Estonian Journal of Engineering, 2010, **16**, 1, 95–106 doi: 10.3176/eng.2010.1.09

Behaviour of uremic toxins and UV absorbance in respect to low and high flux dialyzers

Kai Lauri[a], Jürgen Arund[a], Risto Tanner[a,b], Jana Jerotskaja[a], Merike Luman[a,c] and Ivo Fridolin[a]

[a] Department of Biomedical Engineering, Technomedicum, Tallinn University of Technology, Ehitajate tee 5, 19086 Tallinn, Estonia; kai.lauri@mail.ee
[b] Laboratory of Chemical Physics, National Institute of Chemical Physics and Biophysics, Akadeemia tee 23, 12618 Tallinn, Estonia
[c] Department of Dialysis and Nephrology, North Estonia Medical Centre, Sütiste tee 19, 13419 Tallinn, Estonia

Received 28 November 2009, in revised form 21 January 2010

Abstract. The aim of this study was to investigate the behaviour of uremic toxins and UV absorbance in respect to low and high flux dialyzers during hemodialysis treatments. Ten uremic patients were investigated using online spectrophotometry, with wavelength of 280 nm, over the course of 30 hemodialysis treatments. The polysulphone dialyzers were used. The taken dialysate and blood samples were analysed using standard biochemical methods and reversed phase HPLC. The chromatographic peaks were detected by a UV detector at wavelengths of 254 and 280 nm. Spiking experiments and UV spectra between 200–400 nm allowed to identify predominant uremic toxins in 5 chromatographic peaks identified as creatinine (CR), uric acid (UA), hypoxanthine (HX), indoxyl sulphate (IS), and hippuric acid (HA). Moreover, two persistent, but non-identified peaks, peak 1 (P1) and peak 2 (P2), were detected. There was no significant difference in the reduction ratio of uremic solutes and the UV absorbance between the low and high flux membranes. The reduction ratios, estimated by the total area of HPLC peaks at 254 nm and 280 nm in the serum and by the online UV absorbance at 280 nm, were closest to the removal of small water-soluble non-protein bound solutes urea, creatinine and uric acid. All studied uremic toxins and UV absorbance showed similar reduction for the low and high flux membranes during hemodialysis.

Key words: uremic toxins, hemodialysis, ultraviolet absorption, chromatography, dialysis membrane.

1. INTRODUCTION

Hemodialysis is a treatment that performs the functions of normal kidneys, i.e. removes uremic toxins. To date, a long list of possible uremic toxins has been identified as believed to be responsible for multifactorial and cumulative cause of

uremic toxicity [1]. These toxins have different molecular weights and some of them are bound to proteins. The target of dialysis therapy is adequate solute removal so that plasma concentrations remain at the most non-toxic levels possible. Most common indices of dialysis efficiency are based on the blood urea measurements. Unfortunately, urea is a small molecule weight solute and relying solely on urea may lead to inappropriately short dialysis during high flux or high efficiency dialysis. Thus, urea, the traditional marker for dialysis quality, should not be the only solute used to model the dialysis therapy [2]. At the same time, there is a need for techniques, which can offer a tool for separate monitoring of several compounds, retained in uremic patients and with potential clinical significance. Online monitoring of solute removal by dialysis via an optical UV absorbance-based device represents a valid alternative to the classical and, to date, expensive online urea monitoring devices [3].

For the removal of uremic compounds, different dialysis membranes are available, which will at least partly determine the efficiency of dialysis therapy [4]. Advocacy of the more efficient dialysis modalities with the high flux (HF) and super-flux (SF) membranes stresses the importance to study the behaviour of non-protein-bound and protein-bound uremic toxins in respect to their removal characteristics with different membranes [5]. In this study, the multidimensional effect, how low flux (LF) and HF dialysis membranes can be implicated in the removal of different uremic compounds and measurements of the UV absorbance, was examined. Assuming that the dialysate may be a preferred alternative for continuous monitoring of solute removal and adequacy of dialysis, the HPLC analysis of both serum and dialysate samples would be valuable as a source of information.

The aim of this study was to investigate the behaviour of non-protein-bound and protein-bound uremic toxins and UV absorbance in respect to low and high flux dialyzers during hemodialysis treatments.

2. SUBJECTS AND METHODS

2.1. Subjects and clinical study

This study was performed after the approval of the protocol by the Tallinn Medical Research Ethics Committee at the National Institute for Health Development, Estonia. An informed consent was obtained from all participating patients. Ten patients, mean age 62.6 ± 18.6 years, receiving thrice-weekly hemodialysis, were studied during 30 dialysis sessions (three for each patient). All patients were dialysed with polysulfone membrane dialyzer Fresenius 4008H (Fresenius Medical Care, Germany): (1) 4 patients by low flux dialyzer F8 HPS with an effective membrane area of 1.8 m^2 and an ultrafiltration coefficient of 18 mL/h*mmHg; (2) one patient with a low flux membrane dialyzer F10 HPS with a membrane area of 2.2 m^2, ultrafiltration coefficient of 21 mL/h*mmHg; (3) 5 patients with high flux dialyzer FX 80 with effective membrane area of

1.8 m^2 and ultrafiltration coefficient of 59 mL/h*mmHg. The dialysate flow was 500 mL/min and the blood flow varied from 245 to 350 mL/min. All treatments were monitored optically by a spectrophotometer HR2000 (Ocean Optics, Inc., USA), which was used to determine the UV absorbance with a specially designed optical cuvette, connected to the fluid outlet of the dialysis machine with all spent dialysate passing through during the online experiments. The clinical set-up of the experiments is shown in Fig. 1. The online absorbance curve during a single hemodialysis treatment is also presented. UV absorbance was measured in arbitrary units. The sampling frequency was set to two samples per minute. The obtained UV absorbance values were processed and presented on the computer screen by a PC, connected to the spectrophotometer using the Ocean Optics software (OOIBase32, Ocean Optics, Inc., USA, version 2.0.2.2 for Windows).

2.2. Sampling

Blood and dialysate samples were obtained from dialysis patients. Blood samples were drawn before the start of dialysis (B_{start}) and immediately after the treatment (B_{end}) (Fig. 1) using the slow flow/stop pump sampling technique. Blood was sampled into a BD Vacutainer® Glass Serum Tube (red cup, Beckton Dickinson) and was allowed to clot. After centrifugation at 3000 rpm the serum was separated from the blood cells.

The dialysate samples were taken 10 minutes after the start of the dialysis session (D_{start}), and immediately before the end of dialysis (D_{end} = 210 or 240 min). Also, pure dialysate, used as the reference solution, was collected before each dialysis session, when the dialysis machine was prepared and the conductivity was stable.

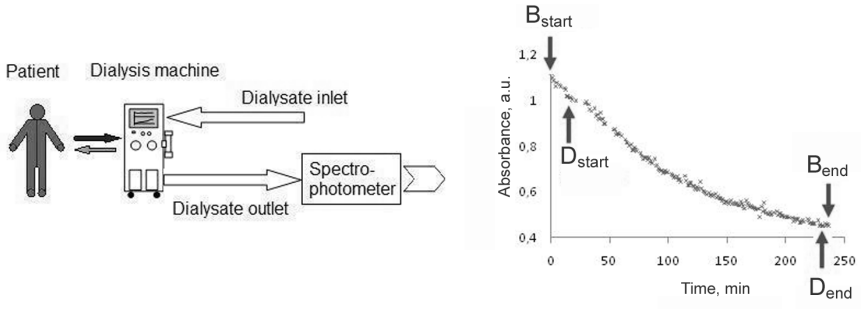

Fig. 1. The clinical set-up of the experiments. Time points when the samples were taken for the later analysis are as follows: B_{start} – blood sample collected before dialysis session, B_{end} – blood sample collected at the end of hemodialysis, D_{start} – dialysate sample collected 10 min after the start of hemodialysis, D_{end} – dialysate sample collected at the end of hemodialysis. The online absorbance measurements after every 30 s are presented by "x".

2.3. Biochemical analyses

The serum and the dialysate samples were analysed immediately at the Clinical Chemistry Laboratory at North Estonia Medical Centre using standardized methods. Urea (UR, MW = 60.06 Da), creatinine (CR, MW = 113.12 Da), and uric acid (UA, MW = 168.11 Da) were measured with a Hitachi 912 autoanalyzer (Roche, Switzerland). The determination of creatinine (μmol/L) in serum based on the Jaffe reaction, the intensity of creatinine complex (with pirate) was measured potentiometrically. Urea (mmol/L) was detected by the kinetic UV assay and uric acid (μmol/L) was detected by the enzymatic colorimetric method. The coefficient variation (CV) of the methods for the determination of different solutes in dialysate and blood were: CR 5%; UA 2%; UR 4%.

2.4. Reversed phase HPLC study

Before the HPLC analysis, the serum samples were purified of proteins by centrifuging with the Microcon centrifugal filters (Millipore, USA) at room temperature. The dialysate samples were acidified down to pH 4.0 with formic acid for conformation with the pH of the chromatographic eluent used.

The HPLC system consisted of a quaternary gradient pump unit, a column oven, and a diode array spectrophotometric detector (DAD, all Series 200 instruments from Perkin Elmer, Norwalk, CT, USA), a manual injector from Rheodyne (Rohnert Park, CA, USA), and a Zorbax C8 4.6×250 mm column from Du Pont Instruments (Wilmington, DE, USA) with a security guard KJO-4282 from Phenomenex (Torrance, CA, USA). The eluent was mixed with 0.05 M formic acid adjusted to pH 4.0 with ammonium hydroxide (A), HPLC grade methanol (B) and HPLC-S grade acetonitrile (C), both from Rathburn (Walkerburn, Scotland), with a six step gradient program as specified in Table 1.

The total flow rate of 1 mL/min was used continuously and the column temperature was adjusted to 30 °C. The UV absorbance was monitored at 280 nm with a measurement interval of 880 ms, spectra registered between 200–400 nm with a time interval of 1.76 s, and data processed respectively by means of Turbochrom WS and Turboscan 200 software from Perkin Elmer. The chromatographic peaks were detected by the UV detector at wavelengths of 254 and 280 nm.

Table 1. Elution program used for HPLC separation of constituents in the dialysate

Step	Time, min	Buffer (A), %	Methanol (B), %	Acetonitrile (C), %	Gradient
0	0	100	0	0	–
1	30	60	36	4	Linear
2	5	10	81	9	Linear
3	4	10	81	9	No grad
4	1	10	0	90	Linear
5	6	10	0	90	No grad

2.5. Data analysis

The reduction ratio (RR) (%) of compounds was defined as a function of pre-dialysis concentration (C_{start}) and concentration at the end of hemodialysis (C_{end}):

$$RR = \frac{C_{start} - C_{end}}{C_{start}} 100\%. \quad (1)$$

C_{start} and C_{end} were replaced by TA $HPLC_{pre}$ and TA $HPLC_{post}$ representing the total area of the HPLC peaks, measured at the start, and the end samples for RR from HPLC, respectively.

The results are presented as mean ± standard deviation. A non-parametric Mann-Whitney U Test and Student's t-test was used to compare groups of values while $p < 0.05$ was considered significant. Two sessions, inadequate due to the technical failure of the spectrophotometer, were excluded. In the case of HPLC analysis only these peaks were taken into account, where separation from neighbouring peaks was confirmed by comparison of UV spectra with those of the reference standards. The number of omitted cases was: IS (4), HA (4), HX (14), P1 (13), P2 (4), TA HPLC 254 nm (3), TA HPLC 280 nm (2), online 280 nm dialysate (4). The data analyses were performed in Statistica 6.0 (Statsoft, Inc. for Windows).

3. RESULTS

Figure 2 shows the representative HPLC chromatogram of the serum, measured at the wavelength of 254 nm. A number of higher prevalent peaks, representing chromophores-uremic toxins, can be observed. Some HPLC peaks were identified, such as creatinine, uric acid (the highest contribution), hypoxanthine, indoxyl sulphate and hippuric acid. Absorbing spectra of two unknown persistent peaks (P1 and P2) were identified at the retention times (RT) of 15.46 and 15.82 min. Additionally, some unknown peaks between the RT of 21–29 min were detected.

The solute concentrations and the UV absorbance values at the start and end of treatment in the serum and online in the spent dialysate for the LF and HF membranes are presented in Table 2. As Table 2 shows, the solute concentrations and the UV-absorbance values are lower at the end of dialysis (C_{end}) compared to the start concentrations (C_{start}). At the same time, the start and end concentrations and UV absorbance values for different membranes were not statistically different ($p < 0.05$).

Table 3 presents the RR (%) of solutes and the total area of the HPLC UV absorbance peaks at wavelengths of 254 and 280 nm in serum, and for the online UV absorbance at 280 nm in the spent dialysate, for different types of membranes. There was no significant difference between the results for RR of LF and HF membranes ($p < 0.05$).

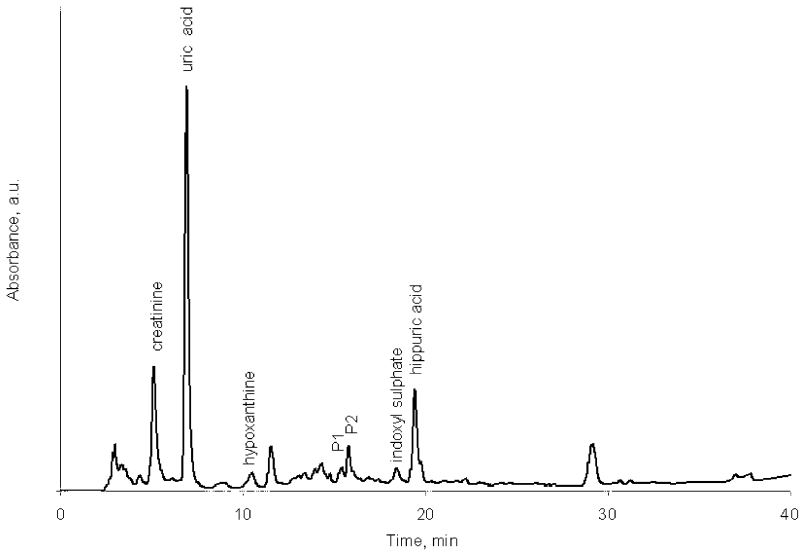

Fig. 2. The representative HPLC chromatogram of the serum, monitored at the wavelength of 254 nm; identified peaks are presented.

Table 2. The start (C_{start}) and end (C_{end}) concentrations of solutes and the total area of the HPLC UV absorbance peaks at the wavelengths of 254 and 280 nm ("TA HPLC 254 nm" and "TA HPLC 280 nm", respectively) in the serum, and the RR of online UV absorbance at 280 nm in the spent dialysate ("Online 280 nm dialysate") for HF and LF membranes; *N* denotes the number of cases

	LF		HF	
	C_{start}	C_{end}	C_{start}	C_{end}
Urea, mmol/L	21.0 ± 4.07 (*N* = 15)	7.03 ± 2.49 (*N* = 15)	19.4 ± 4.48 (*N* = 15)	7.11 ± 1.79 (*N* = 15)
Creatinine, μmol/L	735 ± 172 (*N* = 15)	311 ± 106 (*N* = 15)	591 ± 204 (*N* = 15)	252 ± 83 (*N* = 15)
Uric acid, μmol/L	356 ± 98 (*N* = 15)	116 ± 51 (*N* = 15)	381 ± 62 (*N* = 15)	130 ± 30 (*N* = 15)
Indoxyl sulphate, mg/L	6.20 ± 2.36 (*N* = 15)	3.69 ± 1.74 (*N* = 14)	6.83 ± 3.09 (*N* = 15)	3.76 ± 1.26 (*N* = 13)
Hippuric acid, mg/L	29.9 ± 16.4 (*N* = 15)	6.52 ± 3.95 (*N* = 15)	23.7 ± 28.6 (*N* = 15)	9.22 ± 8.10 (*N* = 11)
Hypoxanthine, mg/L	3.00 ± 2.93 (*N* = 11)	1.42 ± 1.28 (*N* = 14)	2.54 ± 2.34 (*N* = 9)	1.82 ± 2.52 (*N* = 12)
P1, a.u. $\times 10^{-2}$	2.92 ± 1.59 (*N* = 14)	0.99 ± 0.39 (*N* = 12)	2.47 ± 0.70 (*N* = 12)	1.30 ± 0.62 (*N* = 9)
P2, a.u. $\times 10^{-2}$	9.62 ± 5.86 (*N* = 15)	3.99 ± 2.16 (*N* = 14)	7.58 ± 1.73 (*N* = 15)	3.63 ± 1.10 (*N* = 12)
TA HPLC 254 nm, a.u. $\times 10^{7}$	4.00 ± 2.65 (*N* = 14)	1.47 ± 0.98 (*N* = 15)	2.52 ± 0.76 (*N* = 14)	1.16 ± 0.56 (*N* = 14)
TA HPLC 280 nm, a.u. $\times 10^{7}$	3.28 ± 1.42 (*N* = 15)	1.12 ± 0.49 (*N* = 15)	2.89 ± 0.60 (*N* = 14)	1.12 ± 0.21 (*N* = 14)
Online 280 nm dialysate, a.u.	1.53 ± 0.38 (*N* = 15)	0.65 ± 0.22 (*N* = 15)	1.44 ± 0.42 (*N* = 13)	0.60 ± 0.16 (*N* = 13)

Table 3. The RR (%) of solutes and the total area of the HPLC UV absorbance peaks at the wavelengths of 254 and 280 nm ("TA HPLC 254 nm" and "TA HPLC 280 nm", respectively) in the serum, and the RR of online UV absorbance at 280 nm in the spent dialysate ("Online 280 nm dialysate") for different types of membranes; *N* denotes the number of cases

	LF	HF
Urea	67.0 ± 8.7 (*N* = 15)	63.2 ± 5.07 (*N* = 15)
Creatinine	58.2 ± 7.7 (*N* = 15)	56.6 ± 5.4 (*N* = 15)
Uric acid	67.7 ± 8.5 (*N* = 15)	65.6 ± 6.7 (*N* = 15)
Indoxyl sulphate	42.1 ± 18.0 (*N* = 13)	47.8 ± 14.0 (*N* = 12)
Hippuric acid	75.1 ± 11.5 (*N* = 15)	68.1 ± 9.4 (*N* = 10)
Hypoxanthine	42.6 ± 16.0 (*N* = 10)	46.1 ± 18.5 (*N* = 8)
P1	62.1 ± 13.0 (*N* = 12)	61.0 ± 5.3 (*N* = 7)
P2	59.2 ± 17.5 (*N* = 13)	51.6 ± 5.9 (*N* = 12)
TA HPLC 254 nm	60.2 ± 12.5 (*N* = 14)	57.2 ± 7.7 (*N* = 13)
TA HPLC 280 nm	65.2 ± 9.6 (*N* = 15)	60.6 ± 7.9 (*N* = 14)
Online 280 nm dialysate	58.1 ± 8.3 (*N* = 15)	57.0 ± 10.4 (*N* = 13)

Figure 3 presents the RR of the uremic solutes and total area of HPLC peaks (at wavelengths of 254 and 280 nm, "TA HPLC 254 nm" and "TA HPLC 280 nm", respectively) in the serum, and the RR of online UV absorbance at 280 nm in the spent dialysate ("Online 280 nm") combining the results from the start and end samples (Table 3). Three main groups were distinguished according to the average reduction ratio among the studied solutes. The highest RR (>70%) had hippuric acid classified as a "High RR" solute. "Medium RR" solute group ($50\% < RR \leq 70\%$) incorporated the small water soluble compounds uric acid, urea, creatinine, and peaks 1 and 2. The "Low RR" solutes' group ($RR \leq 50\%$) included the protein bound solute indoxyl sulphate and the small water soluble compound hypoxanthine. A statistically dissimilar RR was found between the groups. No statistically different RR was between the solutes within the same group ($p < 0.05$), except for the "Medium RR" group, which could be divided into two subgroups: (1) "Medium 1 RR" solute group ($60\% < RR \leq 70\%$) with uric acid and urea; (2) "Medium 2 RR" group ($50\% < RR \leq 60\%$) with creatinine. The solutes of the subgroups had different removal rates.

All UV absorbance based RR values were higher than the "Low RR" solutes IS, HX, and lower than HA. However, P1 was a "centre-medium peak" removed statistically alike as all "Medium RR" solutes/peaks. The "left side bar" of P1,

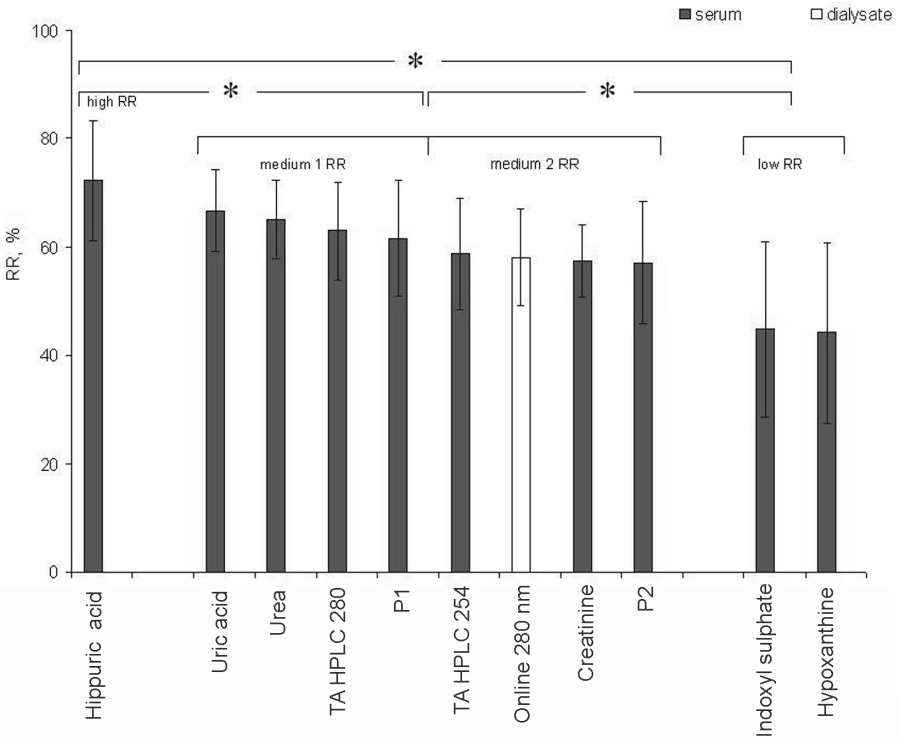

Fig. 3. The RR of solutes and TA HPLC (254 and 280 nm) in the serum and online UV absorbance in the spent dialysate (online 280 nm). Significant differences ($p < 0.05$) are marked with an asterisk (*).

RR of "TA HPLC 280", was similar to UA, UR, "TA HPLC 254", and higher than "Online 280 nm", CR, and P2, whereas the "right side bar" of P1, RR of "TA HPLC 254", was lower than UA, UR, and comparable to all other "Medium RR" solutes/peaks. "Online 280 nm", CR, and P2 were all removed statistically similarly and had lower RR than UA, UR, and "TA HPLC 280".

4. DISCUSSION

The present study investigated behaviour of non-protein-bound and protein-bound uremic toxins and UV absorbance in respect to low and high flux dialyzers during hemodialysis treatment. The results indicated that: (i) the main identified solutes responsible for the UV absorbance at 280 nm are the low molecular weight water-soluble non-protein-bound compounds UA, CR, and the low molecular weight water-soluble protein-bound compounds HA, IS, HX; (ii) two persistent, but non-identified HPLC peaks, P1 and P2, were detected from the HPLC profiles contributing to the UV absorbance, possibly each peak representing a single uremic retention solute; (iii) the LF (F8 HPS, F10 HPS) and HF

(FX80) membranes showed similar start and end concentrations for all studied uremic solutes; (iv) the LF and HF membranes showed comparable RR for all studied uremic solutes, except for P2, having slightly higher RR value for LF membranes ($p = 0.016$); (v) there was no statistical difference between intradialytic start-end values, and removal efficiency for the LF and HF membranes estimated by the total area of HPLC peaks at 254 and 280 nm in the serum and online UV absorbance at 280 nm in the spent dialysate, indicating similar behaviour of the UV absorbance to the uremic toxins.

A number of the higher prevalent peaks on the HPLC profiles of the serum (Fig. 2) indicate that there exists a group of compounds, UV chromophores, which are the main cause of the cumulative and integrated UV absorbance. The results of our study indicate that the main solute, responsible for the UV absorbance, is a low-molecular-weight water-soluble non-protein-bound compound uric acid UA. Four additional uremic retention solutes, creatinine, hypoxanthine, indoxyl sulpate and hippuric acid, were identified from the HPLC profiles contributing to the UV absorbance. Moreover, two persistent, but non-identified HPLC peaks P1 and P2 were identified from the HPLC profiles, contributing to the UV absorbance, possibly each peak representing a single uremic retention solute.

The present randomized trial comparing HD membranes showed no unlike removal of the studied uremic solutes for the LF and HF membranes (Table 3) as presented earlier by Lesaffer et al. [4]. In this study it was found that the cellulose triacetate and polysulphone HF membranes removed similarly classical markers and protein-bound liphophilic solutes as a LF polysulphone membrane. All studied uremic solutes had similar start and end concentrations for different membranes (Table 2). Comparable results were obtained even with the concentrations, corrected by a correction factor, based on the total protein concentration at the start and at the end of dialysis as used by Lesaffer et al. [4]. Furthermore, there was no statistical difference between intradialytic start–end values, and removal efficiency for the LF and HF membranes estimated by the total area of HPLC peaks at 254 and 280 nm in the serum and online UV absorbance at 280 nm in the spent dialysate. This indicates that the UV absorbance is following the behaviour of the UV absorbing compounds – uremic toxins, which are the origin of the total UV absorbance in the serum and in the spent dialysate.

The unaffected removal of the uremic toxins compared to LF and HF membranes can partly be explained by the similar effective blood urea clearance, characterizing the small molecular weight solutes diffusive transport in the dialyzers. The effective blood urea clearance *in vivo* is determined by the dialyzers blood urea clearance *in vitro*, the dialyzer mass transfer area coefficient KoA, and by the blood and dialysate flow rates [6]. There was no statistical difference between the blood and dialysate flow rates, which suppressed the effect of the higher KoA of the HF dialyzer (about 920 mL/min for LF vs 1263 mL/min for HF) yielding almost the same effective blood urea clearances

for both LF (202 ± 25 mL/min) and HF dialyzers (217 ± 25 mL/min). Since the diffusive transport is superior to the convective transport for small non-protein-bound solutes in the dialyzer during conventional hemodialysis, the transport of the studied non-protein-bound solutes in the LF and HF dialyzers was alike regardless of the HF having a higher ultrafiltration coefficient (59 mL/h*mmHg for HF vs 18 and 21 mL/h*mmHg for LF), and slightly higher average total ultrafiltration (UF) (1.95 ± 0.74 l for HF vs 1.57 ± 0.42 l for LF, ns) during the study. Although the mechanism for removal of protein-bound solutes is not well known, diffusion seems to be important [5,7]. This is confirmed by the results from the present study, showing no alteration in the small protein-bound solute removal as observed by LF and HF dialyzers during conventional hemodialysis. Moreover, the RR levels were comparable to those reached by the highly convective hemodiafiltration (HDF) therapies from the study by Meert et al. [7], considering analogous treatment duration, weight loss, and blood and dialysate flow rates in both studies. The removal of low molecular weight proteins (e.g. β2-microglobulin) seems to be dependent on membrane composition and morphology, and the removal of small solutes may be improved by enhancing flow distribution in the dialyser [8].

Some minor effects could arise also from the study design, which benefited from crossover patient dialysing regarding LF and HF membranes. The current study used randomly selected patients with equal sample size for the LF and HF membranes. This caused a slightly higher urea removal rate (URR) value for the LF membrane group (about 4%, but not statistically significant) indicating a somewhat higher delivered dialysis dose for the patients in the LF group. This was primarily due to non-significantly longer dialysis sessions for the patients in the LF group (233 ± 15 min for LF vs 229 ± 17 min for HF) and the dialysis-related difficulties (e.g. non-compliance) in the HF group.

Taking into account the removal efficiency (Fig. 3), a characteristic behaviour can be observed for every uremic toxin group depending on the protein binding. The removal efficiency was highest (except for HA) for the small water-soluble non-protein-bound solutes UA, UR and CR and for the unidentified HPLC peaks P1 and P2, whereas the protein-bound solutes IS and HX had the lowest removal rate. Both HA, being a protein-bound solute, but also the small non-protein-bound compound hypoxanthine, have individual removal patterns, which should be considered when analysing the removal of uremic solutes during hemodialysis. A lower removal of hypoxanthine, compared to urea, is also presented earlier [9]. A slightly higher RR value for LF compared to HF membranes ($p = 0.016$) for P2 was unexpected and the reason must be explained in the future. The total area of HPLC peaks at 254 and 280 nm in the serum and online UV absorbance at 280 nm in the spent dialysate tend to estimate RR levels close to that of the small water-soluble non-protein-bound solutes UA, UR and CR. This can be due to the large contribution of UA and CR to the total UV absorbance at 254 and 280 nm. The variation between the reduction ratio of the total area of HPLC peaks and online UV absorbance at 280 nm can be explained

by a different number of detectable chromophores in the serum and in the spent dialysate and by different sampling times for the serum and the dialysate (the serum was collected before and the dialysate sample 10 min after the start of the dialysis). Due to the characteristic absorbing spectra of the UV-chromophores, the difference in RR at the wavelengths of 254 and 280 nm is seen.

Future studies aim to further identify the remained prevalent peaks on the HPLC UV absorption profiles of uremic fluids, and to investigate the possibility for absolute concentration measurements of the uremic toxins utilizing the UV absorbance.

5. CONCLUSIONS

In conclusion, the present study demonstrated that the studied small water-soluble non-protein-bound solutes UR, CR, UA and HX, and the protein-bound solutes HA and IS showed similar removal efficiency for the LF and HF membranes. Furthermore, the total UV absorbance at 254 and 280 nm seem to estimate removal efficiency levels close to the small water-soluble non-protein-bound solutes.

ACKNOWLEDGEMENTS

The authors wish to thank Galina Velikodneva for her assistance during clinical experiments, Rain Kattai for his skilful technical assistance and also the dialysis patients who so kindly participated in the experiments. This study was supported by the Estonian Science Foundation (grant No. 6936), the Estonian targeted financing project SF0140027s07, and the European Union through the European Regional Development Fund.

REFERENCES

1. Vanholder, R., Glorieux, G., De Smet, R. and Lameire, N. New insights in uremic toxins. *Kidney Int.*, 2003, **63**, 6–10.
2. Gotch, F. A., Sargent, J. A. and Keen, M. L. Whither goest Kt/V? *Kidney Int.*, 2000, **58**, 3–18.
3. Locatelli, F., Buoncristiani, U., Canaud, B., Khler, H., Petitclerc, T. and Zucchelli, P. Hemodialysis with on-line monitoring equipment: tools or toys? *Nephrol. Dial. Transplant.*, 2005, **20**, 22–33.
4. Lesaffer, G., De Smet, R., Lameire, N., Dhondt, A., Duym, P. and Vanholder, R. Intradialytic removal of protein-bound uraemic toxins: role of solute characteristics and of dialyzer membrane. *Nephrol. Dial. Transplant.*, 2000, **15**, 50–57.
5. De Smet, R., Dhondt, A., Eloot, S., Galli, F., Waterloos, M. A. and Vanholder, R. Effect of the super-flux cellulose triacetate dialyzer membrane on the removal of non-protein-bound and protein-bound uraemic solutes. *Nephrol. Dial. Transplant.*, 2007, **22**, 2006–2012.
6. Daugirdas, J. T. and Depner, T. A. A nomogram approach to hemodialysis urea modeling. *Am. J. Kidney Dis.*, 1994, **23**, 33–40.

7. Meert, N., Eloot, S., Waterloo, M. A., Van Landschoot, M., Dhondt, A., Glorieux, G., Ledebo, I. and Vanholder, R. Effective removal of protein-bound uraemic solutes by different convective strategies: a prospective trial. *Nephrol. Dial. Transplant.*, 2009, **24**, 562–570.
8. Ouseph, R., Hutchison, C. and Ward, R. A. Differences in solute removal by two high-flux membranes of nominally similar synthetic polymers. *Nephrol. Dial. Transplant.*, 2008, **23**, 1704–1712.
9. Vanholder, R. C., De Smet, R. V. and Ringoir, S. M. Assessment of urea and other uremic markers for quantification of dialysis efficacy. *Clin. Chem.*, 1992, **38**, 1429–1436.

Ureemiliste toksiinide ja nende UV-absorptsiooni uurimine vedeliku madala ning kõrge läbilaskevõimega dialüsaatorite korral

Kai Lauri, Jürgen Arund, Risto Tanner, Jana Jerotskaja,
Merike Luman ja Ivo Fridolin

Ureemiliste toksiinide elimineerimiseks organismist kasutatakse erinevat tüüpi – madala ja kõrge vedeliku läbilaskevõimega – dialüüsi membraane. Membraani tüübist sõltub osaliselt ka dialüüsiravi efektiivsus. Käesolev uuring näitas, et kasutatud dialüüsravi parameetrite korral elimineerivad madala ja kõrge läbilaskevõimega dialüüsi membraanid sarnaselt nii väikese molekulmassiga vees lahustuvaid valkudega mitte seotud kui ka valkudega seotud aineid. Sarnaselt elimineeritud ureemilistele toksiinidele vähenes ka kõikide kromatograafiliste piikide kogupindala ja UV-absorptsioon.