariates. Mauchly’s test for sphericity was used to test the assumption of equal variances. If Mauchly’s test was significant, signifying non-equal variances, then Greenhouse-Geisser epsilon was used. If a single value of a given variable was missing from the data for an individual patient, then the average of the values preceding and following the missing value was used in the statistical analysis. If the final two observations were missing, the value from month 6 was carried forward. If the final three observations were missing, the patient was dropped from the analysis of that variable. If a single value of a given variable was missing, the value from month 6 was used in the statistical analysis. If the final two values were missing, the average of the values preceding and following the missing value was used in the statistical analysis.

Dialysate quality

To monitor dialysate quality, samples of dialysate were collected each month from 5 to 20 machines selected at random and analysed as described earlier. During the baseline period, 0–50 CFU/ml (median 11 CFU/ml) of bacteria were detected and endotoxin levels were <1 EU/ml. During the ultrafiltered dialysate period no bacteria were detected and endotoxin levels were <0.03 EU/ml.

Inflammation and oxidant stress

There were no significant changes in plasma concentrations of any of the markers of inflammation or oxidant stress over the course of the study (Table 1). However, there was considerable variability in the concentrations of CRP and IL-6, with both markers showing a decrease 3 months after the introduction of

<table>
<thead>
<tr>
<th>Time (months)</th>
<th>CRP (mg/l)</th>
<th>IL-6 (ng/l)</th>
<th>Protein free sulphydryls (mM)</th>
<th>Protein carbonyls (nmol/mg)</th>
</tr>
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<tbody>
<tr>
<td>−3</td>
<td>6.3 (2.7, 15.3)</td>
<td>3.9 (2.4, 8.2)</td>
<td>314 ± 59</td>
<td>0.155 (0.123, 0.200)</td>
</tr>
<tr>
<td>−1</td>
<td>6.5 (1.9, 13.3)</td>
<td>3.8 (2.5, 5.7)</td>
<td>302 ± 56</td>
<td>0.157 (0.119, 0.213)</td>
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<tr>
<td>Introduction of ultrafiltered dialysate</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td>3.4 (1.6, 7.7)</td>
<td>3.7 (2.4, 6.7)</td>
<td>303 ± 51</td>
<td>0.180 (0.135, 0.261)</td>
</tr>
<tr>
<td>6</td>
<td>5.9 (2.1, 13.3)</td>
<td>3.4 (2.5, 6.4)</td>
<td>296 ± 66</td>
<td>0.165 (0.134, 0.197)</td>
</tr>
<tr>
<td>9</td>
<td>5.7 (2.3, 12.4)</td>
<td>4.3 (2.7, 5.9)</td>
<td>248 ± 88</td>
<td>0.153 (0.114, 0.209)</td>
</tr>
<tr>
<td>12</td>
<td>7.3 (3.1, 16.8)</td>
<td>4.4 (2.8, 6.6)</td>
<td>282 ± 62</td>
<td>0.164 (0.133, 0.221)</td>
</tr>
</tbody>
</table>

Note: Data are presented as median (interquartile range) for data not normally distributed (CRP, IL-6, protein carbonyls) or mean ± SD for normally distributed data (protein free sulphydryls).
ultrafiltered dialysate, before rebounding as the study progressed. The CRP level was >10 mg/l at baseline in 42% of patients, while 15% of patients had an IL-6 level >9.7 pg/ml at baseline. When the patients were divided into quartiles based on baseline plasma CRP and IL-6 concentrations, we found a significant decrease in CRP for the quartile with the highest baseline CRP concentrations and a significant increase in CRP and IL-6 for the quartiles with the lowest baseline CRP and IL-6 concentrations (data not shown), consistent with regression to the mean. There was no difference in the concentration of plasma protein free sulphydryl groups or protein carbonyl groups over the course of the study. In contrast to markers of inflammation and oxidant stress, plasma concentrations of β2-microglobulin decreased significantly from 30.9 ± 12.0 to 25.5 ± 8.2 mg/l (Table 2).

**Discussion**

The principal finding of this study is that use of ultrafiltered dialysate may result in improvements in some morbidities associated with inflammation in haemodialysis patients, even in the presence of practices such as dialyser reuse and in the absence of a significant decrease in the plasma concentrations of commonly used markers of inflammation.

Many investigators have shown that haemodialysis patients are in a state of chronic inflammation and oxidant stress. These conditions are believed to contribute to long-term morbidity [1] and it has been hypothesized that reducing the level of inflammation and oxidant stress will reduce long-term morbidity and improve patient outcomes. Inflammation and oxidant stress in haemodialysis patients are multifactorial and appear to be influenced by both the patient’s underlying uraemia [17] and the dialysis procedure [14]. The role of uraemia in the inflammatory process is still poorly understood, making it difficult to develop therapeutic interventions aimed at this source of inflammation. In contrast, features of the dialysis procedure that may contribute to inflammation are better defined and more amenable to change. The bacterial products found in standard dialysate are one stimulus to inflammation associated with the dialysis procedure. Use of ultrapure dialysate is associated with a decrease in the plasma concentration of inflammatory mediators, such as CRP and IL-6 [7–10], and European and Japanese studies suggest that use of ultrapure dialysate is associated with a decrease in vascular events [18], a decrease in the incidence of carpal tunnel syndrome [19], and an improved responsiveness to erythropoietin [8,12,13]. Dialysis practices in the United States differ from those in Europe and Japan in ways that might affect the ability of ultrapure dialysate to reduce morbidity associated with inflammation. Dialyser reuse, for example, is a widespread practice in the United States but is rare in Europe and Japan. The results of our study suggest that the use of ultrapure dialysate can be of benefit to patients, even with these marked differences in practice.

In contrast to some previous studies [7–10] we did not observe a consistent decrease in the plasma levels of inflammatory markers following introduction of ultrafiltered dialysate. There was a transient decrease in plasma levels of CRP, IL-6 and ferritin after 3 months of using ultrafiltered dialysate, but levels rebounded and were not different from baseline at the end of the study. Other investigators also have reported no change in serologic markers of inflammation or a reversion to baseline values over time [11,18,20]. The reasons for our failure to confirm a positive effect of ultrafiltered dialysate on inflammation are unclear. However, there are several potentially confounding factors that may have prevented us from observing an improvement in inflammation with the reduction in contamination achieved by ultrafiltering.
the dialysate. One possibility is limitations in our study design. Randomizing patients to standard or ultrafiltered dialysate would have been a superior design; however, the practicality of randomizing patients within the same dialysis unit to standard or ultrafiltered dialysate for an extended follow-up period without protocol violations is doubtful. Another possibility is that real changes in the plasma concentrations of CRP and IL-6, which were measured only at 3-month intervals, were masked by the high variability in plasma concentrations of both of these markers in haemodialysis patients. Finally, reuse of dialysers and the relatively high incidence of grafts and catheters for blood access may have provided a stimulus to inflammation that made it difficult to detect a reduction in inflammation resulting from the introduction of ultrafiltered dialysate. To examine this last possibility, we performed a subgroup analysis to compare changes in markers of inflammation and oxidant stress in patients who used a fistula for blood access throughout the study with those in patients who used a graft or catheter throughout the study. At entry to the study, there was less oxidant stress in those patients using a fistula than in those using a graft or catheter as demonstrated by a lower level of plasma protein carbonyls and a higher level of plasma protein free sulphhydrals, but no difference in CRP or IL-6 (data not shown). The type of blood access had no impact on changes in the levels of markers of either inflammation or oxidant stress following the introduction of ultrafiltered dialysate.

Oxidant stress is increased in haemodialysis patients and there is evidence of a bidirectional and synergistic link between inflammation and oxidative stress [1]. Plasma protein carbonyls and oxidation of plasma protein thiol groups are markers of oxidant stress in haemodialysis patients. We were unable to demonstrate a change in any of these markers following the introduction of ultrafiltered dialysate. This finding is in contrast to the observations of Furuya et al. [10] and Izuhara et al. [11] who reported a significant decrease in the plasma concentration of pentosidine, a marker of carbonyl stress, in patients treated with ultrapure dialysate. Although we did not measure pentosidine levels, Witko-Sarsat and colleagues have shown a close correlation between pentosidine and advanced oxidation protein products, another marker of oxidant stress [21].

Although we were unable to detect a change in inflammation or oxidant stress, we did observe a significant decrease in the plasma concentration of β2-microglobulin over the period during which ultrafiltered dialysate was used. Other investigators have reported a similar decrease in β2-microglobulin concentration in patients treated with ultrafiltered dialysate [9,10] and use of ultrapure dialysate has been associated with a reduction in the incidence of dialysis-associated β2-microglobulin amyloidosis [19]. Why the use of ultrafiltered dialysate should result in a decrease in β2-microglobulin is unclear. One possibility is that inflammation may play a role in β2-microglobulin production in these patients. Alternatively, the decrease in β2-microglobulin concentration could be unrelated to the use of ultrafiltered dialysate. Use of bleach as a cleaning agent during the processing of dialysers for reuse is known to increase the permeability of some polysulphone membranes to large molecular weight solutes [22]. However, we used Renalin® as a cleaning agent, not bleach, and Renalin® has been reported not to impact membrane permeability [22].

Low albumin levels are associated with an increased relative risk of death in haemodialysis patients [2]. We observed a statistically significant increase in serum albumin from 3.9 ± 0.3 in the baseline period to 4.1 ± 0.4 after 12 months of ultrafiltered dialysate (Table 2). This increase, which is similar in magnitude to that previously reported by Schiff and colleagues [7] and Arizono and colleagues [9], supports the hypothesis that dialysate purity has an impact on the nutritional status of haemodialysis patients. A positive impact of ultrafiltered dialysate on nutritional status is also suggested by the increase in creatinine generation rate observed over the period during which ultrafiltered dialysate was used (Table 2). Since creatinine generation rate reflects muscle mass in haemodialysis patients [3], this observation is consistent with the increases in anthropometric indices of muscle mass reported by Schiff and colleagues [7]. We observed a small decrease in protein catabolic rate over the course of the study. Calculation of protein catabolic rate is based on the net urea generation rate and an assumption of steady state. The increase in creatinine generation rate and serum albumin suggests that our patients were not in steady state and the small decrease in protein catabolic rate may, therefore, reflect the use of nitrogen in forming muscle. The principal cause of a low serum albumin in hypoalbuminemic haemodialysis patients is a decrease in albumin synthesis, which may be related to protein–calorie malnutrition or inflammation [1]. It seems most likely that the increase in serum albumin was the result of decreased inflammation, even though we could not detect a decrease in serologic markers of inflammation.

Unlike in some previous studies [8,12,13], the introduction of ultrafiltered dialysate did not lead to more efficient correction of anaemia by erythropoietin therapy. There was a slight increase in haemoglobin concentration and erythropoietin dose following the introduction of ultrafiltered dialysate; however, these changes were not statistically significant. The failure to find improved anaemia correction following the introduction of ultrafiltered dialysate is consistent with a recent report by Lamas and colleagues [20]. In our study, erythropoietin doses were changed using algorithms that adjusted the dosage based on clinical information and it is possible that this active management of erythropoietin therapy masked small changes resulting from the use of ultrafiltered dialysate. Also, in most previous reports improved anaemia correction was associated with a reduction in inflammation,
as indicated by serum levels of CRP, IL-6 and ferritin [8,12,13]. It may be that the failure to observe a more efficient use of erythropoietin in our study and that of Lamas and colleagues [20] was a consequence of an apparent failure to reduce inflammation.

Interpreting the results of studies such as ours is also confounded by the nature of the microbiological burden in dialysate and potential differences between dialyser membranes, which provide a final barrier between the dialysate and the blood. It is likely that the nature of bacterial contamination varies from dialysis unit to dialysis unit and from time to time. Furthermore, microbiological contamination of dialysate is not only limited to endotoxin, but also includes other bacterial products with pro-inflammatory properties, such as peptidoglycans, β-glucan and fragments of bacterial DNA. Our choice of culturing conditions may have led us to underestimate the level of these other bacterial products in the dialysate. We used the culturing conditions specified in the applicable United States regulations for haemodialysis; that is incubation at 35°C on tryptic soy agar for 48 h [5]. Higher yields of bacteria, which can be obtained using other culture media and incubation at room temperature for longer periods [23], might indicate higher levels of non-endotoxin, pro-inflammatory substances. Finally, different dialyser membranes may have different abilities to prevent these substances from entering the blood [6] and this barrier function may be affected in reprocessed dialysers. Thus, the inflammatory stimulus may vary independently of the endotoxin concentration and this variability may also account for some of the variability in outcomes when ultrafiltered dialysate is introduced.

In spite of our failure to obtain an improvement in inflammation and anaemia correction, our observation of reduction in β2-microglobulin and improvements in nutritional status broadly support the conclusion that improvements in dialysate quality can lead to improved patient outcomes. These improvements were seen even in the presence of other potentially inflammatory stimuli such as dialyser reuse and the use of blood access devices that result in chronic exposure to foreign surfaces. Most of the current generation of dialysate delivery systems can utilize point-of-use dialysate ultrafilters. These devices have been validated for endotoxin removal for a certain number of treatments or period of time when used with dialysate formulated from water and concentrates meeting currently accepted standards of purity. Our results support the use of these ultrafilters for routine haemodialysis.

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