Introduction

High-efficiency and high-flux haemodialysis are clinical treatment modalities that permit short treatment times, since urea and other small-molecular-weight toxins are removed from the patient more rapidly than during conventional haemodialysis. The efficacy of increasing urea clearance to assure the adequacy of dialysis therapy with short treatment times is, however, controversial. For example, an epidemiological survey has found an association between short treatment times and high mortality rates for haemodialysis patients [1], although this relationship may be due to inadequate compensatory increases in small-solute clearance when using short treatment times [2]. Even when urea clearance was appropriately increased, results from the US National Cooperative Dialysis Study demonstrated that short treatment times are associated with higher hospitalization rates [3]. On the other hand, certain dialysis centres have reported good clinical outcome using either high-efficiency [4] or high-flux haemodialysis [5,6] with short treatment times. It is difficult to compare these reports critically because of differences in patient populations, dialysis protocols, and medical practices.

Although appropriate removal of urea and other small-molecular-weight toxins is one important measure of the adequacy of dialysis therapy, other treatment considerations such as the removal of large uraemic toxins (e.g. parathyroid hormone [7], β₂-microglobulin [8], advanced glycosylation end-products [9]) may also be important. Lindsay and Henderson have suggested that adequacy of dialysis therapy should be evaluated using both small- and middle-molecule clearance [10]. Furthermore Henderson has suggested that treatment time could be considered surrogate for middle-molecule clearance and, based on this conjecture, suggested that dialysis with short treatment time is inadequate therapy unless more permeable membranes are employed [11]. Stated in other words, all short treatment strategies should not be considered equal and must be evaluated individually.

The present in vitro study evaluated several com-
monly used clinical treatment strategies for shortening haemodialysis treatment time with respect to the removal of large solutes. Polydisperse neutral dextrans were used as marker macromolecules since the entire molecular weight range of interest could be studied in a single experiment.

Methods

Experimental

In vitro experiments were performed using a Centry® 2 haemodialysis machine (CGH Medical, Lakewood, Colorado, USA) to prepare dialysate from commercial, acetate-buffered concentrate (Erylite®, Erika, Rockleigh, New Jersey, USA) and to pump the dialysate within the test circuit. Outdated human plasma, used as the test solution in all experiments, was circulated from a reservoir through the blood compartment of a haemodialyser at a constant rate (see below) and returned to the reservoir. The reservoir was maintained at 37°C throughout each experiment, using a heated water bath. The volume of the reservoir was maintained approximately constant by manual adjustment of a clamp on the outflow tubing; this procedure limited transmembrane fluid movement during the experiment. A bolus of 1500 U of heparin was initially added to the reservoir, and additional doses (750 U) of heparin were added throughout the experiment as necessary to inhibit clotting in the test circuit.

The following commercial hollow-fibre haemodialysers were employed in these studies: CA110 (1.1 m² of cellulose acetate membrane, ultrafiltration coefficient of 5.3 ml/h/mm Hg; Baxter Healthcare, McGaw Park, Illinois, USA), TAF 175 (1.75 m² of cellulose membrane, ultrafiltration coefficient of 8.8 ml/h/mm Hg; Terumo Medical, Piscataway, New Jersey, USA), CT190G (1.9 m² of cellulose triacetate membrane, ultrafiltration coefficient of 36 ml/h/mm Hg; Baxter), and F60 (1.25 m² of polysulphone membrane, ultrafiltration coefficient of 40 ml/h/mm Hg; Fresenius USA, Walnut Creek, California, USA). CA110 was chosen for study because it is representative of a conventional dialyser. TAF175 is representative of a large-surface-area, high-efficiency dialyser, and CT190G and F60 are representative of high-flux dialysers. Prior to the experiment the blood and dialysate compartments of each dialyser were rinsed with 1-21 of dialysate; then the blood compartment of the dialyser respectively. The average solute concentrations in the inflow and outflow lines to the blood compartment of the dialyser. After 1 h the blood and dialysate pumps were turned off, additional urea and creatinine were added to the reservoir, and the procedure was repeated twice more. Urea and creatinine clearances (K) were calculated assuming no transmembrane ultrafiltration from the following equation

\[ K = Q (C_i - C_o) / C_i \]

where Q denotes the blood flow rate and C_i and C_o denote solute concentrations in the inflow and outflow lines to the blood compartment of the dialyser. The average value from the three separate evaluations was employed to determine urea and creatinine clearances for each experiment.

Analytical

A molecular-weight characterization of dextran was performed in each plasma sample in a manner similar to that described previously using high-performance liquid chromatography [12-14]. Size exclusion chromatography was performed using a TSK-G4000PW column (Kratos Analytical, Ramsey, New Jersey, USA) with a buffer containing 1.0 M ammonium acetate and 0.05 M sodium phosphate, pH 9. A Waters Model 420-E fluorescence detector was employed for monitoring changes in concentration of FITC-labelled dextran in the column effluent, with excitation at 450 nm (bandpass filter) and fluorescence at 500 nm (cut-off filter) being monitored. Column calibration was performed using dextran standards of narrow polydispersity [12] that were labelled with FITC using the method of de Belder and Granath [15]. The concentration of dextran at appropriate molecular weights was determined by the height of the chromatogram at corresponding retention volumes.

Samples analysed for dextran concentration were analysed with no additional preparation. Moreover, no correction for background fluorescence in plasma was necessary at the concentrations employed in these experiments. The molecular radius (R) of dextran in Å was computed using previously reported data [16,17] by the following equation

\[ R = 0.305 M^{0.47} \]

where M denotes dextran molecular weight.

Urea nitrogen and creatinine concentrations were determined by using an automated analyser (Astra-8, Beckman Instruments, Brea, California, USA).
Statistics

All values are expressed as the mean value ± the standard error of the mean (SEM). The significance of changes in reservoir volume as a function of time was assessed using repeated measures analysis of variance [18].

Results

It was not possible to exactly fix the volume of plasma in the reservoir because transmembrane pressure was adjusted manually in these experiments. Nevertheless, close attention during the experiment permitted a relatively constant reservoir volume to be maintained (Figure 1). No changes in reservoir volume were observed except for the CA110 dialyser studied at a blood flow rate of 200 ml/min (P = 0.03). In this case, however, the largest deviation in reservoir volume from its initial value (11) was only 60 ml. Based on these results, the volume of plasma in the reservoir was assumed constant during each experiment; the average volume was calculated from the values recorded at 0, 60, 120, 180 and 240 min.

Figure 2 shows representative results from one experiment using a CT190G dialyser where the logarithm (base 10) of reservoir dextran concentration (as assessed by relative fluorescence) is plotted as a function of time for dextrans with different molecular radii. The concentration of dextrans with molecular radii of 32 and 44 Å decreased only slightly with time since this dialyser is relatively impermeable to macromolecules of this size. For dextrans with smaller radii the reservoir concentration decreased continuously during the experiment; the decrease was greater for smaller molecules. Note that the decrease in reservoir dextran concentration with time was not always linear on this semilogarithmic scale. Since a plot of the logarithm of concentration versus time in these experiments has a slope proportional to the ratio of clearance to the reservoir volume [19], this result demonstrates that dextran clearance decreased during the course of this experiment.

Figures 3A and 3B show the reservoir dextran concentration at 120 and 240 min respectively, normalized by their initial concentrations and plotted as a function of molecular radius. To compare the molecular radii in these plots with those for certain plasma proteins, note that β2-microglobulin has a molecular radius of 17 Å, albumin has a molecular radius of 36 Å, and immunoglobulin G has a molecular radius of 55 Å (as computed from their diffusion coefficients in free solution [20]). For each dialyser studied, normalized dextran concentrations in the reservoir were less, and therefore removal was greater, for smaller macromolecules and for longer times. Removal rates were highest for the CT190G and F60 (high-flux) dialysers, intermediate for the TAF175 (large-surface-area, high-efficiency) dialyser, and lowest for the CA110 dialyser at either blood flow rate. Results for dextran with a molecular radius of 17 Å (molecular radius of β2-microglobulin) are also tabulated (Table 1).

Figure 4 shows the 4-h average dextran clearance (κ, ml/min) as a function of molecular radius computed using the following equation

$$\kappa = - \frac{1}{440} \ln \left( \frac{C(240)}{C(0)} \right)$$

where ln denotes the natural logarithm, V denotes the average reservoir volume (ml), and C denotes the reservoir dextran concentration at the indicated times. Dextran clearance decreased with increasing molecular size and was highest for the CT190G and F60 (high-flux) dialysers, intermediate for the T175 (large-
Fig. 3A. Reservoir dextran concentration after 120 min (C(120)) normalized by the initial reservoir dextran concentration (C(0)) plotted as a function of molecular radius. Results are shown for the CA110 dialyser at a blood flow rate of 200 ml/min (closed circles), the CA110 dialyser at a blood flow rate of 400 ml/min (open circles), the TAF175 dialyser at a blood flow rate of 300 ml/min (triangles), the CT190G dialyser at a blood flow rate of 300 ml/min (squares), and the F60 dialyser at a blood flow rate of 300 ml/min (diamonds). The mean value from three experiments on each dialyser is shown; for clarity, error bars are not shown (see Table 1).

Fig. 3B. Reservoir dextran concentration after 240 min (C(240)) normalized by the initial reservoir dextran concentration (C(0)) plotted as a function of molecular radius. Results are shown for the CA110 dialyser at a blood flow rate of 200 ml/min (closed circles), the CA110 dialyser at a blood flow rate of 400 ml/min (open circles), the TAF175 dialyser at a blood flow rate of 300 ml/min (triangles), the CT190G dialyser at a blood flow rate of 300 ml/min (squares), and the F60 dialyser at a blood flow rate of 300 ml/min (diamonds). The mean value from three experiments on each dialyser is shown; for clarity, error bars are not shown (see Table 1).

Fig. 4. Four-hour average dextran clearance plotted as a function of molecular radius. Results are shown for the CA110 dialyser at a blood flow rate of 200 ml/min (closed circles), the CA110 dialyser at a blood flow rate of 400 ml/min (open circles), the TAF175 dialyser at a blood flow rate of 300 ml/min (triangles), the CT190G dialyser at a blood flow rate of 300 ml/min (squares), and the F60 dialyser at a blood flow rate of 300 ml/min (diamonds). The mean value from three experiments on each dialyser is shown; for clarity, error bars are not shown (see Table 1).

during clinical haemodialysis [21,22]. Further discussion of these data below will employ urea clearances reported by the manufacturers, since they are most often used for prescribing clinical haemodialysis.

Discussion

Despite 30 years experience in treating chronic renal failure patients with haemodialysis, the essential elements of an adequate treatment prescription remain incompletely defined. Results from the US National Cooperative Dialysis Study, the only randomized and prospective clinical trial to date, clearly demonstrated the importance of small uraemic toxin removal (based on urea clearance) on patient morbidity and led to the now common use of urea kinetics to model haemodialysis therapy [23,24]. The importance of other treatment prescription parameters such as haemodialysis membrane biocompatibility, the rate of fluid removal, and the removal of large uraemic toxins in determining long-term patient outcome remains controversial. Nevertheless, limited information to date suggests that removal of large uraemic toxins is important [25].

To decrease treatment time using urea kinetics, it is necessary only to maintain constant the product of urea clearance times treatment time (assuming urea clearance remains constant during the treatment and neglecting multicompartmental effects [26]). Assuming that a 240-min conventional treatment using a CA110 dialyser at a blood flow rate of 200 ml/min represents the targeted therapy, the necessary treatment time to achieve equivalent urea removal for the alternative treatment strategies can be calculated using the urea clearance data supplied by the manufacturers (see
Large solute removal during haemodialysis

Table 1. Reservoir concentrations and 4-h average clearance of dextran with a molecular radius of 17 Å

<table>
<thead>
<tr>
<th>Dialyser</th>
<th>Blood flow rate (ml/min)</th>
<th>C(120)/C(0)</th>
<th>C(240)/C(0)</th>
<th>Clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA110</td>
<td>200</td>
<td>0.687 + 0.039</td>
<td>0.598 + 0.066</td>
<td>1.7 + 0.4</td>
</tr>
<tr>
<td>CA110</td>
<td>400</td>
<td>0.674 + 0.025</td>
<td>0.533 + 0.023</td>
<td>1.8 + 0.1</td>
</tr>
<tr>
<td>TAF175</td>
<td>300</td>
<td>0.392 + 0.037</td>
<td>0.240 + 0.020</td>
<td>4.9 + 0.3</td>
</tr>
<tr>
<td>CT190G</td>
<td>300</td>
<td>0.044 + 0.005</td>
<td>0.029 + 0.003</td>
<td>11.2 + 0.3</td>
</tr>
<tr>
<td>F60</td>
<td>300</td>
<td>0.066 + 0.004</td>
<td>0.028 + 0.004</td>
<td>11.9 + 0.4</td>
</tr>
</tbody>
</table>

Concentrations at 120 (C(120)) and 240 (C(240)) min are normalized by the initial reservoir concentration C(0). Data are shown as mean ± SEM of three experiments.

Table 2. Urea and creatinine clearances reported by the manufacturer and measured in this study

<table>
<thead>
<tr>
<th>Dialyser</th>
<th>Blood flow rate (ml/min)</th>
<th>Manufacturer urea clearance (ml/min)</th>
<th>Manufacturer creatinine clearance (ml/min)</th>
<th>Measured urea clearance (ml/min)</th>
<th>Measured creatinine clearance (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA110</td>
<td>200</td>
<td>176</td>
<td>144</td>
<td>145 ± 6</td>
<td>128 ± 7</td>
</tr>
<tr>
<td>CA110</td>
<td>400</td>
<td>231</td>
<td>164</td>
<td>191 ± 5</td>
<td>151 ± 3</td>
</tr>
<tr>
<td>TAF175</td>
<td>300</td>
<td>259</td>
<td>228</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CT190G</td>
<td>300</td>
<td>256</td>
<td>239</td>
<td>259 ± 7</td>
<td>252 ± 3</td>
</tr>
<tr>
<td>F60</td>
<td>300</td>
<td>244</td>
<td>219</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND, not determined. Data are shown as mean ± SEM of three experiments.

Table 2) as 183 min using a CA110 dialyser at a blood flow rate of 400 ml/min, 163 min using a TAF175 dialyser at a blood flow rate of 300 ml/min, 165 min using a CT190G dialyser at a blood flow rate of 300 ml/min, and 173 min using an F60 dialyser at a blood flow rate of 300 ml/min.

As suggested in the present study, these treatment strategies will have disparate removal rates for large uraemic toxins. To compare large-solute removal rates during haemodialysis, we again assume that a 240-min conventional treatment using a CA110 dialyser at a blood flow rate of 200 ml/min represents the targeted removal of large uraemic toxins. Using dextran with a molecular radius of 17 Å (Table 1) as an example, approximately 40% of solutes of this size are removed from the test circuit by the conventional therapy. For comparison, after only 120 min of treatment using the TAF175, CT190G, or F60 dialysers, considerably more of this macromolecule is removed. (Note that the magnitude of these decreases in solute concentration cannot be directly compared to those expected during clinical haemodialysis since the 1-l volume used in these experiments is considerably smaller than the in vivo distribution volume of relevant solutes). Similar reasoning leads to the same conclusion for all dextrans with molecular radii between 15 and 50 Å. This analysis suggests that a 120-min treatment with high-flux and large-surface-area, high-efficiency dialysers will remove more large uraemic toxins than a 240-min treatment using a CA110 dialyser at a blood flow rate of 200 ml/min. On the contrary, increasing blood flow rate to 400 ml/min using a CA110 dialyser only resulted in increased clearance for urea and creatinine but not for dextrans with molecular radii between 15 and 50 Å.

This result is in accordance with previous work demonstrating that the removal of small solutes during haemodialysis using conventional membranes is highly dependent on blood flow rate, whereas the removal of large solutes is membrane limited and largely independent of blood flow rate [27].

Dextrans are useful markers for characterizing the transport of large solutes during haemodialysis because they are inert and do not significantly interact or bind readily to haemodialysis membranes [28]. While dextrans are useful in describing general transport principles, their rates of removal may not be relevant for a particular uraemic toxin that is significantly bound either to plasma proteins or to haemodialysis membranes. With this in mind, it is relevant to compare the results from this study to previous observations on an example large uraemic toxin, β₂-microglobulin.

Removal rates for β₂-microglobulin during haemodialysis with various high-flux haemodialysers have been recently determined [29–31].

While the removal of β₂-microglobulin from the blood stream depends significantly on its adsorption to certain haemodialysis membranes, the amount of β₂-microglobulin found in the spent dialysate has been reported to be approximately 200 mg for a 4-h treatment using a variety of high-flux dialysers. This corresponds to a clearance of approximately 20 ml/min assuming the plasma concentration of β₂-microglobulin to be 40 mg/l [32] (200 mg/240 min/40 mg/l). While this value is approximately twice that determined in the present study using high-flux dialysers for dextran with a molecular radius equal to that of β₂-microglobulin (Table 1), it should be noted that the present experiments were not conducted under conditions...
identical to those during clinical dialysis. For example, previous studies have shown that the removal of β2-microglobulin during haemodialysis is strongly influenced by transmembrane ultrafiltration [29,33]. It is therefore not surprising that clearance values for macromolecules reported in this study are lower than those previously determined in clinical studies since there was negligible transmembrane ultrafiltration in the present experiments. Moreover, in vitro experiments similar to those described in the present study have reported clearances of β2-microglobulin to be 29.4 and 14.2 ml/min for dialysers similar to the CT190G and F60 dialysers studied herein [34]. We conclude therefore that the results from the present study are consistent with removal rates for β2-microglobulin during clinical haemodialysis and that they likely apply to other large uraemic toxins that do not bind significantly to either plasma proteins or haemodialysis membranes.

It should be emphasized that our in vitro model does not attempt to reproduce all of the complexities of clinical haemodialysis. For example, it is likely that there are intercompartmental resistances to the movement of high-molecular-weight solutes within the patient that limit their removal, and the magnitude of such intercompartmental resistances may vary among different uraemic toxins. Therefore caution should be exercised when extrapolating the results from this study to clinical haemodialysis.

The present experiments were performed without significant net ultrafiltration and accompanying convective solute removal. Nevertheless, additional experiments including these phenomena would probably not lead to alternative conclusions from those reached in the present studies, since convective solute removal would be most significant for the TAF175 and CA110 dialysers but rather unimportant for the TAF175 and CA110 dialysers.

In conclusion, this study suggests that prescribing short treatment times during haemodialysis by increasing blood flow rates using a conventional dialyser does not provide for adequate removal of large uraemic toxins even if sufficient urea is removed. In contrast, prescribing short treatment times by increasing urea clearance using either a high-flux or large-surface-area, high-efficiency dialyser are more effective strategies for maintaining the removal of large uraemic toxins. Verification of these principles will require additional studies using identified uraemic toxins of large molecular weight [35]. Interpretation of results from long-term clinical trials comparing various treatment modalities should take into account these differences in large uraemic solute removal rates.

Acknowledgements: This work was supported by DVA Medical Research Funds and the Dialysis Research Foundation, Ogden, Utah, USA. The authors thank Janice F. Gilson for her technical assistance.

References


29. Flöege J, Granolleras C, Deschodt G et al. High-flux synthetic versus cellulose membranes for β2-microglobulin removal during
Large solute removal during haemodialysis


Received for publication: 24.3.95
Accepted in revised form: 22.9.95