Vascular Reactivity During Combined Ultrafiltration-Hemodialysis: Influence of Dialysate-Derived Contaminants

W.H.M. van Kuijk, W.A. Buurman, P.G.G. Gerlag, and K.M.L. Leunissen

ABSTRACT

It has been suggested that hemodynamic instability and impaired vascular reactivity during combined ultrafiltration-hemodialysis are related to bioincompatibility factors such as dialysate-derived contaminants or the dialyzer. The study presented here investigated whether vascular reactivity could be improved by the use of sterile dialysate. Forearm vascular resistance and venous tone (measured by strain-gauge plethysmography) as well as arterial blood pressure (by Dinamap) and heart rate (by electrocardiogram) were measured in ten stable dialysis patients (age range, 28 to 71 yr) during 2 h of combined ultrafiltration-hemodialysis (bicarbonate; ultrafiltration rate 1.0 L/h). In addition, a dialysate sample was obtained for culture and ilmus amebocyte lysate testing while blood was withdrawn for the estimation of plasma bactericidal/permeability increasing factor (measured by ELISA) and the soluble tumor necrosis factor receptor p75 (measured by ELISA). Patients served as their own control, comparing dialysis with nonsterile and sterile dialysate. No bacterial growth was observed in sterile dialysate, whereas all samples were positive for Pseudomonas in culture in nonsterile dialysis. All ilmus amebocyte lysate tests were negative. Bactericidal/permeability increasing factor tended to increase during nonsterile dialysis (F = 0.063) and remained unchanged during sterile dialysis. In both treatments, tumor necrosis fac-

tor receptor p75 increased significantly (P < 0.01). There were no significant differences in hemodynamic parameters between the treatment modalities. Despite use of sterile dialysate, forearm vascular resistance remained unchanged whereas venous tone decreased significantly. These results indicate that vascular reactivity during combined ultrafiltration-hemodialysis is not improved by the use of sterile dialysate.

Key Words: Forearm vascular resistance, forearm venous tone, pyrogen-free dialysate, BPI, sTNF-Rp75

One of the main goals of dialysis therapy is the withdrawal of excess fluid by ultrafiltration, which might result in hemodynamic instability with symptomatic hypotension under certain conditions. When blood volume decreases, blood pressure stability is especially dependent on an adequate increase in peripheral vascular tone. In this respect, fluid withdrawal during isolated ultrafiltration and hemofiltration has been shown to result in an adequate increase in peripheral vascular tone, although in contrast, peripheral vascular reactivity is impaired during combined ultrafiltration-hemodialysis (1,2). A number of factors, such as differences in plasma osmolality, acid-base status, or extracorporeal blood temperature, have been suggested to be responsible for these differences in vascular reactivity, of which the latter seems to be most important (3–6).

Henderson and colleagues hypothesized in their “Interleukin Hypothesis” that hemodynamic instability during combined ultrafiltration-hemodialysis could be related to the release of cytokines by blood mononuclear cells after stimulation by pyrogenic materials derived from dialysate or as a result of complement activation because of bioincompatibility of the dialyzer. Better hemodynamic stability as observed during isolated ultrafiltration and hemofiltration would then reflect the absence of dialysate (and contaminants) in both treatment modalities and the use of more biocompatible membranes in hemofiltration (7). In this respect, dialysate contaminants are now considered to be the most important bioincompatibility factor (8,9), whereas most dialysis membranes have been shown to be permeable for pyrogens derived from dialysate (10,11). Conflicting data exist whether plasma cytokine levels increase during dialysis (12–14). However, monocytes have been shown to be stimulated by a single dialysis treatment in which dialysate-derived pyrogens play an essential role (9,15). Concerning hemodynamic instability, Beasly and
Brenner have proposed a possible role for cytokine-induced activation of nitric oxide synthesis within vascular smooth muscle cells (16). Finally, recent data indicate that the hemodynamic response during dialysis is independent of membrane bioincompatibility (17).

However, the clinical importance of monocytic cell stimulation in relation to dialysate-derived contaminants remains to be established. Especially with respect to hemodynamics during dialysis, the "Interleukin Hypothesis" is still a hypothesis. In the study presented here, therefore, we investigated whether impaired vascular reactivity as observed during combined ultrafiltration-hemodialysis could be improved by the use of pyrogen-free dialysate.

METHODS

Subjects and Dialysis

All patients signed an informed consent for participation in the study that had been approved by the Ethics Committee of the St. Joseph Hospital, Veldhoven, The Netherlands. Two women and eight men on intermittent hemodialysis were included, with a mean age of 51 yr (range, 28 to 71 yr) and a mean time on hemodialysis of 99 months (range, 15 to 260 months). All patients were hemodynamically stable patients who rarely suffered from intradialytic hypotension. Exclusion criteria were severe coronary (New York Heart Association Class II or more) or valvular heart disease, compromised left ventricular function (ejection fraction of 30% or less), diabetes mellitus, and a donor kidney in situ. Patients were studied on the regular day of his or her dialysis schedule. By adjusting fluid intake, patients were instructed to achieve a predialysis weight at approximately 2.0 kilograms above their dry-weight, which was estimated by echography of the inferior caval vein (18). All vasoactive medication was stopped 48 h before the study, and patients were not allowed to smoke or drink caffeine-containing beverages during the last 12 h before the study.

Each patient served as his or her own control and was studied during two standardized dialysis treatments of 2 h combined ultrafiltration-hemodialysis (UF + HD) at an ultrafiltration rate of 1 L/h using either nonsterile (NS-UF + HD) or sterile dialysate (S-UF + HD). The order of treatments was randomized. Nonsterile dialysate was routinely prepared from a liquid bicarbonate component while the dialysis monitor (AK-100; Gambro, Lund, Sweden) was sterilized overnight by heat disinfection. Sterile dialysate was prepared from a Biocart module while two 1.4-m² Polyamide (U-7000) membranes were used for filtration of dialysate (AK-100 Ultra; Gambro). Overnight, the monitor was sterilized with peracetic acid (Dialox; Seppic, France). In all treatments, a hemophane dialyzer (GFS-16; Gambro) was used. The composition of dialysate was: sodium, 141 mmol/L; potassium, 2.0 mmol/L; calcium, 1.75 mmol/L; bicarbonate, 34 mmol/L; acetate, 3 mmol/L; magnesium, 0.5 mmol/L; and chloride, 108 mmol/L. The dialysate temperature was 37.5°C. Blood flow rate was 250 mL/min and dialysate flow rate was 500 mL/min.

Dialysate Microbiology

Before each dialysis treatment, a sample of 250 mL dialysate was obtained under aseptic conditions and directly transported to the laboratory for immediate microbiological analysis. Each sample was filtered through 0.45 μm filters. The microfilters were incubated for 3 days at 30°C on PCA (plate count agar)-agar plates, after which the number of colony-forming units (CFU) per filter was estimated. In addition, samples of 5 mL dialysate were obtained in pyrogen-free tubes and directly frozen at −20°C for limulus amebocyte lysate (LAL) testing. LPS was measured with a chromogenic Limulus assay (Coastal endotoxin; Kabivitrum, Stockholm, Sweden) according to the manufacturer's instructions. The detection limit was 10 pg/mL. In addition, data of routine cultures were used to estimate the bacterial content of RO (reverse osmosis) water.

Bactericidal/Permeability Increasing Factor (BPI) and Soluble Tumor Necrosis Factor Receptor p75

Before dialysis, as well as at 30, 60, and 120 min, arterial afferent blood was obtained for the estimation of the plasma levels of bactericidal/permeability increasing factor (BPI) and the soluble tumor necrosis factor receptor p75 (sTNF-Rp75). Blood was withdrawn in ice-chilled tubes (EDTA) and immediately centrifuged. Plasma was obtained without disturbing theuffy coat, and stored at −20°C.

BPI and sTNF-Rp75 were determined by ELISA. In short, 96-well plates (Immu-no-Maxisorp; Nunc, Roskilde, Denmark) were coated overnight at 4°C with human rBPI-specific monoclonal antibody 4E3 or MR2-2 specific for TNF-Rp75. Free sites were blocked by 1 h of incubation with phosphate-buffered saline (PBS) plus 1% BSA at room temperature. Human rBPI and rsTNF-Rp75 were used as standards. Samples were incubated for 2 h at room temperature. Next, biotinylated polyclonal rabbit anti-human BPI immunoglobulin (Ig) G and anti-human TNF-Rp75 were added and incubated for 1 h at room temperature. Peroxidase-conjugated streptavidin (Dakopatts, Glostrup, Denmark) diluted in PBS plus 0.1% BSA was added; after 1 h of incubation, plates were washed with distilled water containing 0.1% Tween 20. The substrate for peroxidase was 3,3',5,5'-tetramethylbenzidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD). Spectrophotometry (450 nm) was done with a micro-ELISA autoreader. The detection limits for BPI and sTNF-Rp75 in plasma were 100 and 500 pg/mL, respectively (19,20).

Hemodynamics

All hemodynamic measurements were performed before (pretreatment) and every 30 min during dialysis. Using strain-gauge plethysmography, forearm vascular reactivity was assessed at the non-fistula arm that was positioned just above heart level (Periflow; Janssen Scientific Instruments, Turnhout, Belgium) (21). A cuff was applied to the upper arm while the mercury-filled strain gauge was positioned at the thickest part of the forearm. In addition, an antecubital vein was cannulated (Venflon, 1 mm; Ohmeda AB, Helsingborg, Sweden) for the recording of direct intravenous pressure (Hewlett-Packard 78205C pressure monitor).

Changes in venous tone (VT) (active venous constriction) were estimated by recording the pressure/volume ratio (mm Hg/mL per 100 mL) at a cuff pressure of 40 mm Hg (22). Pressure was applied until both intravenous pressure and arm volume reached a plateau. Changes in pressure and volume were estimated directly after the deflation of the upper arm cuff to minimize the influence of capillary filtration on assessing volume change. To measure forearm blood flow, the upper arm cuff was rapidly inflated 5 ms after the R-top of the electrocardiogram at a cuff pressure of 50 mm
Hg. The inflation/deflation ratio was 3:2 heart beats. The
hand circulation was occluded from 1 min before and during
flow measurements. Forearm blood flow was estimated using
a computerized integrator. Forearm vascular resistance (FVR) was calculated by dividing mean arterial pressure by
forearm blood flow (mm Hg/mL per 100 mL/s). Blood pres-
sure was measured with an automatic blood pressure mon-
itor (Dinamap 1486 SX; Critikon Inc., Tampa, FL). The
average of four consecutive measurements was recorded.
Continuous heart rate was derived from an electrocardio-
gram.

Blood Volume

Changes in relative blood volume were measured continu-
ously and noninvasively by means of an optical reflec-
tion method that operates at a wavelength of 950 nm. The optical
sensor was clipped on the arterial line (Haemoguard 2000;
Sanofi Sante, Maassluis, The Netherlands) (23). The baseline
value was obtained after 2 min of extracorporeal circulation
at a blood flow rate of 250 mL/min without ultrafiltration, to
exclude the influence of saline (recirculation) present in the
blood lines at the start of dialysis.

Blood Temperature

Luminal blood temperatures were measured in the arterial
and venous lines with needle thermometers (Hewlett-Pack-
ard 78214C temperature monitor). Connectors with a
built-in adaptor were placed in the arterial and venous lines
under sterile conditions at a distance of about 15 cm from
the patient. There was no direct contact between blood and
thermometer. Blood temperatures were measured to exclude
in vivo temperature differences between the AK-100 and the
AK-100 Ultra monitor that may interfere with the obtained
results.

Laboratory

Before as well as after 2 h of UF+HD, blood was withdrawn
for the estimation of sodium, potassium, total CO₂, urea,
osmolality (vapour pressure osmometer), and colloid osmot-
cal pressure (COP). In addition, blood was withdrawn in ice-
chilled tubes and directly centrifuged at a temperature of 4°C
for the estimation of plasma catecholamines (by HPLC and
fluorescence detection).

Statistical Analysis

Changes in hemodynamic parameters within each treat-
ment as well as differences between treatments were ana-
yzed by repeated measurements multivariate analysis of
variance (MANOVA). If the sphericity of the variance-covari-
ance matrix appeared to be violated, degrees of freedom in
the univariate MANOVA tests were corrected by the Green-
house-Geisser epsilon to avoid Type I error in testing the F
ratio. All laboratory parameters were analyzed by Friedman's
ANOVA and, if appropriate, by the Wilcoxon's signed-rank
test.

RESULTS

Dialysate samples of 250 mL obtained before
S-UF+HD showed no bacterial growth in culture, with
the exception of two samples. Four CFU were counted
in both samples, identified as respectively Actinetobac-
ter and Bacillus species. All dialysate samples were
positive in culture in NS-UF+HD, identified as pri-
marily Pseudomonas species. Because of confluence
of colonies, it was not possible to reliably count the
number of CFU in NS-UF+HD. All LAL tests were
negative in both treatment modalities. RO-water con-
tained 201 ± 209 CFU per mL (N = 30).

The immunological data are presented in Figures 1
and 2. Although not statistically significant, BPI
tended to increase during NS-UF+HD (P = 0.063 at
time t = 60 min). In S-UF+HD, BPI remained un-
changed. The sTNF-Rp75 increased significantly in
both treatments (P < 0.01). There were no significant
differences in BPI or sTNF-Rp75 between the two
treatment modalities.

The predialysis weights in NS-UF+HD and
S-UF+HD were, respectively, 69.1 ± 6.6 kg and
69.1 ± 6.5 kg (not significant [ns]). The hemodynamic
data are presented in Table 1 and Figures 3 and 4.
Systolic blood pressure decreased significantly in
S-UF+HD (−11 ± 9 mm Hg, P < 0.05), although dia-
stolic and mean arterial pressure remained un-
changed. There were no significant changes in arteri-
al BP during S-UF+HD. Heart rate increased signifi-
cantly during both NS-UF+HD and S-UF+HD with
6 ± 8 (P < 0.05) and 6 ± 7 (P < 0.01) beats/min,
respectively. Concerning vascular reactivity, FVR did

![Graph](image-url)
TABLE 1. Hemodynamic data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NS-UF + HD</th>
<th>S-UF + HD</th>
<th>Changes (NS versus S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>151 (16)</td>
<td>141 (11)b</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>84 (8)</td>
<td>85 (9)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean Arterial BP (mm Hg)</td>
<td>111 (13)</td>
<td>108 (9)</td>
<td>NS</td>
</tr>
<tr>
<td>Heart Rate (beats/min)</td>
<td>72 (11)</td>
<td>78 (12)c</td>
<td>NS</td>
</tr>
<tr>
<td>FVR (mm Hg/mL per 100 mL/s)</td>
<td>2769 (1300)</td>
<td>3440 (979)</td>
<td>NS</td>
</tr>
<tr>
<td>VT (mm Hg/mL per 100 mL)</td>
<td>17.5 (5.5)</td>
<td>15.2 (3.9)c</td>
<td>NS</td>
</tr>
</tbody>
</table>

a NS-UF + HD, nonsterile ultrafiltration-hemodialysis; S-UF + HD, sterile ultrafiltration-hemodialysis; Pre, before treatment; Post, after treatment; NS, not statistically significant; S, statistically significant; BP, blood pressure; FVR, forearm vascular resistance; VT, venous tone.

not change significantly during both treatments. VT decreased significantly during NS-UF+HD and S-UF+HD with \(-2.4 \pm 3.6\) (P < 0.05) and \(-3.1 \pm 3.5\) (P < 0.01) mm Hg/mL per 100 mL, respectively. There were no significant differences in hemodynamic parameters comparing NS-UF+HD with S-UF+HD.

The laboratory data are presented in Table 2. Changes in the different laboratory parameters were comparable between NS-UF+HD and S-UF+HD. Plasma adrenaline remained unchanged during both treatment modalities. During NS-UF+HD, plasma noradrenaline increased significantly (P = 0.050), whereas during S-UF+HD the increase did not reach statistical significance (P = 0.059). Blood volume decreased respectively to \(84 \pm 6\) and \(86 \pm 7\%\) (ns).

The blood temperatures are presented in Figures 5 and 6. The extracorporeal blood temperatures were comparable with no significant changes during either treatment.

**DISCUSSION**

To study the role of bacteriological contamination of dialysate in the hemodynamic response during dialysis, this study investigated whether vascular reactivity during UF+HD could be improved by the use of sterile dialysate. The primary conclusion of our results is that both arteriolar and venous reactivity during UF+HD are not improved when sterile dialysate is used.

Ultrapure dialysate was prepared by ultrafiltration of dialysate through two polyamide membranes. The physicochemical characteristics of this hydrophilic membrane have been shown to completely remove pyrogenic, cytokine-inducing substances from dialysate (24,25). In agreement, only two of ten cultures were positive in S-UF+HD, with a very low count of CFU, whereas all LAL tests were negative. Identification of *Acinetobacter* and *Bacillus* species in these two positive samples is highly suggestive for contamination after the sample was obtained. Whereas it was not possible to reliably count the number of CFU, samples obtained in NS-UF+HD were all positive in culture, with *Pseudomonas* species being the most dominant genus, present in all samples. Based on the number of CFU in RO-water, at least 200 CFU per mL can be expected in dialysate. Although all LAL tests were negative in NS-UF+HD, presence of pyrogens other than LPS or lipid A that might pass the dialysis membrane (such as LPS fragments, peptidoglycans, and muramylpeptides) is not excluded (11). This is not possible in S-UF+HD, because monocytes in culture are not stimulated by contaminated dialysate after filtration of dialysate through polyamide membranes (25). Moreover, exposition to pyrogens in NS-UF+HD is further suggested by a trend toward an increase in
TABLE 2. Laboratory data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NS-UF + HD</th>
<th>S-UF + HD</th>
<th>Changes (NS versus S)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>137.7 (2.5)</td>
<td>140.2 (1.6)(^b)</td>
<td>138.1 (2.1)</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>5.7 (0.6)</td>
<td>4.2 (0.4)(^b)</td>
<td>5.6 (0.5)</td>
</tr>
<tr>
<td>Total CO(_2) (mmol/L)</td>
<td>21.7 (2.4)</td>
<td>24.3 (2.1)(^b)</td>
<td>21.7 (3.0)</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>22.7 (4.9)</td>
<td>11.3 (3.4)(^b)</td>
<td>23.4 (2.8)</td>
</tr>
<tr>
<td>Osmolarity (mosmol/kg)</td>
<td>300.7 (10.1)</td>
<td>289.6 (8.4)(^b)</td>
<td>298.4 (7.1)</td>
</tr>
<tr>
<td>COP(^a) (KPa)</td>
<td>3.4 (0.3)</td>
<td>4.2 (0.5)(^b)</td>
<td>3.4 (0.3)</td>
</tr>
<tr>
<td>Noradrenaline (nmol/L)</td>
<td>1.84 (0.62)</td>
<td>2.15 (0.58)(^c)</td>
<td>1.74 (0.76)</td>
</tr>
<tr>
<td>Adrenaline (nmol/L)</td>
<td>0.18 (0.08)</td>
<td>0.18 (0.12)</td>
<td>0.19 (0.08)</td>
</tr>
</tbody>
</table>

\(^a\) COP, colloid osmotic pressure. All other abbreviations are defined in the footnote to Table 1.

\(^b\) \(P < 0.01\), Post versus Pre.

\(^c\) \(P < 0.05\), Post versus Pre.

BPI, which remained unchanged during S-UF+HD. BPI is released from polymorphonuclear leukocytes after stimulation by LPS or cytokines. BPI has been shown to neutralize LPS activity in vitro and in vivo (26). In agreement with our results, Schindler et al. found an increase BPI during dialysis with either a cellulose or polysulfone dialyzer. The microbiological contamination of dialysate in their study varied between 20 and 150 CFU per mL (27).

Although only to a very small extent, the sTNF-Rp75 increased significantly during both treatment modalities, which suggests that stimuli other than pyrogens such as the dialysis membrane could play a role in the release of the sTNF-Rp75 from mononuclear and endothelial cells during dialysis.

Hemodynamics were measured under strictly standardized conditions with comparable predialysis weights and changes in relative blood volume. In addition, changes in laboratory parameters as well as in extracorporeal blood temperature were also comparable. Only in NS-UF+HD did the decrease in systolic blood pressure reach statistical significance. However, there were no significant differences in either arterial blood pressure, heart rate, or vascular reactivity between NS-UF+HD and S-UF+HD. Despite the use of sterile dialysate, UF+HD was still associated with a decrease in VT, whereas FVR remained unchanged. In addition, HR increased. In previous studies, comparable hemodynamic responses have been observed in UF+HD using nonsterile dialysate, which contrasts with a clear increase in peripheral vascular tone, as observed during hemofiltration and isolated ultrafiltration (1,2,6). Thus, our results show that moderate dialysate contamination is not responsible for the impaired vascular response during UF+HD as compared with hemofiltration and isolated ultrafiltration. However, we cannot completely exclude the possibility that high levels of dialysate contamination might lead to a further impairment of the vascular response in UF+HD.

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