On the Balance Between Albumin Loss and Removal of Middle Molecules in Dialyzers

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BACKGROUND

Uremic toxins can be divided into small water-soluble molecules (< 0.5 kD), middle molecules (≥0.5 kD) and protein-bound solutes. Middle molecules have been classified according to weight as small-middle molecules (0.5 - 15 kDa), medium-middle molecules (> 15 - 25 kDa), and large-middle molecules (> 25 - 58 kDa). Retention of middle molecules may lead to negative outcomes, and it is believed that removing them leads to improved outcomes in patients on dialysis.

In addition to dialyzer mode, the dialyzer hollow fiber membranes play an extensive role in toxin removal. Membranes differ in mean pore radius, pore size distribution, and pure water permeability. The pore size distribution of hemodialysis membranes is essential for selectivity. Tailoring the membrane's molecular weight cut-off appropriately balances the removal of middle-molecular weight uremic toxins while avoiding albumin loss.

Low and high flux membranes are used to remove small water-soluble molecules, but do not effectively eliminate larger toxins. High cut-off membranes with larger pores than low and high flux membranes remove toxins up to 50 kDa, but due to their large pore size distribution also allow unwanted albumin loss, which could lead to hypoalbuminemia. Medium cut-off membranes are designed to remove middle molecules up to 50 kDa, but due to their sharper pore size distribution, they can offer lower albumin loss.

Use of medium cut-off membranes with hemodialysis or expanded hemodialysis therapy has been investigated in numerous clinical studies with the overall consensus that expanded hemodialysis therapy improves the clearance of a wide range of middle molecules, and can improve quality of life, morbidity, and mortality. Various medium cut-off membranes are available from different manufacturers with potentially different properties.

OBJECTIVE

The current study aims to determine the plasma clearance of middle molecules (12 - 52 kDa) and the albumin loss in four commercially available dialyzers:

- **Theranova** 500 dialyzer (Gambro Dialysatoren GmbH)
- Phylther HF20SD from Bellco Mirandola
- VitE 21 X (Asahi Kasei Medical Co., Ltd)
- Elisio 19 HX (Nipro Medical Corp.)

Five dialyzers from each type were tested.

METHODS

This study was a comprehensive ex-vivo investigation of performance characteristics of four different commercial dialyzers. Membrane pore characteristics were analyzed, ex-vivo experiments were performed to evaluate the clearances of significant middle molecules, and experiments at different blood flow rates quantified the influence on middle molecule clearance and albumin loss.

Dextran sieving experiments

Dextran sieving experiments were done to characterize the membranes. From the resulting sieving curves, the retention and the pore size distribution can be derived. In order to provide a comparable result, measurements were performed according to Boschetti-de Fierro et al. (2013)[1] using different dextran fractions. Dextran concentrations were measured with an Agilent HPLC 1200 equipped with a TSK gel column from Tosoh Bioscience. The pore size distribution was then calculated from the sieving curves.

Plasma clearance

Anti-coagulated (citrate) human plasma Octaplas from Octapharma with a protein concentration of 55 ± 10 g/L was used, and the following extrinsic marker substances were spiked in the plasma to measure clearance: 5 mg/L of LEE Biosolutions' β2 microglobulin (β2m) (M_w = 12 kDa) and Myoglobin (Myo) (M_w = 17 kDa). YKL-40 (MW = 40 kDa) is an intrinsic marker, therefore the initial concentration might vary for different plasma charges. The plasma volume loss due to ultrafiltration was compensated by substituting dialysate into the plasma pool, and the temperature was set to 37 °C to mimic the human body. Set-up is shown in Figure 1.

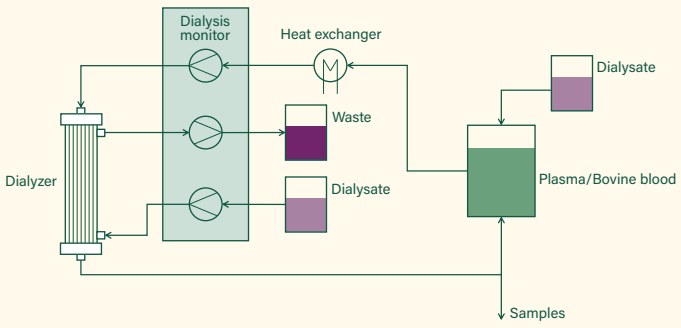


FIGURE 1. Experimental setup for the plasma clearance and albumin loss measurements.

The dialyzer was rinsed and vented with 0.9 % saline solution (blood side: 5 min, dialysate side: 1 min). After replacing the saline solution with plasma and dialysate, the clearance tests started, beginning with a recirculation phase during which the membrane was saturated and adsorption of intrinsic plasma components took place. After 55 min, the extrinsic markers, β2m, and Myo were added, and the recirculation was continued for another 5 min. After that, the 60 min measuring phase started.

Clearance tests were performed at three different flow rate combinations, consisting of the plasma flow rate (QB), the dialysate flow rate (QD) and the flux over the membrane (UF):

(1) QB = 200 mL/min, QD = 500 mL/min and UF = 10 mL/min;

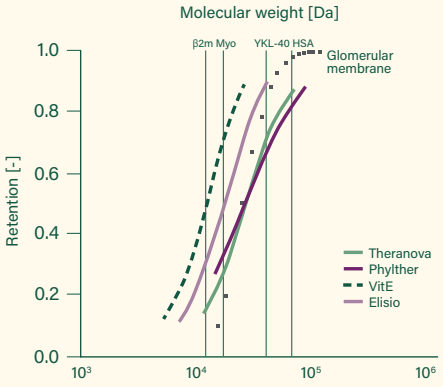
(2) QB = 300 mL/min, QD = 500 mL/min and UF = 10 mL/min;

(3) QB = 400 mL/min, QD = 600 127 mL/min and UF = 10 mL/min.

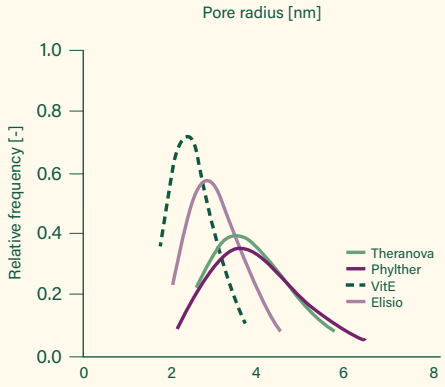
Marker concentrations were determined and clearance for each tracer was calculated.

Albumin loss

Experiments were run with bovine whole blood with protein concentration 60 ± 5 g/L, hematocrit 32 ± 3%, and viscosity 1.50 to 1.64 mm/s^2. The concentration of bovine serum albumin (BSA) in the blood and the dialysate was monitored. The experimental set-up was similar to Figure 1 with the addition of a sampling device installed in the dialysate drain line to collect spent dialysate continuously. The rinsing procedure for the dialyzer was the same as described for clearance testing. The albumin loss was analyzed for 240 min at a flow rate combination of QB = 300 mL/min, QD = 500 mL/min, and UF = 10 mL/min.

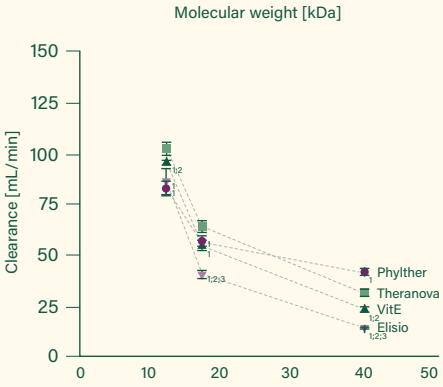


(a) Experimental dextran retention curve.

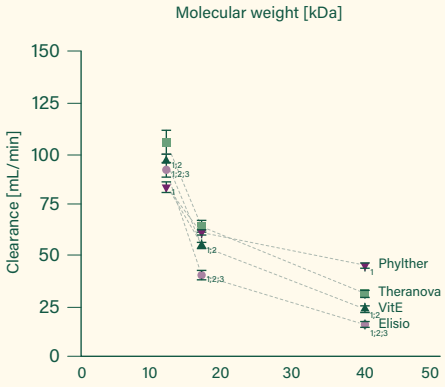


(b) Pore size distributions estimated from the retention curves using Equation SI.1.

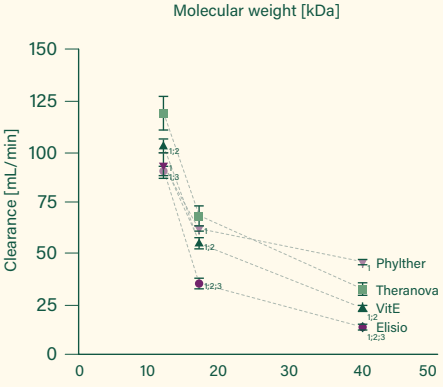
FIGURE 2. Solute transport characteristics of the 4 investigated dialyzers. Retention of the glomerular membrane taken from Axelsson et al. (2009). The VitE membrane results need to be taken with care as membrane drying might have affected the pore structure.



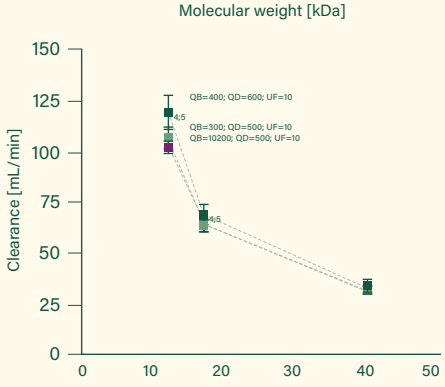
(a) Flow rates [mL/min]: QB=200 QD=500 UF=10.



(b) Flow rates [mL/min]: QB=300 QD=500 UF=10.



(c) Flow rates [mL/min]: QB=400 QD=600 UF=10.



(d) Impact of the flow rate, shown for Theranova.

FIGURE 3. Plasma clearance data for different middle molecules (β2 microglobulin (12 kDa), myoglobin (17 kDa) and YKL-40 (40 kDa)), dialyzer types and flow rate configurations. (1) is tested vs. **Theranova**, (2) vs. Phylther, (3) vs. ViE21X with p<0.05. (4) is tested vs. QB=200; QD=500; UF=10 and (5) vs.QB=300; QD=500; UF=10 with p<0.05.

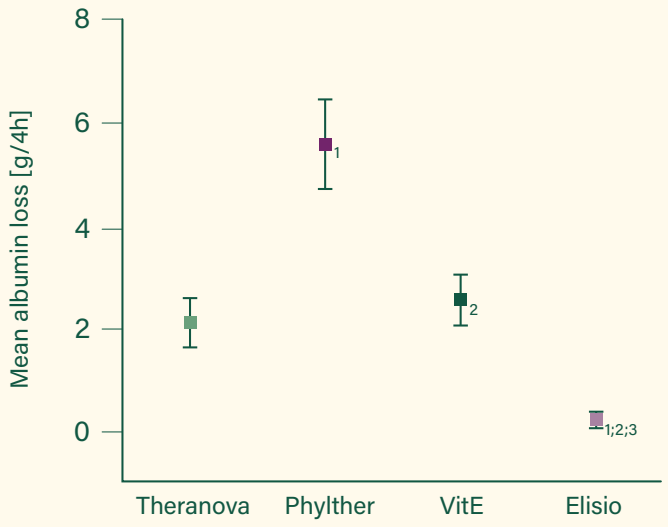


FIGURE 4. The mean albumin loss during a 4 h simulated treatment with bovine blood. The flow rate was set to QB=300 mL/min, QD = 500 mL/min, and UF = 10 mL/min. (1) is tested vs. **Theranova**, (2) vs. Phylther, (3) vs. VitE with $p<0.05$.

RESULTS

Dextran sieving experiments

Dextran retention curves along with the glomerular membrane retention curve are shown in Figure 2a, and pore size distributions in Figure 2b.

The Elisio retention curve is to the left of the glomerular membrane, meaning it retained smaller dextrans than the kidney's retention curve. The **Theranova** dialyzer and Phylther curves cross the glomerular membrane, indicating there are pores large enough to let albumin pass. Compared to the **Theranova** dialyzer and Phylther, the retention curve for Elisio is sharper, which results in a more precise cut-off, however the Elisio curve is not suitable for removing molecules larger than 30 kDa. The membranes in the VitE dialyzer were dried to ensure potting adhesion in preparation of the mini-modules for sieving coefficient experiments, and it is assumed that this procedure significantly changed the pore structure therefore conclusions cannot be drawn from the results.

Plasma clearance

The clearance for the different molecules across dialyzer types and flow rates are shown in Figures 3a-c. The **Theranova** 500 dialyzer showed the highest clearance for β2 microglobulin (12 kDa) and myoglobin (17 kDa), and the Phylther dialyzer showed the highest clearance for YKL-40 marker (40 kDa).

Figure 3d shows the impact of flow rate on clearance for the **Theranova** dialyzer. It shows that the flow rate influences the clearance of smaller middle molecules, but for molecules larger than 40 kDa the impact of the flow rate is negligible.

Albumin loss

Literature reports that the acceptable threshold of albumin loss is less than 5 g per dialysis session.[3,4] The Phylther dialyzer exceeded this limit (5.62 g/4h). The **Theranova** dialyzer and VitE have significantly lower albumin loss compared to Phylther, and the Elisio dialyzer had the lowest albumin loss (Figure 4).

DISCUSSION AND CONCLUSION

The current study investigated the plasma clearance of middle molecules and the albumin loss in four commercially available dialyzers under carefully controlled and identical conditions. A previous study investigating four medium cut-off dialyzer membranes reported reduction ratios for various middle molecules and found no significant difference between the dialyzers.[2] In the current study, similar results were found by calculating reduction ratios. However, clearance should be used rather than reduction ratios to characterize the removal capacity of a dialyzer as clearance takes into account efficiency and blood flow rate. The reduction ratio neglects the filtration duration, thus a long filtration time will result in a high reduction ratio value, impairing the comparison across literature.

The Elisio dialyzer membrane has the sharpest distribution but the lowest mean pore radius. It showed lower clearance values for all tested markers and barely any albumin loss. The **Theranova** dialyzer and Phylther membranes have larger mean pore sizes. For clearance of small-middle molecules, the **Theranova** dialyzer was superior to Phylther, whereas, for clearance of large-middle molecules, Phylther was superior to the **Theranova** dialyzer. However, this higher clearance of large-middle molecules by the Phylther dialyzer comes with albumin loss that exceeds the recommended limit of 5 g per treatment, which could expose patients to the risk of hypoalbuminemia.

The **Theranova** dialyzer demonstrates the best compromise between low albumin loss and good clearance of middle molecules.

Limitations

This was an ex-vivo study, and additional research is needed to compare the study results to real dialysis conditions.

Among four dialyzer membranes, the Theranova dialyzer offers the best compromise and good clearance of middle molecules and low albumin loss.

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