Evaluation of an \textit{in vitro} dialysis system to predict drug removal

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Abstract

**Background.** Variation in the extent of drug removal under different dialysis conditions presents a challenge for prediction of drug elimination and dosage regimen adjustment during haemodialysis (HD). Dependence on clinical pharmacokinetic studies in HD patients for dosing guidelines is problematic given the increasing number of dialysers with variable rates of drug removal. Thus, the purpose of this study was to characterize drug removal using an \textit{in vitro} system and to evaluate its reliability to predict \textit{in vivo} elimination by HD using vancomycin (VANC) as a model drug.

**Methods.** \textit{In vitro} dialysis was performed for 2 h (volume 4.0 l normal saline, initial VANC concentration 30 mg/l, flow rate 300 ml/min, dialysate flow 800 ml/min) using four different dialysers: polymethylmethacrylate (BK-2.1 U), polysulfone (F-80), AN69 (Filtral-20) and hemophan (COBE 700HE). The \textit{in vitro} dialysis clearance for VANC (CL\textsubscript{D}) for the polysulfone dialyser was compared with values determined in eight HD patients. \textit{In vitro} VANC CL\textsubscript{D} for all dialysers was compared with the clearance and KoA for B12 reported for each dialyser.

**Results.** \textit{In vitro} VANC CL\textsubscript{D} values were 93±11 ml/min for the polymethylmethacrylate BK-2.1, 136±7 ml/min for the AN69, 65±9 ml/min for the hemophan COBE 700HE and 143±10 ml/min for the polysulfone F80. The CL\textsubscript{D} for the polysulfone F80 was not statistically different from the \textit{in vivo} CL\textsubscript{D} of 135±18 ml/min (\textit{P}=0.48). \textit{In vitro} VANC CL\textsubscript{D} correlated with the B12 CL\textsubscript{D} (\(r^2=0.77\)) and the B12 KoA (\(r^2=0.63\)) reported for each dialyser.

**Conclusion.** VANC CL\textsubscript{D} in HD patients for the polysulfone dialyser was correctly predicted using the \textit{in vitro} dialysis system. Use of this system may be superior to estimations of drug CL\textsubscript{D} based on dialyser information provided by the manufacturer for compounds of similar molecular weight.

**Keywords:** dialysis; drug removal; haemodialysis; pharmacokinetics; vancomycin

Introduction

Renal replacement therapies including haemodialysis (HD) have evolved substantially as essential treatment modalities for patients with acute and chronic kidney failure. While technological advances in these modalities have resulted in increased clearance of uraemic toxins and other endogenous substances, the effect of such changes on drug elimination has not been adequately studied.

Drug removal by HD is primarily diffusive and dependent on both the characteristics of the drug and the dialysis process. Variations in process-specific factors, such as blood and dialysate flow rates, type of dialyser, and duration of dialysis substantially alter solute and drug clearances [1,2]. Drug characteristics affecting clearance include molecular size, steric hindrance, protein binding and distribution [3]. Recent advancements in HD techniques that employ higher blood flow rates and dialysers with larger pore sizes, i.e. high flux HD, substantially increase intradialytic drug removal when compared with conventional HD [1,4].

Variation in the extent of drug removal under different dialysis conditions presents a challenge for prediction of drug elimination and dosage regimen adjustment during HD. Clinical studies of intradialytic drug removal report drug clearances for specific dialysers and conditions, usually in a small number of patients. This limited approach provides information on intradialytic drug disposition specific to the dialyser and dialysis conditions tested.

An alternative strategy to assess drug elimination during dialysis is through an \textit{in vitro} approach to characterize solute removal during renal replacement.

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therapies. There are several reports of drug removal using *in vitro* dialysis systems [5–9]. These techniques allow rapid and cost-effective evaluation of drug removal under varying dialysis conditions. However, there is little information on the reliability of extrapolating from an *in vitro* technique of drug removal to patients undergoing dialysis. Thus, the purpose of this study was to characterize drug removal using an *in vitro* system and to evaluate its reliability to predict *in vivo* elimination by HD. Vancomycin (VANC) was chosen as the model drug for evaluation as its removal differs significantly between conventional and newer high flux dialysers [1,4].

**Subjects and methods**

*In vitro dialysis system*

A 5-l Erlenmeyer flask with 4 l of normal saline served as the drug reservoir with an initial VANC concentration of 30 mg/l representing a typical peak plasma concentration. The solution was maintained at 37°C during the procedure and continuously mixed using a magnetic stirrer. Polyvinyl chloride tubing from the reservoir to the dialyser served as the ‘arterial line’, and the return to the reservoir as the ‘venous line’, providing a closed-loop, fixed-volume system. Dialysate lines were connected from the dialysate supply (standard bicarbonate dialysate) to the dialyser and from the dialyser into a container for collection of the dialysate. The system was primed with normal saline prior to initiation of the dialysis procedure (see Figure 1).

Dialysis was performed using four different dialysers: polymethylmethacrylate BK-2.1 U (Toray Inc., New York, NY), polysulfone F80 (Fresenius, Walnut Creek, CA), AN69 Filtral-20 (Gambro/Hospal, Lakewood, CO) and hemophan COBE 700HE (Gambro, Lakewood, CO). Dialysis conditions for each procedure were as follows: duration of dialysis = 120 min, reservoir solution flow rate \( Q_a \) = 300 ml/min, and dialysate flow rate \( Q_d \) = 800 ml/min. The solution flow rate of 300 ml/min was set to simulate the plasma flow rate achieved for a typical patient with a blood flow of 450 ml/min and a haematocrit (HCT) of 34%. Ultrafiltration was set to zero. ‘Arterial’ and ‘venous’ samples (~2 ml) were collected at the start of dialysis, and at 20, 40, 60, 80, 100 and 120 min. All samples were stored at –20°C until analysis.

VANC concentrations were measured using fluorescence polarization immunoassay (TDx: Abbott Diagnostics, North Chicago, IL). Low controls (5.5–8.5 mg/l, mean 7.0 mg/l), and medium controls (30.0–40.0 mg/l, mean 35.0 mg/l) were used. Inter-assay coefficients of variation were 1.6% for the medium control (32.7 mg/l) and 5.9% for the low control (7.0 mg/l). The lower limit of quantification for the assay was 1.0 mg/l.

**In vitro drug clearance**

VANC clearance during the dialysis procedure (CLD) was calculated for each dialyser as:

\[
\text{CLD} = \left( \frac{Q_a (C_a - C_v)}{C_a} \right) / C_a
\]

where \( C_a \) = ‘arterial’ VANC concentration and \( C_v \) = ‘venous’ VANC concentration. The CLD reported is the mean of the CLD calculated at each sampling time point. Methods to calculate VANC CLD that incorporate dialysate concentrations were not used as dialysate concentrations of VANC were below the lower limit of quantification for the assay. The VANC CLD values for all dialysers were compared with the dialyser clearance of B12 (1354 Da) and the KoA (mass transfer coefficient) of B12 reported for each dialyser.

**In vivo dialysis**

VANC disposition and clearance by HD were evaluated in eight adult patients undergoing chronic HD three times weekly at an outpatient dialysis centre. All patients were dialysed using the polysulfone F60 or F80 dialyser (1.3 m², 1.8 m², respectively) according to their individual dialysis prescription. A 1-g dose of VANC was infused intravenously (i.v.) over 1 h on a non-dialysis day (study day 1). Blood samples were collected at 0, 1, 1.5, 2, 3, 4, 6 and 8 h following completion of the infusion. During the next HD session (study day 2), samples were collected immediately prior to dialysis, at 1 and 3 h during HD (simultaneous arterial and venous samples), and at 0, 0.5, 1, 1.5, 2, 3 and 4 h following completion of HD. Pre-dialysis samples were collected immediately before the next three scheduled HD sessions (study days 4, 6 and 9). Serum and dialysate VANC concentrations were measured using the Emit® (Syva Corporation, San Jose, CA) VANC immunoassay method.

Fig. 1. *In vitro* dialysis system.
which measures VANC concentrations between 1.0 and 50 mg/l in human serum or plasma. The HCT values for each blood sample collected on day 2 were measured using a Hematostat II® (Separation Technologies, Inc., Altamonte Springs, FL).

VANC clearance during HD was determined using arterial and venous VANC concentrations measured during the procedure (at 1 and 3 h) and plasma flow rates. Arterial plasma flow ($Q_{pa}$) was calculated as:

$$Q_{pa} = Q_a \times (1 - \text{HCT}_a)$$

where $Q_a$ is the arterial blood flow and HCTa the arterial HCT. Venous plasma flow ($Q_{pv}$) was determined by:

$$Q_{pv} = Q_{pa} - \text{UFR}$$

where UFR is the ultrafiltration rate (ml/min). HD clearance (CLD) was calculated using the HD flow model:

$$\text{CLD} = \left[\frac{(Q_{pa} \times C_a) - (Q_{pv} - C_v)}{C_a}\right]$$

where $C_a$ and $C_v$ are the arterial and venous plasma VANC concentrations, respectively.

The in vitro VANC CLD for the polysulfone F80 dialyser was compared with the in vivo VANC CLD for this dialyser using the Student’s $t$-test. A $P < 0.05$ was considered significant.

## Results

### In vitro experiments

All samples were collected according to the designated sampling schedule during the 2-h dialysis procedure. The VANC ‘arterial’ concentration vs time profiles for all dialysers are shown in Figure 2. The CLD of VANC ranged from 65 ml/min for the hemophan COBE 700 HE dialyser to 143 ml/min for the polysulfone F80 dialyser (see Table 1), and correlated with the clearance ($r^2 = 0.77$, $P = 0.12$) and the KoA of B12 ($r^2 = 0.63$, $P = 0.21$) (see Table 1 and Figure 3) reported for each dialyser [10].

### In vivo dialysis

A total of four males and four females were enrolled with a mean age of 38.8 ± 5.4 years (range 20–69), mean time on dialysis of 58.1 ± 24.6 months (range 2–210) with hypertension as the cause of their end-stage renal disease. All eight patients were anuric. The mean blood flow was 463 ± 69 ml/min, mean dialysate flow 605 ± 15 ml/min, and the duration of dialysis 3.6 ± 0.42 h. Three subjects were dialysed using the F60 and five using the F80 polysulfone membranes.

The observed mean VANC concentration vs time data from the in vivo study are shown in Figure 4. The interdialytic elimination rate constant ($k_e$) was 0.014 ± 0.01 h−1, half-life ($t_{1/2}$) = 49.1 ± 21.4 h, interdialytic clearance 9.2 ± 0.24 ml/min and the CLD 135 ± 18 ml/min. No differences were observed between VANC clearances for the F60 (139 ± 14 ml/min) and the F80 (133 ± 21 ml/min) dialysers ($P = 0.67$).

### Discussion

VANC is used extensively in the HD population and was selected as a model drug to assess in vitro dialysis clearance as an estimate of in vivo drug removal in HD patients [1,4,11–13]. The VANC CLD determined from the in vitro system for the polysulfone F80 dialyser was a good estimate of VANC CLD in patients undergoing dialysis, 143 ± 10 vs 135 ± 18 ml/min, respectively. The slightly greater CLD using this in vitro procedure may be due to the absence of protein binding in this system. A broad range of protein binding with VANC has been observed in patients with normal renal function (27–62%), which significantly correlates with the concentration of α1-acid glycoprotein [14]. Protein binding may decrease the extent of VANC removal by dialysis, although this effect is variable with such a wide range of binding percentage. Decreased protein binding reported for VANC in patients with end-stage renal
disease suggests more unbound drug will be available for removal by dialysis in the HD population [15]. The CLD values reported for the polysulfone F80 dialyser in other in vivo studies under similar dialysis conditions are listed in Table 2 [1,4,11–13]. DeSoi et al. [1] observed a CLD of 123±51 ml/min and Foote et al. [11] determined a VANC CLD of 131±30 ml/min. Other investigators have reported VANC clearances for the F80 of 120±59 ml/min when similar blood and dialysate flow rates were used [12]. The unbound CLD determined in this study for the F80 is within the range of in vivo VANC dialysis clearance values reported with this dialyser.

The dialysers selected for the in vitro study are composed of materials that vary in porosity (hemo- phan < synthetic dialysers such as polysulfone). Hemo- phan dialysers are composed of a cellulosynthetic material, more porous than conventional cuprophane dialysers yet not of the clearance capacity of synthetic membranes such as polysulfone, which are the most porous. Thus, the potential for a substance of relatively large molecular weight, such as VANC, to traverse the pores of a cellulose or hemophan dialyser is less than that for the synthetic materials. A VANC dosing interval appropriate for patients dialysed using a cuprophan dialyser is suboptimal in patients dialysed using a synthetic dialyser due to more extensive VANC removal [4]. The in vitro dialysis system correctly predicts the increased elimination of VANC with these synthetic dialysers compared with the hemophan dialyser.

For each dialyser, clearance values and the KoA (mass transfer coefficient) are reported for substances of varying molecular weights, usually urea, creatinine, phosphate and B12 to indicate how well a given dialyser removes small vs large solutes. The KoA indicates the efficiency of a dialyser in removing a given solute and is essentially the maximum possible clearance of that solute without blood or dialysate flow constraints. Vitamin B12 is a compound with a molecular weight similar to VANC (1354 vs 1448 Da, respectively). Therefore, VANC clearance would be expected to follow a trend similar to the B12 clearance reported for a dialyser. A correlation was observed between the in vitro VANC CLD and the reported B12 CLD, $r^2 = 0.77$. However, if the B12 CLD were to be used as a means of predicting VANC CLD, it would be expected that the clearances for the polymethylmethacrylate (PMMA) dialyser and the AN69 would be similar since the B12 CLD reported for these dialysers is identical (125 ml/min). This was not the case as the VANC CLD for the AN69 was greater than the PMMA (136±7 and 93±11 ml/min, respectively). A confounding factor is the variation in the flow rates for the in vitro system tested in this study and the system used to determine dialyser information (see Table 1). Use of the KoA permits a comparison between dialysers without interference from variations in flow rates and ultrafiltration; however, there was a poorer correlation between the B12 KoA and VANC CLD $(r^2 = 0.63)$. Thus, although VANC and B12 have similar molecular weights, the correlations between the VANC CLD and B12 CLD and between VANC CLD and B12 KoA are inadequate.

### Table 2. In vivo VANC CLD for the F80 dialyser reported under similar conditions

<table>
<thead>
<tr>
<th>Reference</th>
<th>VANC CLD (ml/min)</th>
<th>$Q_b$ (ml/min)</th>
<th>$Q_d$ (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DeSoi [1]</td>
<td>123 ± 51</td>
<td>400–450</td>
<td>570–620</td>
</tr>
<tr>
<td>Lanese [4]</td>
<td>85 ± 7</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>Pollard [12]</td>
<td>120 ± 59</td>
<td>417</td>
<td>800</td>
</tr>
</tbody>
</table>

aVANC CLD reported as mean ± SD; $Q_b$ = blood flow rate; $Q_d$ = dialysate flow rate.
to predict VANC CLD. Differences in other physicochemical properties (including protein binding) between VANC and B12 may account for the observed discrepancy in dialysis clearance. The extent of differences that exist between most drugs and the four standard compounds for which dialyzer clearance data are reported (urea, creatinine, phosphate, B12) support use of the in vitro method over use of dialyzer information alone.

Use of a protein-free drug reservoir eliminates protein binding and provides for estimation of unbound CLD. Incorporating whole blood into the system would account for protein binding. This system could also be used to address the issue of dialyzer reuse. VANC binds to polyacrylonitrile membranes as reported in previous in vitro dialysis studies [16]. Quantifying drug adsorption to dialysis membranes and the effect on CLD could be determined in an in vitro system [17,18]. The use of whole blood, plasma or albumin could be used as the ‘arterial’ fluid allowing estimation of CLD that includes protein binding.

While diffusion is the primary transport mechanism by which solutes are removed with dialysis, convection does play a role, particularly for larger molecular weight substances such as VANC [7]. Convection occurs when solutes contained within plasma water are transferred across the dialyzer. The transmembrane pressure in the in vitro dialysis study was set at zero to minimize the contribution of convection to VANC transport across the dialysers tested. Under these conditions, however, there remains some degree of backfiltration that may result in the transport of fluid and dissolved solutes from dialysate back to the reservoir solution, a phenomenon most notable with high-flux dialysers [19]. Changes in the ultrafiltration rate for the in vitro system in subsequent studies could address the contribution of the convective component. Other dialysis conditions that may alter drug clearance include drug reservoir and dialysate flow rates. The flow rates used for this in vitro process were set at typical flow rates used in the clinical setting (blood flow 250–450 ml/min, dialysate flow 500–800 ml/min) and were similar to the in vivo dialysis conditions.

Recirculation occurs when the volume returned from the dialyzer (venous side) is immediately pumped back through the dialyzer (arterial side), a situation that contributes to inefficient dialysis under in vivo conditions. To minimize the risk of recirculation in this study the ‘arterial’ and ‘venous’ lines were separated within the flask. The reservoir solution in the in vitro system was also confined to the space of the reservoir container, a condition that differs from the circulating blood volume in vivo. A magnetic stirrer was used to allow mixing of the solution during the procedure.

An in vitro system such as this does not account for distribution of drug into the tissue, a drug characteristic that may result in a rebound of drug concentrations post-dialysis. Rebound occurs when the elimination rate of a solute by dialysis exceeds the rate of transfer of drug from the tissue to the plasma compartment. There is an increase (or rebound) in drug concentration post-dialysis due to re-equilibration between peripheral and plasma compartments (see Figure 4). This in vitro system was not designed to characterize rebound; however, clearance data determined for individual dialysers using this method may be used in conjunction with other pharmacokinetic parameters to predict the likelihood of rebound.

Similar in vitro studies have been performed based on advantages of maintaining controlled conditions of dialysis to evaluate drug removal [5–9]. Other studies have incorporated mathematical modelling to characterize drug disposition in patients undergoing dialysis. Pallone et al. [5] developed a mathematical model to predict urea clearance with continuous arteriovenous HD at various dialysate flow rates with and without net filtration. This model was tested using an in vitro procedure to study the relative contributions of diffusion and convection to overall urea elimination with varying ultrafiltration and dialysis conditions. Spivey et al. [6] found their model useful in predicting rebound and drug removal by HD and in estimating pharmacokinetic parameters. These studies demonstrate the practical use of in vitro systems and mathematical modelling to predict solute disposition during dialysis procedures, although they lack validation by comparisons with in vivo elimination.

The intent of the approach presented here is to provide a simple in vitro method for the rapid, quantitative assessment of HD drug elimination with validation through comparison of in vitro and in vivo CLD. Recent technical advances in the design of HD systems and dialysers has increased the complexity of drug disposition during renal replacement therapy. Clinical pharmacokinetic studies to address this question are costly and often impractical. A model system such as the one used in this study incorporating in vitro data is needed and would serve as a valuable screening tool for assessment of drug removal by HD. Validation is needed to determine its predictive ability with other drugs and the effect of altering dialysis conditions, such as flow rates and filter selection.

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Conflict of interest statement. None declared.

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