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Hepatotoxic substance(s) removed by high-flux membranes enhances the positive acute phase response

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Hepatotoxic substance(s) removed by high-flux membranes enhances the positive acute phase response.

Background. Acute phase proteins (APPs) are enhanced in end-stage renal disease patients (ESRD) requiring dialysis treatment. They are involved in a variety of pathologic processes like muscle proteolysis, cachexia, regulation of appetite, and atherosclerosis. They are predictive for mortality. APPs are not only makers but also active substances. They are mainly produced in liver cells and are primarily, but not exclusively, regulated by proinflammatory cytokines. To what extent hepatic APPs are influenced by uremic toxins is still unclear. Therefore, we investigated the effects of different ultrafiltrates (UFs) on the synthesis of α_1-acid glycoprotein (AGP) in HepG2 cells.

Methods. A cross-sectional as well as a crossover study with high-/low-flux membranes was conducted to investigate the impact of UFs on bioactivity of liver cell cultures. Metabolic activity (MTT test), cytotoxicity (lactate dehydrogenase release), and the positive APP AGP were measured in HepG2 cells.

Results. Cultured hepatocytes treated with UFs from high-flux membranes exhibited a higher cytotoxicity (18.6 ± 0.3% high-flux vs. 13.9 ± 0.2% low-flux, $P < 0.001$) and a lower metabolic activity (29.3% high-flux vs. 50.3% low-flux, $P < 0.001$) in comparison with low-flux UFs. In addition, enhanced APP secretion could be observed under costimulatory conditions (high-flux 5.0 ± 0.7 vs. low-flux 3.1 ± 0.6 ng/μg protein, $P < 0.05$). The effects of high- and low-flux UFs were strongly expressed at the beginning and were still significantly different after 120 minutes of hemodialysis (HD) treatment. The crossover experiments confirmed that UFs collected during high-flux HD had a higher capacity to stimulate AGP synthesis in liver cells.

Conclusion. The effects of UFs from dialysis patients demonstrate that hepatotoxic substances can be removed by dialysis. Stimulating the acute phase response UF collected during high-flux HD had a higher impact on liver cells in comparison with low-flux UF. These substances are putative cofactors involved in cytokine regulation.

End-stage renal disease (ESRD) is associated with a variety of pathological processes, for example, muscle proteolysis, cachexia, lower appetite, and atherosclerosis. Theses diseases are supposed to be promoted by proinflammatory mediators, mainly interleukin-6 (IL-6) and IL-1β [1–3].

The generation of these cytokines again stimulates the synthesis of acute phase proteins (APPs), which are linked with mortality and morbidity in ESRD patients. Low serum albumin is supposed to be one of the most consistent predictors of mortality in patients undergoing hemodialysis (HD) and is regarded not only as a marker of nutrition, but also of the negative acute phase response [4–6]. Albumin and C-reactive protein (CRP), the most prominent markers of acute phase reaction, are regulated in the liver. It is well known that proinflammatory cytokines such as IL-1 and IL-6 are able to stimulate APP, but the complete regulatory process is not fully understood.

Therefore, the question remains as to why the inflammatory process is active in patients with ESRD.

On one hand, membrane contact of circulating blood components and contaminated dialysate play an important role in initiating inflammatory reactions. On the other hand, these effects may be attributed to stimulatory or costimulatory substances present in the uremic plasma (uremic toxins) [7–11].

Therefore, this study was undertaken to investigate whether ultrafiltrate (UF) of dialysis patients is able to affect hepatocyte function, particularly the synthesis of APP.

METHODS

Cross-sectional and crossover study design

The cross-sectional evaluation compared the effects of UFs from patients treated by high-flux (polyamide) versus low-flux membranes (hemophan); that is, the influence on hepatocyte activity was tested by incubating cultured liver cells (HepG2) with UFs.

In the following crossover study with a treatment period of one week, the reversibility of putative hepatocyte substance elimination was examined.

Key words: end-stage renal disease, acute phase proteins, uremic toxin, dialysis, hepatocytes, liver, metabolism.

Cross-sectional evaluation

The hepatoactive effects of UFs were determined by cell culture technique. Pure UFs of 13 patients routinely treated (longer than 4 weeks) with high-flux membrane dialyzer (H) and UFs of 14 patients usually treated with low-flux dialyzer (L) were collected for testing metabolic activity (MTT) and cell integrity [lactate dehydrogenase (LDH)] in HepG2 cells.

Additionally, the effects of UFs, diluted 1:1 with culture medium, on metabolic activity were examined.

Moreover, the time course for elimination of hepatoactive substances during HD treatment was evaluated. UFs from high-flux (H) or low-flux (L) patients were collected at different time points during HD treatment and were investigated in further detail. The time points were 10 minutes (defined as $t = 0$) and 1, 2, and 4 hours during a single dialysis treatment for each patient.

Crossover study

The crossover study was investigated to confirm the results determined in the pilot study. Patients routinely dialyzed with high-flux filters were changed to low-flux and vice versa.

Seven patients of each group (H and L) were randomly assigned for a crossover protocol. Patients belonging to group H followed the scheme high/low/high flux for one week on each treatment mode. Patients belonging to the low-flux group followed the crossover protocol low/high/low flux. However, one patient of group L suffered from gall stones and needed acute operative intervention, and therefore was excluded from the crossover study.

Membranes

The dialysis membranes used were polyamide (Polyflux®) for high-flux and hemophan (GFSplus20®) for low-flux treatment. Both membranes were purchased from Gambro (Hechingen, Germany).

Patient characteristics

Patients treated by high-flux or low-flux HD for at least four weeks were randomly assigned to two different groups to exclude any bias related to diagnosis of primary kidney disease or comorbidity conditions. The composition of the dialysate bath was kept constant during the study according to the individual prescription.

Participants in the different study parts are characterized in detail in Table 1.

There was no difference between the treatment groups concerning mean age and body weight. The distribution of diabetics was equivalent to the other patient groups (8 out of 13 in group H vs. 7 out of 14 in group L). The number of hypertensive patients was higher in the low-flux group, with 12 out of 14 in group L versus 7 out of 13 in group H (high-flux). Erythropoietin medication was also higher for the low-flux group. Residual renal function measured by the amount of daily urine production was significantly different for both groups during the crossover protocol. The time of dialysis and Kt/V, calculated by double-pool model according to Daugirdas formula, was comparable within each group (Table 1). Liver-specific parameters, as measured by alanine-aminotransferase (ALAT) and cholinesterase (CHE), were within the normal range (Table 1). The metabolic profile is demonstrated in Table 2. Patients of both groups were not significantly different with respect to metabolic parameters. Albumin and transferrin serving as nutritional or negative acute phase parameters were in the normal range as well as total protein concentration.

Ultrafiltrate collection and treatment protocol

Vascular access was provided by a single-lumen dialysis catheter of the jugular vein or venoarterial shunt. Each filter and blood lines were initially rinsed with 1 to 2 L of normal saline solution. Patients were connected directly to the extracorporeal circuit. Blood flow was of 250 to 300 mL/min, and the dialysate flow rate was 500 mL/min for all patients. After the start of dialysis treatment, the dialysate efflux side was discontinued. The first 50 mL of fluid were removed in order to avoid contamination of UF with dialysate and with rinsing solution. Thereafter, 100 mL of pure UF (without rinsing solution, without dialysate) were collected for analysis. Samples were cooled on ice, sterile filtered (0.2 μm) aliquoted under a laminar flow hood, and stored at −20°C until further use. For time-course experiments, UFs were collected after different time points (1, 2, and 4 hours) as described previously in this article.

Sampling procedure during the crossover portion of the study. Samples were taken during the third dialysis treatment of each week (sampling procedure as described earlier).

Culture technique

Hepatoma-derived cell line HepG2 (ATCC: HB-8065) was routinely cultured in RPMI growth medium [RPMI 1640 (Biochrom Co., Berlin, Germany) containing 10% fetal calf serum (FCS), 1% glutamine, and 1% streptomycin/penicillin]. Cells were subcultured (1:4) weekly and were seeded in 96- or 24-well plates (Greiner Co., Frickenhausen, Germany) in a density of 100,000 cells/well or 500,000 cells/well, respectively. After 24-hour growth, medium was removed, and cells were incubated for 48 hours with pure UFs or with UFs diluted 1:1 in culture medium.

All media had a low endotoxin level (0.2 EU/mL). Vitality of cells as assessed by trypan blue exclusion test was always greater than 95%.

Table 1. Clinical data and concomitant medication

	Cross-sectional		Crossover	
	H (N = 13)	L (N = 14)	H (N = 7)	L (N = 6)
Age	59.2 ± 3.3	62.0 ± 3.5	55.1 ± 5.2	69.5 ± 5.0
Body weight *kg*	64.4 ± 3.9	64.5 ± 2.5	70.3 ± 5.3	62.9 ± 4.8
Diabetes	8	7	5	4
Hypertension	7	12	4	5
Serum creatinine *mg/dL*	8.8 ± 0.8	8.4 ± 1.1	10.2 ± 1.1	7.9 ± 1.2
ALAT *U/L*	11.8 ± 2.6	7.1 ± 1.2	16.4 ± 4.4	11.2 ± 3.0
CHE *kU/L*	4.5 ± 0.4	3.9 ± 0.3	4.8 ± 0.6	4.5 ± 0.4
Erythropoietin *U/week*	5000 ± 1297	5140 ± 1114	4500 ± 1701	1000 ± 316
Renal residual function *mL/day*	281.8 ± 111.0	520.0 ± 131.5	314.3 ± 159.9[a]	600.0 ± 86.6
Time of dialysis *hours*	4.0 ± 0.07	3.8 ± 0.11	4.0 ± 0.11	3.8 ± 0.17
Kt/V	1.2 ± 0.3	1.1 ± 0.2	1.2 ± 0.1	1.1 ± 0.2

Abbreviations are: ALAT, alanine aminotransferase; CHE, cholinesterase; Kt/V, dialysis dose; H, high-flux; L, low-flux.

Data are given as mean ± SEM or number of patients belonging to the groups. Statistical differences in the cross-sectional study were tested by Student's t-test for independent data sets. In the crossover study, the differences between high- and low-flux membranes were analyzed by Student's t-test for paired data sets.

[a] $P < 0.05$, residual renal function H vs. L

Table 2. Analysis of metabolic parameters in serum of patients participating in the crossover study

	High-flux	Low-flux
Albumin *g/L*	43.0 ± 1.4	45.3 ± 0.9
AGP *g/L*	1.1 ± 0.1	1.4 ± 0.1
Transferrin *g/L*	1.8 ± 0.2	1.8 ± 0.2
Total protein *g/L*	67.1 ± 2.5	69.7 ± 1.7
Triglyceride *mg/dL*	175.8 ± 22.3	124.0 ± 15.4
Cholesterol *mg/dL*	200.8 ± 15.7	198.8 ± 18.4
ApoA *mg/dL*	121.8 ± 9.3	100.6 ± 14.2
ApoB *mg/dL*	109.8 ± 11.1	97.8 ± 17.0

Data are given as mean ± SEM of N = 7 patients using high-flux and N = 6 low-flux membranes. Statistical differences between the groups were tested by Student's t-test for independent data sets.

Interleukins

Interleukin-1β and IL-6 were purchased from Strathmann Biotech GmbH (Hannover, Germany). Final concentrations in assays were 10 ng/mL for IL-1β and 100 U/mL for IL-6.

Analysis methods

MTT test. The overall activity of liver cells was assessed by the MTT test. MTT tetrazolium salt was reduced in metabolic active cells, predominantly by nicotinamide adenine dinucleotide-dependent enzymes of the endoplasmatic reticulum and by succinate dehydrogenase in mitochondria to formazan crystals [12]. After incubation of filtrate over 48 hours, supernatants were removed, and cells were incubated for two hours with 1 mg/mL MTT solution (Sigma Co., Deisenhofen, Germany) at 37°C. The cells were lyzed, and the dark blue formazan crystals were dissolved with 10% sodium dodecyl sulfate/17% N,N-dimethylformamide (DMF)/0.6% acetic acid for at least two hours. The optical density was measured (570 nm vs. 630 nm; Dynatech MR5000 microplate reader; Dynatech, Denkendorf, Germany). Medium controls were arbitrarily set to 100%.

LDH assay. Cytotoxicity of UFs was assessed by LDH release of hepatocytes. The measurements were performed by a commercially available assay (Roche Molecular Biochemicals, Mannheim, Germany) according to the instructions of the manufacturer.

pH value and osmolality. The pH of the UFs was measured after two minutes of electrode contact using standard equipment (pH-Meter CG825; Schott, Mainz, Germany). The osmolality of the different UFs was determined using the osmometer (Knauer, type ML, Bad Homburg, Germany).

α_1-Acid glycoprotein assay. Ninety-six–well immunoplates (NUNC, Wiesbaden, Germany) were coated overnight with 1 μg/mL of mouse anti-human α_1-acid glycoprotein (AGP; Quartett Immundiagnostika, Berlin, Germany)

Phosphate-buffered saline containing 0.05% Tween 20 was used for washing between each step. One hundred microliters of antigen [calibrator: AGP standard (Quartett) or cell supernatant] were added to each well and incubated for one hour at room temperature. Enzyme-linked immunosorbent assay (ELISA) sandwich was completed by incubation with horseradish peroxidase-conjugated antibodies (sheep antihuman AGP; Quartett). OPD (o-phenylendiamine dihydrochloride; Sigma Co.) in 0.1 mol/L phosphate/citrate buffer was used as chromogenic substrate. The reaction was stopped by the addition of 50 μL of 4 mol/L sulfuric acid, and the optical density was measured with a Dynatech microplate reader at dual wavelength (490 vs. 630 nm). The intra-assay and interassay coefficient of variation were 7.3 and 9.6%, respectively. The sensitivity of the assay was 20 ng/mL in the lower range and 700 ng/mL in the upper range. The results were calculated by non linear regression analysis with GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA).

Protein detection in cell culture assay. Cells were lyzed in 0.1 mol/L NaOH by overnight incubation at room temperature. The total protein concentration was measured with a slightly modified Bradford assay using bovine serum albumin for calibration.

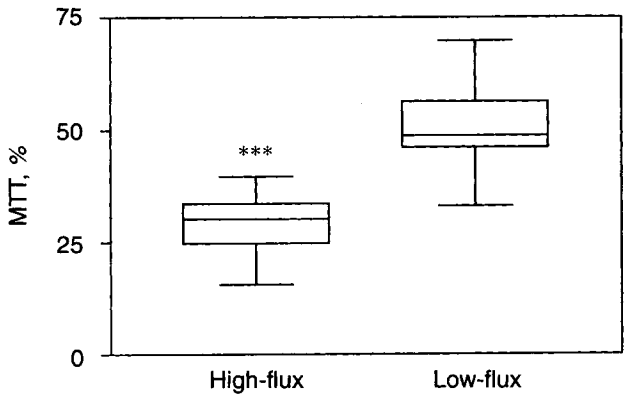

Fig. 1. Metabolic activity as a function of membrane type. Metabolic activity of hepatocytes (HepG2 culture) incubated for 48 hours with pure ultrafiltrates [UF; high-flux (H), $N = 13$, and low-flux (L), $N = 14$, each UF was analyzed in triplicate] collected immediately after beginning of hemodialysis (HD) treatment. Data are presented as box-plot diagrams, with the box encompassing the range of values from the 25th percentile (lower bar) to the 75th percentile (upper bar). The horizontal line within the box represents the median, and the lines above and below the box signify the maximum and minimum values, respectively. Statistical differences were analyzed by the Student t test for independent samples; H vs. L, *** $P < 0.001$.

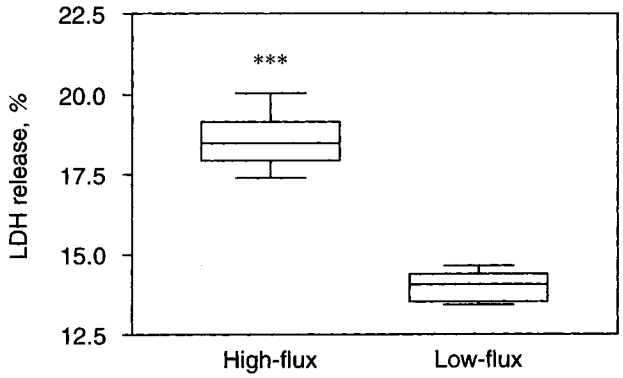

Fig. 2. Cytotoxicity as a function of membrane type. HepG2 cells were incubated with different UFs for 48 hours (high-flux, $N = 14$, low-flux, $N = 13$). Lactate dehydrogenase (LDH) release was determined as described in the experimental procedures. Data are presented as box-plot diagrams, with the box encompassing the range of values from the 25th percentile (lower bar) to the 75th percentile (upper bar). The horizontal line within the box represents the median, and the lines above and below the box signify the maximum and minimum values, respectively. Statistical differences were analyzed by the Student t test for independent samples; H vs. L, *** $P < 0.001$.

Serum protein concentration. Serum samples were taken during the crossover study, directly at the beginning of the treatment. Analysis was performed routinely in a laboratory of the hospital.

Statistics

Data are presented as means ± SEM. Statistical differences between the two groups were tested by Student's t test for independent data sets in the cross-sectional study or by Student t test for paired data sets in the crossover study. One-way analysis of variance (ANOVA) was performed when several groups were compared. The correlation between MTT and LDH was calculated by linear regression analysis. $P < 0.05$ was considered significant.

RESULTS

pH and osmolality in ultrafiltrates

Neither osmolality nor pH of high-flux and low-flux UFs differed significantly. Because of the presence of bicarbonate in the patients' dialysate bath, the pH of the collected UFs was alkaline (high-flux, 8.13 ± 0.05; low-flux, pH 8.09 ± 0.06, P = NS). By incubating UFs in a 5% CO_2 atmosphere for three hours, the pH decreased to 7.50 ± 0.06 (high-flux) and 7.46 ± 0.05 (low-flux), respectively. The pH remained stable for the entire incubation period.

In dilution experiments (1:1 dilution with culture medium), the pH measured in cell supernatants at the end of the entire experiment (48 hours) was pH 7.37 ± 0.04 for high-flux and pH 7.30 ± 0.03 for low-flux.

Effects of ultrafiltrates on metabolic activity and cytotoxicity of HepG2 cells (cross-sectional study)

The MTT and LDH assays were performed to investigate UF-mediated influence on HepG2 cells. On one hand, bioreduction of MTT tetrazolium salt was significantly inhibited by high-flux UF (high-flux, 29.3% ± 1.8; low-flux 50.3% ± 2.6, $P < 0.001$; Fig. 1). On the other hand, LDH leakage of HepG2 cells was significantly lower if cells were incubated with low-flux UFs (high-flux, 18.6% ± 0.3; low-flux 13.9% ± 0.2, $P < 0.001$; Fig. 2). There is a significant correlation between these two parameters ($R = 0.72$, $P = 0.002$).

Either diluted or pure UFs (1:1 dilution with culture medium) had similar effects on metabolic activity of hepatoma cells. While the MTT signals were shifted to higher activity levels by supplementation of UFs with culture medium, the difference in metabolic activity of cells incubated with high- or low-flux UFs persisted (high-flux, 51.3% ± 1.2; low-flux, 87.8% ± 0.9, $P < 0.001$; Fig. 3).

Time course for the elimination of hepatoactive substance during hemodialysis treatment

HepG2 cells were incubated UF samples taken at different times during HD treatment (0, 1, 2, and 4 h) to examine the time course for elimination of hepatoactive substances.

While MTT signals exerted by low-flux UFs remained nearly on the same level for the whole four-hour low-flux treatment, UFs collected during high-flux treatment resulted in a significant decreased metabolic activity [high-flux vs. low-flux: 51.1% ± 1.2 vs. 87.8% ± 0.9, $P <$

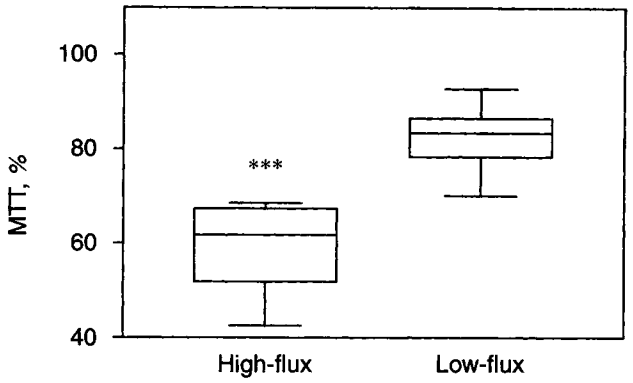

Fig. 3. Effect of medium supplementation of ultrafiltrates (UFs) on metabolic activity. HepG2 cells were incubated for 48 hours with high-flux or low-flux UFs 1:1 supplemented with culture medium [high-flux (H), $N = 7$; low-flux (L), $N = 7$]. Data of three different incubation experiments are presented as box-plot diagrams, with the box encompassing the range of values from the 25th percentile (lower bar) to the 75th percentile (upper bar). The horizontal line within the box represents the median, and the lines above and below the box signify the maximum and minimum values, respectively. Statistical differences were analyzed by the Student t test for independent samples; H vs. L, *** $P <$ 0.001.

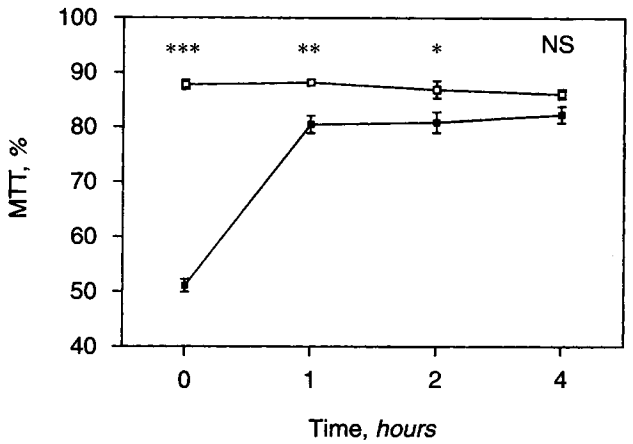

Fig. 4. Time course for the removal of hepatoactive substance during hemodialysis treatment. HepG2 cells were incubated for 48 hours with ultrafiltrates (UFs) collected at different time points during HD treatment (0, 1, 2, and 4 h). Incubation medium was composed of 50% culture medium and 50% UF. Symbols are: (■) high-flux UF; (□) low-flux UF. Data were given as mean ± SEM, where a triplicate measurement of each patient filtrate (H, $N = 5$; L, $N = 5$) was performed. Statistical differences were analyzed by one-way ANOVA. ***$P <$ 0.001, t = 0, H vs. L; **$P < 0.01$, t = 1 hour, H vs. L; *$P < 0.05$, t = 2 hours, H vs. L; P = NS, t = 4 h, H vs. L.

0.001 (t = 0); 80.5% ± 1.6 vs. 88.2% ± 0.6, $P < 0.01$ (t = 1 h); 80.9% ± 1.9 vs. 86.9% ± 0.6, $P < 0.05$ (t = 2 h)]. Only high- and low-flux UFs collected after four hours of treatment did not influence HepG2 cells significantly different with respect to MTT assay [82.3% ± 1.5 vs. 86.1% ± 0.6, P = NS (t = 4 h); Fig. 4].

Secretion of α_1-acid glycoprotein in cell supernatants of HepG2 cells

The positive acute phase protein AGP was measured in supernatants of cells under basal (without interleukins) and under stimulating conditions (stimulation with mediators of the hepatic acute phase response, that is, IL-6 and IL-1β).

Incubation of cells with high-/low-flux UFs had no significant influence on AGP accumulation in HepG2 cell supernatants (high-flux 1.4 ± 0.2 vs. low-flux 1.5 ± 0.3 ng/μg protein; Fig. 5).

Stimulation of cells with a combination of IL-1β and IL-6 increased secretion of AGP, while cells that were incubated with high-flux UFs and costimulated with interleukins exhibited significant higher AGP levels in the cell supernatants compared with cells incubated with low-flux UFs (high-flux 5.0 ± 0.7 vs. low-flux 3.1 ± 0.6 ng/μg protein, $P < 0.01$; Fig. 5).

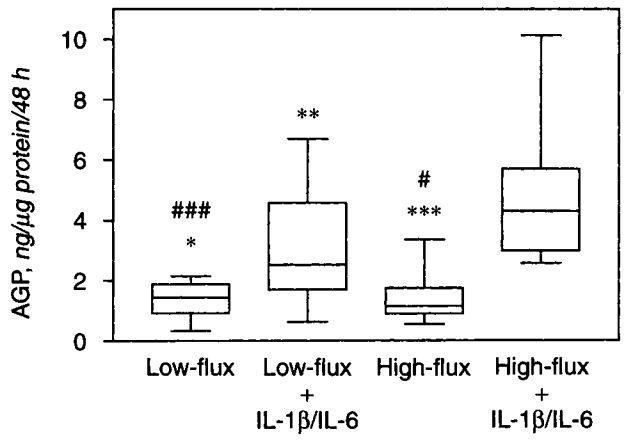

Fig. 5. Effect of ultrafiltrates (UFs) on α_1-glycoprotein (AGP) secretion by HepG2 cells. Cells were incubated with both pure UFs (H, high-flux; L, low-flux) and with UFs and interleukins [H(s) plus IL-1β and IL-6; L(s) plus IL-1β and IL-6] for 48 hours (H, $N = 14$; H, $N = 13$). Secreted AGP was measured by ELISA. Data (ng/μg total protein) of three different incubation experiments are presented as box-plot diagrams, with the box encompassing the range of values from the 25th percentile (lower bar) to the 75th percentile (upper bar). The horizontal line within the box represents the median, and the lines above and below the box signify the maximum and minimum values, respectively. Statistical differences were analyzed by one-way ANOVA. H vs. H(s), ***$P < 0.001$; H vs. L(s), #$P < 0.05$; L vs. L(s), *$P < 0.05$; L vs. H(s), ###$P < 0.001$; L(s) vs. H(s), **$P < 0.01$.

Effects ultrafiltrates on metabolic activity and AGP secretion of HepG2 cells (crossover study)

To confirm the results from the cross-sectional study, a crossover design was conducted to assess the effects of different membrane types.

Patients routinely hemodialyzed with high-flux or low-flux membranes were changed to the other type of membrane for one week and were then switched back to the original membrane type. High-flux UFs always exhibited a significant decrease in metabolic activity compared

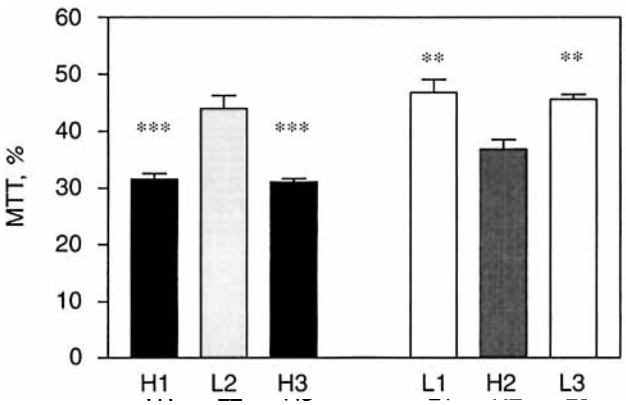

Fig. 6. Metabolic activity as a function of membrane type in the crossover study design. HepG2 cells were incubated for 48 hours with undiluted ultrafiltrates. H (N = 7 patients) and L (N = 6 patients) represent the type of dialyzer membrane used; the numerals (1, 2, 3) represent the week of treatment during the crossover experiment. As described in the **Methods** section, patients usually treated with high-flux dialyzers underwent high/low/high-flux treatment; patients usually treated with low-flux dialyzers followed the other modality (low/high/low-flux). Data were given as mean ± SEM. Each UF was analyzed in triplicate. Statistical analysis was performed using one-way ANOVA. ***$P < 0.001$, L2 vs. H1, H3; **$P < 0.01$, H2 vs. L1, L2.

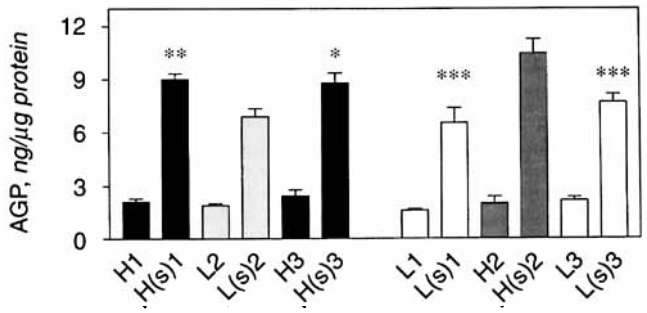

Fig. 7. Effect of UFs on AGP secretion by HepG2 cells in the crossover study design. Cells were incubated with both pure UFs or with UFs and interleukins [H(s): high-flux and IL-1β, IL-6; L(s): low-flux and IL-1β, IL-6] for 48 hours (H, N = 14; L, N = 13). Secreted AGP was measured by ELISA. Data (ng/µg total protein) of three different incubation experiments are presented as mean ± SEM. The week of treatment during the crossover experiment is represented by numerals (1, 2, 3). As described in the **Methods** section, patients usually treated with high-flux dialyzers underwent high/low/high-flux treatment; patients usually treated with low-flux dialyzers followed the other modality (low/high/low-flux). Statistical differences were analyzed by one-way ANOVA. **$P < 0.01$, H(s)1 vs. L(s)2; *$P < 0.05$, H(s)3 vs. L(s)2; ***$P < 0.001$L(s)1, L(s)3 vs. H(s)2.

with filtrates from low-flux dialyzers. Figure 6 represents the data of MTT activity in hepatocytes incubated with UFs out of the sequence high-flux (H1)/low-flux (L2)/high-flux (H3) (31.6% ± 1.0/44.0% ± 2.3/31.1% ± 0.6, L2 vs. H1, H3, $P < 0.001$) in relationship to the corresponding group treated with low-flux (L1)/high-flux (H2)/low-flux (L3) (46.8% ± 2.3/36.9% ± 3.3/45.6% ± 0.8, H2 vs. L1 and L3, $P < 0.01$; Fig. 7).

As determined in the cross-sectional study, AGP secretion of HepG2 cells was not significantly different when influenced by incubation of high- or low-flux UFs.

Significant higher AGP concentrations (ng/µg protein/48 h), however, were measured in supernatants of cells incubated with high-flux UF and costimulated with IL-1β and IL-6, irrespective of whether patients first underwent high-flux or low-flux treatment in the triple treatment series: (*1*) high-flux (H1)/low-flux (L2)/high-flux (H3) (9.0 ± 0.3/6.9 ± 0.4/8.8 ± 0.6, L2 vs. H1, $P < 0.01$; L2 vs. H3, $P < 0.05$); (2) low-flux (L1)/high-flux (H2)/low-flux (L3) (6.5 ± 0.8/10.5 ± 0.8/7.7 ± 0.5, H2 vs. L1, L3, $P < 0.01$).

DISCUSSION

Serum albumin is an important predictor of morbidity and mortality in HD patients [3, 6, 13, 14]. For adequacy of HD treatment, not only albumin but also other negative (apoB) and positive (CRP, α_2-macroglobulin) APPs have become areas of increased interest (abstract; Kaysen et al, *J Am Soc Nephrol* 7:1486, 1996). These proteins are primarily produced in the liver.

Commonly used liver function markers, that is, transaminases, are not sensitive enough to assess hepatic disturbances [15]. Therefore, we tested an in vitro system, that is, cultured liver cells, as an indicator system to assess the elimination of substances by dialysis treatment with high-flux or low-flux membranes.

It seems highly reasonable that toxic substances, including hepatotoxic molecules, can be removed by HD treatment. Concentration-dependent effects (serial dilution of UF result in decreased metabolic activity; data not shown), time-dependent effects (higher activity of UF collected at the start of HD treatment in comparison to samples taken after 4 h of HD), and membrane cut-off effects (stronger impact of high-flux vs. low-flux UF) provide evidence for the previously mentioned thesis.

The pathophysiological events exerted by substances removed during HD may be of multifarious nature. Our results suggest that UFs of HD patients contain substances that are able to amplify an acute phase response in HepG2 cells stimulated with IL-1β and IL-6. A considerable number of HD patients exhibit an activated acute phase response that is closely related to high levels of atherogenic vascular risk factors and cardiovascular death [16]. Therefore, it seemed convincing to us to investigate the effects of UFs in HepG2 by costimulating cells with interleukins.

The positive acute phase protein AGP is raised in serum and urine not only by a variety of inflammatory diseases, but also by uremia, HD, and glomerular failure [17, 18]. The pathophysiological role of this acute phase protein is not precisely known, but it is believed to be a nonspecific immunosuppressant and a carrier of cationic ligands [19]. Our AGP data tempt us to speculate that

a costimulatory substance is present in high-flux UFs that is removed from plasma by high-flux treatment.

At the moment, the nature of the stimulatory substance is speculative. It is well established that IL-1, tumor necrosis factor-α, and IL-6 are the main components stimulating AGP production. In our experimental setting, however, primary factors, that is, cytokines, can be excluded as effectors because incubation of cells with pure UFs shows no effect, in contrast to experiments comprising incubation periods with UFs and cytokines. However, there are factors other than cytokines, cytokine inhibitors, and cytokine receptors that are constituents of the network-controlling synthesis of APPs. These are the so-called cofactors, corticosteroids (for example, dexamethasone), which are the best studied and known to be potent enhancers of the acute phase response [20]. To our knowledge, none of the patients in this study were given a glucocorticoid regimen, and it is also unknown whether the kind of membrane has any influence on steroid hormone synthesis and/or secretion of patients. Thus, the nature of the costimulatory substance as well as possible clinical consequences of the magnitude of activated AGP response are currently unknown. However, one can assume that cytotoxic factors eliminated by high-flux HD have not only effects on hepatic acute phase proteins, but also on different metabolic pathways in the liver. Several studies revealed that hyperglyceridemia, one of various atherogenic risk factors in chronic HD patients, can be decreased by high-flux dialysis [21–23]. The characteristics of our patients are not in strong accordance with that which is most likely due to the short periods of treatment either with high- or with low-flux membranes (Table 2).

In summary, our findings clearly indicate that global liver functions as well as the acute phase protein AGP were influenced by high- and low-flux UFs in a different manner. Cytotoxic substances were removed by high-flux treatment, which affects the secretion of the positive acute phase protein AGP under stimulated conditions in the hepatoma-derived cell line HepG2. Further studies are necessary to elucidate more in detail hepatoactivity/hepatotoxicity of uremic toxins and whether there are more acute phase proteins besides AGP that are modulated by these toxins. Therapeutic pathways preventing these effects remain to be revealed. With regard to the strong impact of metabolic parameters on long-term outcome of dialysis patients, an in vitro system is required that permits the analysis of the important role of the liver during dialysis treatment.

In our minds, liver cells are an interesting target cell to assess biocompatibility as well as adequacy of different dialysis modalities and their effect on hepatic impairment related to uremia and ESRD therapy.

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