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Removal of uraemic retention solutes in standard bicarbonate haemodialysis and long-hour slow-flow bicarbonate haemodialysis

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Abstract

Background. Several studies already stressed the importance of haemodialysis (HD) time in the removal of uraemic toxins. In those studies, however, also the amount of dialysate and/or processed blood was altered. The present study aimed to investigate the isolated effect of the factor time *t* (by processing the same total blood and dialysate volume in two different time schedules) on the removal and kinetic behaviour of some small, middle and protein-bound molecules.

Methods. The present study had a crossover design: 11 stable anuric HD patients underwent two bicarbonate HD sessions (~4 and ~8 h) in a random sequence, at least 1 week apart. The GENIUS® single-pass batch dialysis system and the high-flux FX80 dialysers (Fresenius Medical Care, Bad Homburg, Germany) were used. The volume of blood and dialysate processed, volume of ultrafiltration, and dialysate composition were prescribed to be the same. For each patient, blood was sampled from the arterial line at 0, 60, 120, 180 and 240 min (all sessions), and at 360 and 480 min (8-h sessions). Dialysate was sampled at the end of HD from the dialysate tank. The following solutes were investigated: (i) small molecules: urea, creatinine, phosphorus and uric acid; (ii) middle molecule: β_2M; and (iii) protein-bound molecules: homocysteine, hippuric acid, indole-3-acetic acid and indoxyl sulphate. Total solute removals (solute concentration in the spent dialysate of each analyte × 90 L − the volume of dialysate) (TSR), clearances (TSR of a solute/area under the plasma water concentration time curve of the solute) (*K*), total cleared volumes (*K* × dialysis time) (TCV), and dialyser extraction ratios (*K*/blood flow rate) (ER) were determined. The percent differences of TSR, *K*, TCV and ER between 4- and 8-h dialyses were calculated. Single-pool *Kt*/*V*urea, and post-dialysis percent rebounds of urea, creatinine and β_2M were computed.

Results. TSR, TCV and ER were statistically significantly larger during prolonged HD for all small and middle molecules (at least, $P < 0.01$). Specifically, the percent increases of TSR (8 h vs 4 h) were: for urea 22.6% ($P < 0.003$), for creatinine 24.8% ($P < 0.002$), for phosphorus 26.6% ($P < 0.001$), and for β_2M 39.2% ($P < 0.005$). No statistically significant difference was observed for protein-bound solutes in any of the parameters being studied. Single-pool *Kt*/*V*urea was 1.41 ± 0.19 for the 4-h dialysis sessions and 1.80 ± 0.29 for the 8-h ones. The difference was statistically significant ($P < 0.0001$). Post-dialysis percent rebounds of urea, creatinine and β_2M were statistically significantly greater in the 4-h dialysis sessions (at least, $P < 0.0002$).

Conclusions. The present controlled study using a crossover design indicates that small and middle molecules are removed more adequately from the deeper compartments when performing a prolonged HD, even if blood and dialysate volumes are kept constant. Hence, factor time *t* is very important for these retention solutes. The kinetic be-

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haviour of protein-bound solutes is completely different from that of small and middle molecules, mainly because of the strength of their protein binding.

Keywords: haemodialysis; middle molecules; protein-bound solutes; small molecules; uraemia

Introduction

One of the major aims of renal replacement therapy is to remove uraemic waste products. The quantification of this removal is an important parameter in the assessment of adequacy of renal replacement therapy [1]. Urea is currently used as the standard marker for dialysis adequacy, by the calculation of the clearance index *Kt*/*V*urea. It depends on two separately modifiable factors: dialyser clearance *K* and dialysis time *t*. Eloot *et al*. showed the independent effect of the factor time *t* on the adequacy of haemodialysis (HD) in a simple and elegant way [1]. In fact, Eloot *et al*. were able to show that, by performing HD treatments of 4, 6 and 8 h in nine stable uraemic patients, processing the same total blood and dialysate volume by the GENIUS® batch dialysis system and the high-flux FX80 dialysers (Fresenius Medical Care, Bad Homburg, Germany), the total solute removals (TSR), the dialyser extraction ratios (ER) and total cleared volumes (TCV) were significantly larger during prolonged dialysis for urea, creatinine, phosphorus and β_2-microglobulin (β_2M) [1].

It should be realized that removal of the above solutes is not representative for many other molecules, and this is especially the case for 'difficult to remove' molecules, such as protein-bound solutes. Many studies suggested a correlation between plasma concentration of some protein-bound compounds and many severe clinical outcomes [2–6]. Protein-bound compounds are poorly cleared by conventional HD treatments because protein binding limits the 'free' solute concentration driving diffusion. Thus, dialysis removal largely depends on the equilibrium between bound and free fractions. To the best of our knowledge, no study has been yet performed to assess the removal of protein-bound solutes by means of long-hour (8 h) slow-flow HD treatments.

In this crossover study, we investigated the isolated effect of the factor time *t* (by processing the same total blood and dialysate volume in two different time schedules) on the removal and kinetic behaviour of some small molecules such as urea, creatinine, uric acid and phosphorus; middle molecules such as β_2M; and some protein-bound solutes, namely hippuric acid, homocysteine, indole-3-acetic acid and indoxyl sulphate.

Materials and methods

Design of the study

The study was approved by our institutional review board and was conducted in accordance with good clinical practice guidelines and ethical principles of the Helsinki Declaration. The inclusion criteria were (i) standard bicarbonate HD treatment since at least 6 months and (ii) uncomplicated HD sessions. A group of 11 stable Caucasian prevalent uraemic patients (nine males and two females, mean age 54.1 ± 17.8 SD years, dialysis vintage 78.0 ± 60.2 months) was enrolled. After obtaining informed consent, they underwent one standard (~4 h) and one long-hour (~8 h) slow-flow bicarbonate HD session in a random sequence, always at the same interdialytic interval, at least 1 week apart. The experimental HD sessions utilized the GENIUS® single-pass batch dialysis system [1]. It provides 90 L of bicarbonate dialysate per dialysis session. The characteristics of the GENIUS® dialysis system are described elsewhere [7]. The sessions were pair-matched as far as the dialysate and blood volume processed (90 L) and volume of ultrafiltration (V_{UF}) are concerned. Thus, the duration of treatment was dictated by the achievement of the target dialysate and blood volume processed (i.e. 90 L): for this reason, it was slightly different from 4 and 8 h (Table 1). Worth noting, the same dialysis machine and high-flux FX80 dialysers were used in all sessions. The characteristics of the dialyser are described elsewhere [1]. Dialysate composition was as follows: ionized calcium (Ca++) 1.5 mmol/L, magnesium 0.5 mmol/L, K+ 2 mmol/L, Na+ 140 mmol/L, bicarbonate 35 mmol/L, chloride 113 mmol/L, glucose 5.55 mmol/L and citrate 0.10 mmol/L.

Blood and dialysate sampling

Blood samples were taken from the inlet blood lines immediately before the onset of dialysis and at 60, 120, 180 and 240 min during the 4- and 8-h sessions. Additional samples were taken at 360 and 480 min during the 8-h sessions. Furthermore, at the end of dialysis, two samples were taken, respectively, from the ultrafiltrate recipient and from the dialysate tank, after thorough mixing, in order to quantify solute concentration in total spent dialysate. Particular attention was paid to the thorough mixing of the spent dialysate, strictly adhering to the ad hoc instructions present in the GENIUS® operator's manual.

All blood samples were drawn 1 min after decreasing the pump speed to 50 mL/min, which actually decreases also the dialysate flow rate to 50 mL/min in the case of the GENIUS® machine. After collection, blood and dialysate samples were immediately placed on ice. Blood samples were centrifuged at 3000 rpm (4235A Centrifuge, ALC, Milan, Italy), and all samples were stored at −80°C until analysis.

Measurements

Small molecules. Urea (MW: 60.1 Da), creatinine (MW: 113.1 Da), phosphorus (MW: 96 Da) and uric acid (MW: 168 Da) concentrations were measured by standard laboratory methods.

Middle molecules. β_2M (MW: 11 800 Da) concentrations were quantified using a two-step enzyme immunoassay sandwich method with a final fluorescent detection (bio-Mérieux SA, Marcy-l'Etoile, France).

Protein-bound molecules. To establish the total (the sum of free and bound fractions) concentration of hippuric acid (MW: 179.2 Da, protein binding ±50%), indole-3-acetic acid (MW: 175.2 Da, protein binding ± 65%) and indoxyl sulphate (MW: 212.1 Da, protein binding ±90%), plasma samples were deproteinized with acetonitrile. The supernatant was evaporated to dryness. The residue was reconstituted with mobile phase and analysed by high-performance liquid chromatography (HPLC) on a reverse-phase column (C18) using a diode array detector at 254 nm. In order to measure homocysteine (MW: 135.2 Da, protein binding ±70%)

Table 1. Dialysis data of the 11 patients undergoing two experimental HD sessions (4 and 8 h)

Parameter	4 h	8 h	P[a]
Treatment time (min)	257.7 (1.1)	469.1 (2.8)	<0.0001
Blood flow rate (mL/min)	350 (0)	190 (0)	<0.0001
Dialysate flow rate (mL/min)	350 (0)	190 (0)	<0.0001
Pre-dialysis body weight (kg)	72.2 (10.8)	71.9 (10.7)	0.205
Post-dialysis body weight (kg)	69.2 (10.3)	69.1 (10.1)	0.458
V_{UF} (L)	2.9 (0.8)	2.9 (0.9)	0.851
UF rate (mL/h/kg)	9.9 (2.2)	5.5 (1.5)	<0.0001

Mean (SD).
[a]Student's *t*-test for unpaired data.

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levels, plasma and dialysate samples were processed according to the Bio-Rad test (IVD and CE kit). The quantitative analysis was performed by HPLC using an isocratic system on a reverse-phase cartridge and a fluorescence detector (excitation: 385 nm; emission: 515 nm).

The precision of the measurements of the four protein-bound solutes by HPLC had been evaluated in a preliminary study based on the NCCLS EP5-A guideline 'Evaluation of Precision Performance of Clinical Chemistry Devices' [8]. In this study, two runs were performed daily over 20 working days. In each run, aliquots with low, mid and high concentrations of the four protein-bound solutes were analysed in duplicates. In order to determine linearity and detection limits of the four protein-bound solutes by HPLC, vials with five different concentrations of the four protein-bound solutes were prepared. Four replicates of each vial were analysed. In order to determine the recovery of the four protein-bound solutes by HPLC, sera with known concentrations of the four protein-bound solutes were spiked with a defined amount of the four protein-bound solutes.

Blood samples collected at the time points described above were utilized also for the measurement of plasma total protein (TP) concentrations by means of routine automated methods.

Calculations

(1) Plasma water concentration of the solutes was calculated as follows:

$$\text{Cpw} = \text{Cp}/(1-\alpha\text{TP}),$$

where Cpw is the plasma water solute concentration, Cp is the plasma solute concentration and α is a factor to calculate the protocrit from plasma TP ($\alpha = 0.00107$ L/g) [9].

(2) Solute concentration in total spent dialysate was measured as described for all the analytes under study. TSR was calculated as follows:

$$\text{TSR} = \text{solute concentration in the total spent dialysate of each analyte} \times 90\ \text{L(the volume of dialysate)}.$$

(3) Clearances (K) of all analytes were calculated as follows (calculation of Kurea is shown as an example):

$$K\ \text{urea} = \text{TSR}/\text{AUC urea},$$

where AUC urea is the area under the plasma water urea concentration time curve. AUCs were calculated by using the trapezoidal rule [10].

(4) TCV (millilitre) were calculated as a function of K and dialysis duration t (t_d) (minute):

$$\text{TCV} = Kt_d$$

(5) To obtain an idea about the removal capacity of the dialyser irrespective of the dialyser blood flow rate (Q_B), we considered the dialysed ER (dimensionless), defined as follows:

$$\text{ER} = K/Q_B(1)$$

(6) Reduction ratio (RR) (percent) of all the solutes was defined as a function of the pre-dialysis Cpw (preCpw) and concentration at different time points during dialysis (txCpw) of samples taken at the dialyser inlet:

$$\text{RR} = \frac{\text{preCpw} - \text{txCpw}}{\text{preCpw}}$$

(7) Single-pool Kt/Vurea was computed using the second-generation Daugirdas equation [11].

(8) The percent differences of TSR, K, TCV, ER and RR between 4- and 8-h dialyses were also calculated.

(9) Post-dialysis rebounds of urea, creatinine and β_2M were computed according to Tattersall *et al*. [12,13]: the equilibrated post-dialysis concentration (eqCpw) for a variety of solutes (urea, creatinine and β_2M) can be predicted from the measured preCpw of the solute, the immediate post-dialysis concentration (tfCpw) of the solute,

Table 2. Efficacy parameters of small solutes and the middle molecule β_2M during the 4- and 8-h dialysis sessions

	4 h	8 h	% increase (8 h vs 4 h)	P[a]
Total solute removal (mg)				
Urea	31 248 (12 239)	38 299 (17 132)	22.6	<0.003
Creatinine	1402 (685)	1750 (808)	24.8	<0.002
Uric acid	1135 (311)	1328 (310)	17.0	<0.002
Phosphorus	977 (218)	1237 (315)	26.6	<0.001
β_2M	181 (76)	252 (105)	39.2	<0.005
Clearance (mL/min)	4 h	8 h	% decrease (8 h vs 4 h)	P[a]
Urea	141 (41)	102 (28)	−27.7	<0.0001
Creatinine	109 (29)	92 (22)	−15.6	<0.001
Uric acid	98 (28)	67 (22)	−31.6	<0.0001
Phosphorus	112 (27)	86 (22)	−23.2	<0.01
β_2M	27 (6)	26 (7)	−3.7	0.458
Total cleared volume (mL)	4 h	8 h	% increase (8 h vs 4 h)	P[a]
Urea	36 186 (10 594)	47 558 (13 186)	31.4	<0.0001
Creatinine	28 013 (7489)	44 160 (10 169)	42.4	<0.0001
Uric acid	25 186 (7908.0)	32 160 (9982.7)	27.7	<0.0001
Phosphorus	28 769 (6965)	40 340 (10 166)	40.2	<0.003
β_2M	8201 (2117)	12 049 (3439)	46.9	<0.001
Extraction ratio	4 h	8 h	% increase (8 h vs 4 h)	P[a]
Urea	0.40 (0.12)	0.54 (0.15)	35.0	<0.0001
Creatinine	0.31 (0.08)	0.48 (0.11)	54.8	<0.0001
Uric acid	0.28 (0.08)	0.35 (0.1)	25.0	<0.0001
Phosphorus	0.32 (0.08)	0.45 (0.11)	40.6	<0.002
β_2M	0.08 (0.02)	0.13 (0.04)	62.5	<0.01

Means (SD).
[a]Student's *t*-test for unpaired data.

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Table 3. Efficacy parameters of protein-bound solutes during the 4- and 8-h dialysis sessions

	4 h	8 h	% difference (8 h vs 4 h)	P[a]
Total solute removal				
Homocysteine (μmol)	259 (233)	301 (238)	16.2	0.357
Hippuric acid (mg)	378 (62)	303 (71)	−19.4	0.177
Indole-3-acetic acid (μg)	44 (23)	50 (20)	13.6	0.541
Indoxyl sulphate (mg)	46 (17)	45 (17)	−2.2	0.921
Clearance (mL/min)	4 h	8 h	% difference (8 h vs 4 h)	P[a]
Homocysteine	23 (9)	18 (7)	−21.7	0.118
Hippuric acid	33 (5)	18 (4)	−45.5	0.004
Indole-3-acetic acid	13 (15)	10 (8)	−23.1	0.444
Indoxyl sulphate	13 (5)	9 (4)	−30.8	0.122
Total cleared volume (mL)	4 h	8 h	% difference (8 h vs 4 h)	P[a]
Homocysteine	6013 (2334)	8292 (3401)	37.9	0.08
Hippuric acid	8504 (1029)	8443 (1283)	−0.2	0.989
Indole-3-acetic acid	3476 (3855)	4537 (3635)	30.5	0.489
Indoxyl sulphate	3298 (1371)	4189 (1821)	27.0	0.285
Extraction ratio	4 h	8 h	% increase (8 h vs 4 h)	P[a]
Homocysteine	0.07 (0.03)	0.09 (0.04)	28.6	0.07
Hippuric acid	0.09 (0.02)	0.10 (0.02)	1.0	0.940
Indole-3-acetic acid	0.04 (0.04)	0.05 (0.04)	25.0	0.467
Indoxyl sulphate	0.04 (0.02)	0.05 (0.02)	25.0	0.261

Means (SD).
[a]Student's *t*-test for unpaired data.

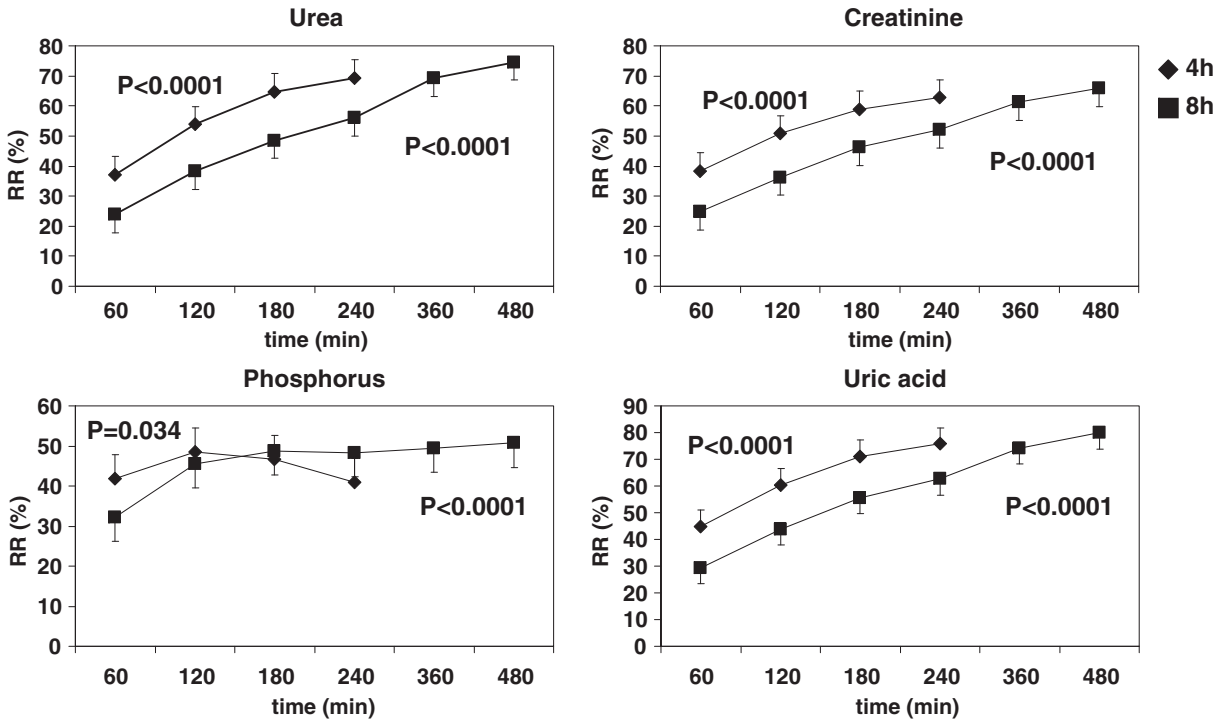

Fig. 1. Reduction ratios (RRs) at different time points during the 4- and 8-h dialysis sessions for small solutes. RRs increased over time, and the cumulative changes (differences between t60 and t240 or t480) were statistically significant for urea, creatinine, uric acid (in both the 4- and 8-h sessions) and phosphorus (only in the 8-h sessions); RR of phosphorus decreased significantly over time during the 4-h sessions. P-values are related to the difference between RR values at t60 and t240 or t480 in each session.

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session length (t_d) and the theoretical patient clearance time (pct) value:

$$\text{eqCpw} = \text{preCpw} \times (\text{tfCpw}/\text{preCpw})^{td/td+pct}$$

where pct, which can be defined as the time needed to clear all body compartments when the dialyser clearance is infinite, is 35, 70 and 110 min for urea, creatinine and β_2M, respectively [12,13].

This allows computing the percent rebound for the above solutes, as follows:

$$\%\ \text{rebound} = (\text{eqCpw} - \text{tfCpw})/\text{tfCpw} \times 100$$

Statistical analyses

Data are reported as means (SD). The normality of distribution of the data was ascertained by means of the Kolmogorov–Smirnov test. The values of all the parameters at the start and at the end of each HD session were compared by means of Student's *t*-test for unpaired data. Then, the trend analysis for the parameters studied during the HD sessions was made by using the repeated measures ANOVA with the Wilks lambda as multivariate test for the significance of each effect studied and with the Mauchly test of sphericity of the matrix. Correlation coefficients were determined for preCpw and TSR of all analytes according to Pearson's correlation coefficient. All the statistical inferences were made by means of SPSS 11.0 software (SPSS Inc., Chicago, IL, USA), and a P-value <0.05 was considered for the statistical significance.

Results

TSR, *K*, TCV and the dialyser ER of the small solutes and of the middle molecule β_2M are shown in Table 2. TSR, TCV and ER were statistically significantly larger during prolonged HD for all small and middle molecules. *K* of the 8-h dialysis sessions was statistically significantly less than *K* of the 4-h dialysis sessions, except for β_2M. The percent decrease of *K* of prolonged HD sessions was statistically significantly less (Table 2).

TSR, *K*, TCV and the dialyser ER of the protein-bound solutes are shown in Table 3. No statistically significant difference was observed for protein-bound solutes in any of the parameters being studied, except for hippuric acid where a difference in *K* was found (P < 0.004).

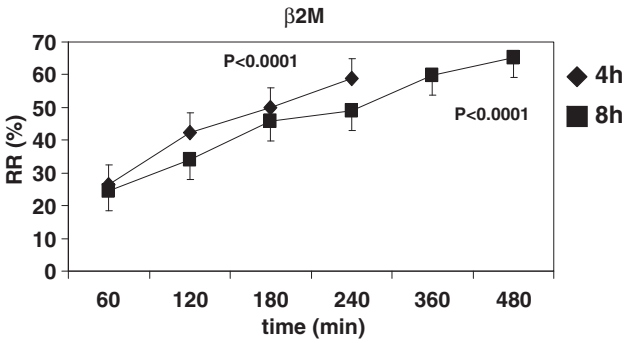

Fig. 2. Reduction ratios (RRs) at different time points during the 4- and 8-h dialysis sessions for middle molecule β_2M. RRs increased over time, and the cumulative changes (differences between t60 and t240 or t480) were statistically significant. P-values are related to the difference between RR values at t60 and t240 or t480 in each session.

RRs at different time points during the 4- and 8-h dialysis sessions are shown in Figures 1 (small solutes), 2 (β_2M) and 3 (protein-bound solutes). The RRs increased over time, and the cumulative changes (differences between t60 and t240 or t480) were statistically significant for urea, creatinine, uric acid, β_2M and indoxyl sulphate (in both the 4- and 8-h sessions) (Figures 1–3). The RR of phosphorus increased over time, and the cumulative changes were statistically significant only in the 8-h sessions (Figure 1). The RR of phosphorus significantly decreased over time during the 4-h sessions (Figure 1). No statistically significant changes over time were observed for the RRs of the other protein-bound solutes (Figure 3).

Single-pool *Kt*/*V*urea was 1.41 ± 0.19 for the 4-h dialysis sessions and 1.80 ± 0.29 for the 8-h ones. The difference was statistically significant (P < 0.0001).

Table 4 shows the correlations between pre-dialysis plasma water concentrations and TSRs for all the solutes under study during a dialysis session of 4 and 8 h. Many statistically significant correlations could be demonstrated not allowing, however, to identify class relationships among the categories of molecules under study: for example, as far as the small molecules are concerned, the correlation existed for urea and creatinine, but not for phosphorus and uric acid; as far as the protein-bound solutes are concerned, the correlation existed for homocysteine, but not for hippuric acid, indole-3-acetic acid and indoxyl sulphate.

The percent differences in the efficacy parameters under study (TSR, *K*, TCV and ER) between the 4- and 8-h dialysis sessions are shown in Table 2 (small solutes and β_2M), in Table 3 (protein-bound solutes) and in Table 5. Specifically, the latter shows the comparison between the RRs of post-dialysis versus pre-dialysis values of the small solutes, β_2M and protein-bound solutes. The RRs of the 8-h dialysis sessions were statistically significantly larger than the corresponding RRs of the 4-h dialysis sessions for the small solutes and β_2M. No difference was observed as far as protein-bound solutes are concerned.

Finally, post-dialysis percent rebounds of urea, creatinine and β_2M were statistically significantly greater in the 4-h dialysis sessions, when compared with the 8-h ones (P < 0.0001, P < 0.0001 and P < 0.0002, respectively) (Table 6).

Discussion

Dialysis is a lifesaving but imperfect technique; if it were less imperfect, survival would be almost certainly much improved. The lacking elements are both quantitative and qualitative [9]. Among the former, what is currently under investigation is whether longer dialysis times and/or more frequent dialysis can improve outcomes. The theoretical reasons behind these treatments are (i) the increased removal of substances whose clearance is hindered due to their size or binding to plasma proteins and (ii) opportunities to remove more gently (i.e. with fewer side effects and, hence, more efficiently) extracellular fluid and reach dry weight [14]. To some extent, the qualitative/quantitative distinction is arbitrary. Impaired removal of substances >5000 Da clearly has a qualitative

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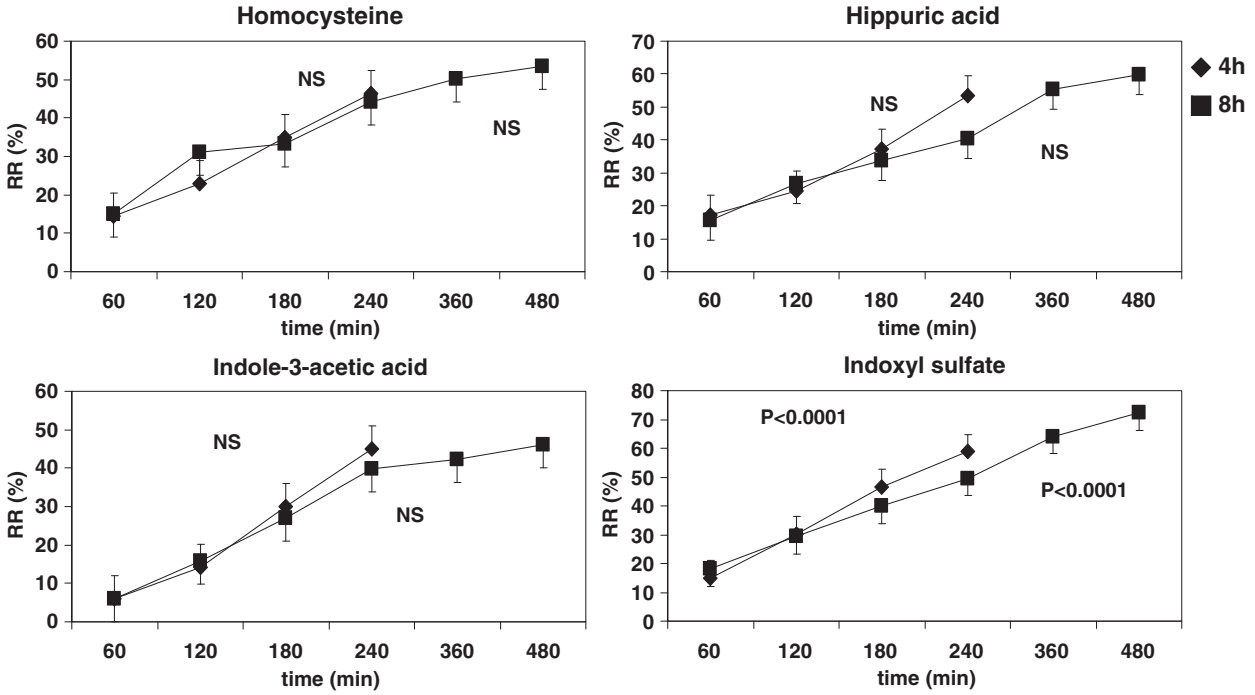

Fig. 3. Reduction ratios (RRs) at different time points during the 4- and 8-h dialysis sessions for protein-bound solutes. RRs increased over time, and the cumulative changes (differences between t60 and t240 or t480) were statistically significant only for indoxyl sulphate. No statistically significant changes over time were observed for the RRs of the other protein-bound solutes. P-values are related to the difference between RR values at t60 and t240 or t480 in each session.

aspect to it, as current high-flux dialysis membranes are deliberately designed to limit albumin losses and, as a consequence, restrict effectively the removal of molecules much larger than β_2M [14]. This will inevitably impair clearance of small proteins known to be 'toxic' and their glycated, oxidized, sulphated, and nitrated end products [14]. Another principal qualitative shortcoming of current standard or high-flux HD therapy is the poor removal of the many substances identified as uraemic toxins which are bound to plasma proteins, particularly to albumin.

The present study was designed to investigate the isolated effect of the factor time *t* on the removal and kinetic behaviour of some uraemic retention solutes. However, the total amount of blood and dialysate crossing the dialyser over the entire session was the same for both strategies. By maintaining at the same level the blood and dialysate volumes processed during long-hour dialyses, it may have limited the effect of prolonging time on the removal of these solutes.

The main findings of our study can be summarized into two points:

(1) a statistically significant increase in TSR, TCV and dialyser ER of small and middle molecules during prolonged dialysis when compared with standard dialysis; and

(2) no statistically significant difference in TSR, TCV and dialyser ER of protein-bound uraemic molecules during prolonged dialysis when compared with standard dialysis.

As far as the first point is concerned, our findings are not novel: in fact, they confirm, even though not completely, those by Eloot *et al*. [1]. There are two main points of discrepancies with the study by Eloot *et al*. [1]: at variance with our data, they did not find any difference in *Kt*/*V*urea and RRs. The reasons for these discrepancies are far from being clear. However, the main message is common to the two studies, i.e. small and middle molecules are removed more adequately from the deeper compartments when performing a prolonged HD, even if blood and dialysate volumes are kept constant. Worth noting, post-dialysis percent rebounds of urea, creatinine and β_2M in the 8-h dialysis sessions decreased by 33.3%, 35.6% and 24.7%, respectively, when compared with the 4-h ones, despite the fact that their TSRs increased by 22.6%, 24.8% and

Table 4. Correlations between pre-dialysis plasma water concentrations and total solute removal for all the solutes under study during a dialysis session of 4 and 8 h

	4 h		8 h	
	R	P[a]	*R*	P[a]
Urea	0.561	0.072	0.806	<0.003
Creatinine	0.628	0.043	0.617	<0.047
Phosphorus	0.636	0.039	0.491	0.115
Uric acid	0.455	0.150	0.331	0.327
β_2M	0.787	0.003	0.774	<0.004
Homocysteine	0.780	0.005	0.942	<0.0001
Hippuric acid	−0.209	0.541	0.445	0.171
Indole-3-acetic acid	−0.092	0.804	0.455	0.164
Indoxyl sulphate	0.145	0.656	0.009	0.991

[a]Pearson's correlation coefficients.

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Table 5. Reduction ratios (RRs) at the end of the 4- and 8-h dialysis sessions compared to the pre-dialysis values

	RR 4 h (%)	RR 8 h (%)	% increase (8 h vs 4 h)	P[a]
Urea	69.3 (4.9)	74.6 (4.8)	7.6	<0.0004
Creatinine	62.7 (6.7)	65.9 (5.7)	5.1	<0.03
Uric acid	75.8 (6.4)	79.9 (5.3)	5.4	<0.002
Phosphorus	40.9 (12.5)	50.7 (13.8)	23.9	<0.01
β_2M	58.9 (8.4)	65.3 (8.3)	10.9	<0.01
Homocysteine	45 (22.2)	51 (15.8)	13.3	0.185
Hippuric acid	53.3 (10.4)	57 (13.5)	7.5	0.428
Indole-3-acetic acid	44.7 (9.4)	46.5 (11.3)	4.9	0.651
Indoxyl sulphate	61.4 (17.0)	71.5 (19.6)	16.3	0.102

Means (SD).
[a]Student's *t*-test for unpaired data.

39.2%, respectively. Once again, these data confirm the crucial role played by the factor time *t* in the adequacy of HD. Even though the importance of *t* had already been stressed [15,16], its crucial role had always escaped the experimental verification because of the difficulty in separating the role of *t* from that of other variables. Thus, the diffusive mechanism turns out to be more efficient simply because of an increase in *t*: consequently, it turns out to be more effective clinically because it increases the TSR and TCV of urea, creatinine, uric acid, phosphorus and β_2M in a statistically significant way. As far as the improved dialyser ER (8-h sessions) of urea, creatinine, uric acid, phosphorus and β_2M is concerned, it is not necessarily due to the longer treatment duration. In fact, it is well known that *K* of a given dialyser is not linear to Q_B and dialysate flow rate. Therefore, an effect of the reduced flow rates is more likely.

As far as the second point is concerned, our findings are completely new. They show no difference between standard and prolonged dialysis. Many could be the reasons why prolonged dialysis did not generally result in improved protein-bound toxin removal: the low patient number and statistical power of the study, compartmental behaviour, and regeneration of solute pools over time versus dialyser clearance at given Q_B. However, we think that the main explanation resides in the strength of the protein binding in plasma of these molecules. The latter is influenced by several chemical and physical conditions (e.g. blood pH, electrical charges of molecules, amount of plasma TP, nutrition, etc.) which induce different degrees of protein binding. Therefore, it is reasonable to have observed the differences in the kinetics of these toxins as we did in our study: a statistically significant RR in long dialysis for indoxyl sulphate, at variance with the other protein-bound molecules.

Table 6. Comparison of post-dialysis percent rebounds of urea, creatinine and β_2M between the 4- and 8-h dialysis sessions

	4 h	8 h	% decrease (8 h vs 4 h)	P[a]
Urea	15.0 (2.4)	10.0 (1.6)	−33.3	<0.0001
Creatinine	23.3 (5.4)	15.0 (3.0)	−35.6	<0.0001
β_2M	30.3 (7.4)	22.8 (6.2)	−24.7	<0.0002

Means (SD).
[a]Student's *t*-test for unpaired data.

A very recent paper comparing the protein-bound uraemic toxin removal in HD and post-dilution haemodiafiltration showed that the elimination of these molecules is governed particularly by diffusion [17]. Actually, another recent paper showed that the increase of dialyser mass transfer area coefficient and dialysate flow rate has a positive effect on clearances of protein-bound solutes [18]. The data by Krieter *et al*. [17] confirm previous *in vitro* studies on continuous venovenous haemofiltration and haemodiafiltration, which suggest that an increase of protein-bound toxin removal by higher ultrafiltration rates (i.e. convection) is scarcely effective [19]. Furthermore, a previous trial, which had reported that convection can provide superior protein-bound solute removal compared with high-flux HD, did also suggest that diffusion is the most important mechanism governing the transport of (the free portion of) protein-bound solutes [20]. So, in this respect, it does not contradict the results of Krieter *et al*. [17]. As a matter of fact, the best performance of haemodiafiltration in the paper by Bammens *et al*. was in a pre-dilutional mode with high substitution volumes [20]. This again points to the importance of creating a large dilution gradient for the free portion of the protein-bound solutes. Thus, the question remains open on how to improve clearance of these retention solutes, even though the findings suggest that removal of protein-bound solutes during HD and haemodiafiltration with non-protein-leaking membranes is almost exclusively caused by elimination of their unbound fraction [20]. Alternative strategies promise to be more efficient therapy forms. These could comprise adsorptive measures, either applied orally [21,22] or during extracorporeal therapy [23,24], which interact with protein-bound compounds or their precursors, and techniques, which alter the strength of the protein binding in plasma of these molecules.

Some limitations of the study must be acknowledged: only the total concentration was evaluated as far as the protein-bound solutes are concerned, whereas it is the free fraction that probably exerts toxicity. Thus, one cannot exclude the possibility that, whereas total concentration did not differ between both treatments, such a difference would well exist for the free fraction.

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In conclusion, the present controlled study using a crossover design indicates that small and middle molecules are removed more adequately from the deeper compartments when performing a prolonged dialysis, even if blood and dialysate volumes are kept constant. Hence, factor time *t* is very important when comparing different HD durations. The kinetic behaviour of protein-bound solutes is completely different from that of small and middle molecules, mainly because of the strength of their protein binding.

Conflict of interest statement. None declared.

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