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Influence of Hemodialysis Membranes on Gene Expression and Plasma Levels of Interleukin-1β

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Abstract: Plasma levels of interleukin-1β (IL-1β) were measured in 10 normal subjects, in 11 nondialyzed end-stage renal failure (ESRD) patients, and in 22 hemodialysis (HD) patients. Of the HD patients, 7 were dialyzed with Cuprophan (CU), 7 with polymethylmethacrylate (PMMA), and 8 with polysulphone (PS) dialyzers. In normal controls, nondialyzed ESRD patients, and HD equipped with CU, PAMM, and PS dialyzers, plasma levels of IL-1β were 10.73 ± 5.24 pg/ml, 9.97 ± 3.61 pg/ml, 13.17 ± 4.04 pg/ml, 15.16 ± 6.16 pg/ml, and 13.96 ± 5.47 pg/ml, respectively. There were no statistically significant differences among the groups ($p \geq 0.05$). In contrast, the gene expression of IL-1β for peripheral blood mononuclear cells (PBMC) by in situ hybridization showed differences among the groups. The gene for IL-1β for PBMC appears in HD equipped with different membranes, but not in cases of nondialyzed uremic patients and normal subjects. With computer imaging analysis, we carried out quantitative analysis of cells in in situ hybridization with an area of positive spots to an area of total cells. In HD with CU, PMMA, and PS, the results were 10.64 ± 1.07, 3.34 ± 0.74, and 3.27 ± 0.64%, respectively. The levels of IL-1β gene expression in CU were higher than that in PMMA or PS. There were statistically significant differences ($p \leq 0.001$) between CU and PMMA or PS and no significant difference between the PMMA and PS ($p \geq 0.05$). We suggest measuring the gene expression of cytokines for PBMC and which may be better than measuring cytokine levels only for investigating the blood compatibility of dialyzers, which may help in understanding chronic complications of the dialysis procedure. **Key Words:** Interleukin-1β—Gene expression—Hemodialysis—Membrane—Blood compatibility.

Interleukin-1 (IL-1) is a member of the monokines that are produced by a variety of cells, particularly monocytes and macrophages (1). It has a number of biological effects that are often indistinguishable from the signs observed after microbial invasion and injury, including T-cell activation, hemodynamic shock, and tissue damage. The induction of IL-1 from peripheral blood mononuclear cells (PBMC) during hemodialysis (HD) may be an important mechanism responsible for acute and/or chronic pathological changes associated with HD therapy (2,3). The clinical relevance of IL-1 in HD is supported by the observation that plasma levels of IL-1 are elevated in HD patients. Moreover, plasma IL-1 activity increases during a single hemodialysis session (4).

In vitro studies have demonstrated possible mechanisms of cytokine induction during HD by activation of complement of the alternative pathway, direct contact between PBMC and the membrane, and bacterial contamination of the dialysate. In the past few years, there have been a number of studies investigating the induction of cytokine during HD. In vivo evidence supporting this hypothesis is difficult to obtain. One study reported that cell-associated levels of IL-1 bioactivity were elevated even in uremic patients not on renal replacement therapy (5) while other studies did not confirm this finding (6). Therefore, it is possible that increased cell-associated levels of IL-1 are influenced not only by the dialysis procedure itself, but also by unrelated dialysis factors. Measurement of cytokine in plasma or serum in HD patients gives conflicting results. Some studies reported that circulating levels of IL-1 in patients were elevated compared with normal subjects (4,7,8) while other studies did not confirm this (9–11). Furthermore, in most studies

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there was no or only a small rise in circulating IL-1 levels during HD (9–12). A possible explanation for the difficulties of detecting cytokines in the circulation is the variety of inhibitors and binding proteins present in plasma, which may also be produced in cell culture supernatants, for instance, tumor necrosis factor (TNF)-α binding protein, IL-1 receptor antagonist, soluble IL-1 receptor, autoantibodies or nonspecific binding protein, and IL-6 inhibitors (7).

Recently, some studies reported that hemodialysis induced cytokine transcription but not synthesis (13). Therefore, in the present study, we investigated plasma IL-1β levels and the gene expression of IL-1β in PBMC during HD to determine the consequences of blood/membrane interactions in HD with Cuprophan (CU), polymethylmethacrylate (PMMA), and polysulphone (PS) dialyzers.

MATERIALS AND METHODS

Patients and controls

Group 1

Twenty-two patients (15 male, 7 female, aged 31–74 years) with end-stage renal disease (ESRD) (on regular acetate HD therapy for less then 6 months) were studied. The etiology of renal failure was chronic glomerulonephritis in 12, polycystic kidney disease in 1, interstitial nephritis in 2, chronic pyelonephritis in 2, and unknown etiology in 5. Patients with intercurrent infection, blood transfusion, or immunosuppressive therapy prior to 2 months, systemic lupus erythematosus, or diabetes mellitus were excluded from participation in the study.

Group 2

Eleven uremic patients (3 male, 8 female, aged 32–74 years, serum creatinine 444 to 1,486 μmol/L), not on HD, served as the patient control group.

Group 3

Ten healthy volunteers (5 male, 5 female, aged 24–40 years) served as the normal control group.

Three types of dialyzers of comparable surface area (1.2 m^2) were used. Seven patients were dialyzed with Cuprophan, 7 with PMMA, and 8 with PS. There were no changes in the following dialysis parameters throughout the study: blood flow of 200 ml/min, dialysate flow of 500 ml/min, and dialysate composition.

Plasma and cell preparation

Blood samples were drawn from the efferent blood tubing at the indicated time points during HD into heparinized (10 μm/ml) syringes in Group 1. In Groups 2 and 3, blood samples were obtained by cubital venous puncture. After centrifugation (2,000 rpm, 4 min), the plasma was separated into aliquots and frozen at −20°C. Chloroform extraction was not carried out on the samples, and all assays were carried out on samples thawed only once.

Ten milliliters of whole blood was carefully layered over lymphocyte separation medium (Shanghai Chemical Reagent Factory, Shanghai, China), allowing gradient density centrifugation (3,000 rpm, 20 min). Fresh cells were immediately painted on the slide for in situ hybridization.

Assay for IL-1β

IL-1β levels were measured by using an enzyme immunoassay for human IL-1β (Quantikine, R&D Systems, Inc., Minneapolis, MN) and expressed in picograms per milliliter. The Quantikine IL-1β immunoassay is a solid-phase ELISA that measures IL-1β in less than 4.5 h for serum and/or plasma samples. It contains recombinant human IL-1β and antibodies raised against recombinant human IL-1β. It can be used to determine relative mass values for natural IL-1β and the levels of activity of IL-1β present in biological fluids. This assay employed the quantitative "sandwich" enzyme immunoassay. Briefly, a monoclonal antibody specific for IL-1β had been already coated onto 96-well flat-bottomed microtiter plates. Samples were then pipetted into the wells, and IL-1β, if any, was bound by the antibody. Subsequently, an enzyme-linked polyclonal antibody specific for IL-1β was added to complete the "sandwich." A substrate solution was then added, and the optical density was measured with a spectrophotometer (Bio-Rad model 450, Hercules, CA) set as 450 nm.

Its performance characteristics included four samples of known concentrations assayed in replicates of 20 to assess intraassay precision, and three samples of known concentrations were assayed 20 times to assess interassay precision. The lowest concentration of IL-1β detectable from the zero levels was 3.9 pg/ml.

PBMC in situ hybridization

A 5 μl of fresh PBMC solution was painted onto the slide treated by poly-L-lysine to adhere the cells. When cells became dry, they were fixed on the slide by 3% polyformaldehyde diluted with diethyl pyrocarbonate (DEPC) water. We synthesized IL-1β cDNA oligonucleotide with the DNA autosynthesizer (381A type) and labeled it with a digoxigenin (DIG) oligonucleotide 3′-end labeling kit (Boehringer, Mannheim Biochemicals, Germany). Northern analysis involved the following.

TABLE 1. *Plasma IL-1β levels among the different groups*

Group	Number	Plasma IL-1β (mean ± SD, pg/ml)
1 (HD group)		
CU	7	13.17 ± 4.04
PMMA	7	15.16 ± 6.16
PS	8	13.96 ± 5.47
2 (NHD group)		
NHD	11	9.97 ± 3.61
3 (Normal group)		
NC	10	10.73 ± 5.24
P		>0.05[a]

CU, Cuprophan; PMMA, polymethylmethacrylate; PS, polysulphone; NHD, nonhemodialysis; NC, normal control.
* Comparisons between CU:PMMA, CU:PS, CU:NHD, CU:NC, PMMA:PS, PMMA:NHD, PMMA:NC, PS: NHD, PS: NC, and NHD:NC.

First, a prehybridization solution (4× SSC, 2 ml; 40% Formamide, 4 ml; 1× Denhardt's solution, 200 μl; 5% dextran sulfate, 2.5 ml; 0.25 mg/ml yeast tRNA, 500 μl; 0.5 mg/ml of denatured salmon sperm DNA, 500 μl; and DEPC water, 0.3 ml) was denatured for 10 min at 100°C. Second, a 1 ml prehybridization solution was added to the cell on the slide to hybridize in 1 h at room temperature. Third, a 0.8–1.0 ng/ml labeling probe in 1 ml of prehybridization solution was added to the slide and laid in a damp box at 37°C over night. After hybridization, the slide was washed on a shaker in 2× SSC (pH 7.0) for 15 min at room temperature, and then the procedure was repeated 1× SSC. Subsequently, the slide was washed in 0.5× SSC at 37°C for 15 min, and again at room temperature. Fourth, buffer 1 and 2% normal sheep serum (NSS) were added to the slide for 30 min at room temperature to enhance the following immune reaction. Fifth, 1 μl anti-DIG antibody diluted with 1 ml of buffer 1 and 1 μl of NSS was added to the slide for 2 h at room temperature (DIG nucleic acid detection kit, Boehringer Mannheim Biochemicals). Sixth, the slide was washed with buffer 1 twice to remove unbinding antibodies. Seventh, it was washed in buffer 3 for 2 min at room temperature to buffer the following color reaction. Eighth, 3.5 μl of X-P and 4.5 μl of nitro blue tetrazolium (NBT) mixed in 1 ml of buffer 3 was added to the slide to carry out the color reaction at 37°C for 5 h in dark incubation. Ninth, buffer 4 was used to stop the color reaction. Finally, the slide was dried. Further, the samples needed to be dehydrated and discolored in ascending ethanol series (70, 96, and 100%) and xylene. The signal of hybridization was taken under microscopy and processed by computer imaging analysis (VIDAS, Opton, Oberkochen, Germany).

Statistics

Data are expressed as mean ± SD. Students *t*-test (SAS software package, USA) was used with statistical significance established at $p \leqslant 0.05$.

RESULTS

Plasma levels of IL-1β

Samples of all patients in Group 1 were tested at the beginning and after 5, 30, and 180 min and 5 h of hemodialysis using ELISA. Table 1 shows that plasma levels of IL-1β in each group were almost similar. The differences of IL-1β levels among Groups 1, 2, and 3 were not significant ($p \geqslant 0.05$).

IL-1β gene expression by in situ hybridization

Because the maximal accumulation of IL-1β messenger RNA (mRNA) was consistently observed in blood samples taken at 5 min from the venous line, we took samples at this point. PBMC analyzed in Group 1 contained mRNA for IL-1β but not Groups 2 or 3. After using CU, PMMA, and PS as dialyzer membranes, significant gene expression of IL-1β was observed in each of the 3 membranes. The β-actin gene expressed in HD patients represented the positive control. The amount of mRNA induction during HD was relatively quantitated by computer imaging analysis. The results are shown in Table 2. We found a greater increase of IL-1β mRNA expression in HD with CU compared with HD with PMMA and PS; a statistical significance was found ($p \leqslant 0.001$).

TABLE 2. *The relative amount of IL-1β gene expression in PBMC on HD with CU, PMMA and PS (mean ± SD)*

Membrane types	Patients	Area of positive spots (μm^2) / Area of total cells (μm^2) (%)	Number of positive spots / Area of total cells (μm^2) (single/μm^2)
CU	7	10.64 ± 1.07[a]	1.58 ± 0.13[b]
PMMA	7	3.34 ± 0.74[c]	0.52 ± 0.10[c]
PS	8	3.27 ± 0.64	0.51 ± 0.14

[a] $p < 0.001$ CU versus PMMA or PS.
[b] $p < 0.005$ CU versus PMMA or PS.
[c] $p > 0.05$ PMMA versus PS.

DISCUSSION

A variety of stimuli on HD can activate the monocyte by inducing gene expression for one or more cytokines. This "priming" leads to enhanced gene transcription and mRNA accumulation in the monocytes. Subsequently, the activated monocytes are returned from blood to the patient's body. If the activated monocytes are subjected to stimulation of "second messenger," including membranes per se, endotoxin and acetate in the dialysate, etc., they would synthesize and secrete cytokine. In the case of hemodialysis, chronically and frequently the plasma IL-1β levels should be higher compared with nonhemodialyzed uremic patients and normal subjects. However, measurement of plasma IL-1 levels gave conflicting results. The inconsistent results may be due to the different assay methods for IL-1; a significant proportion of the IL-1 synthesized remains intracellular and is not released into plasma (14); poor nutrition and anemia in renal failure and HD are associated with decreased cytokine production. In fact, in HD patients treated with erythropoietin, there was a 5-fold increase in the production of TNF, inteferon, and colony stimulated factor in vitro (15); measured cytokine values reflected a time-varying balance between generation rate, as influenced by both modes of dialysis and patient variables, and removal rate by various mechanisms, including residual kidney clearance, internalization and clearance by tissue, the transport properties of the membrane and plasma soluble receptors (16); and the detectable time point may miss the peak point of cytokine production.

Haubitz et al. (17) reported that cell associated levels of IL-1β in PBMC following incubation in tissue culture medium were also low and not different comparing cells of normal controls with those of HD patients treated with Cuprophan. They demonstrated that the plasma IL-1β levels in normal controls did not differ from those in HD patients. Our results concur. In addition, that no difference in plasma IL-1β levels was observed between the nonhemodialyzed uremic patients and the HD patients may be due to increased production and decreased clearance in renal insufficiency. Some studies revealed IL-1 secretions in the urine of healthy individuals (18), but its clearance by different membranes was little influenced (7), suggesting that the kidney has an important role in the metabolism and clearance of cytokines. Our study demonstrated no difference among ESRD, HD patients, and normal subjects.

Pereira et al. (7) reported there were no significant differences in the plasma levels of IL-1β and TNF-α among nondialyzed ESRD, HD patients, and normal subjects, but plasma levels of IL-1 receptor antagonist (IL-1Ra) and TNF soluble receptors (TNFsR) in nondialyzed ESRD and HD patients were significantly higher than those in normal controls.

An increasing interest is devoted to the understanding of the consequences of blood/membrane interaction in hemodialysis with different membranes. Some found that plasma levels of IL-1 increased with both cellulose or synthetic membranes (12) while others reported that plasma IL-1 activity rose with the use of Cuphrophan and Hemophan but was unchanged or reduced with the use of PS (19). Our in vivo study did not find differences of IL-1β levels among groups of hemodialysis patients treated with Cuprophan, PMMA, or PS membranes. This is in agreement with other reports (20–22). It has been demonstrated that whereas polymorphonuclear cells (PMNC) produce IL-1 when subjected to in vitro dialysis, no IL-1 is released into the circulating medium. Even after 24 h of incubation, most of the IL-1 produced is still confined to the intracellular compartment. Haeffner-Cavaillon et al. (23), measuring intramonocytic IL-1 activity, also found that both CU and polyacrylonitrile (PAN)-treated patients displayed increased IL-1 activity and that no significant difference could be detected between the 2 groups of patients. The detection of plasma IL-1β levels may not reflect the relative production of this protein by cytokine-producing cells and has its limitation.

In our study, the transcription of IL-1β mRNA in PBMC after HD with CU, PMMA, and PS dialyzers was determined. The results showed an increase of gene expression by in situ hybridization but not in the nondialyzed ESRD or normal controls. Quantitative analysis of the cells in situ hybridization signal showed that there are significant differences between CU, PMMA, and PS membranes (as shown in Table 2). Schindler et al. (13,24) reported that only complement-activating membranes can prime PBMC by inducing the transcription of IL-1β mRNA by the reverse transcription polymerase chain reaction (RT-PCR) technique, which results in more accumulation of IL-1β mRNA at 5 min in the same patient than the use of membranes (PAN, PS, polyamide, and Hemophan) that activate less complement, suggesting that complement triggers IL-1β mRNA. Others reported that IL-1β mRNA transcription may be induced even in the absence of complement (25). The stimulus that promotes cytokine secretion in hemodialysis remains uncertain.

Clearly, release of mature IL-1β is not a simple direct consequence of transcription of the procytokine gene.

The number of hybridized positive cells was not great. In addition, to contain 30–60% PMN by Fiocoll/Hypague preparations, mononuclear cells include not only monocytes and macrophages but also a large number of lymphocytes. Blood-membrane contact selectively activates monocytes (13). Therefore, few positive cells were found under microscopy.

CONCLUSION

Our study showed that hemodialysis with different membranes is undifferentiated to stimulate the release of IL-1β, but the induced transcription of IL-1β in PBMC with the Cuprophan dialyzer is significantly higher than those with the PMMA or PS dialyzers. We suggest that measuring the gene expression of cytokines in PBMC may be better than only measuring cytokine levels for investigating the blood compatibility of dialyzers, which may help in understanding the chronic complications of the dialysis procedure. Interpretation of data from these highly sensitive techniques must be regarded cautiously. Further investigation must be carried out by a long-term multiple center study.

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