An in vitro study of elimination of oseltamivir carboxylate by haemofiltration.

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Abstract:
Objective: To determine the in vitro adsorption and sieving coefficient of oseltamivir carboxylate using two types of haemofilter
Design: In vitro model of continuous venovenous haemofiltration (CVVH)
Setting: The Chinese University of Hong Kong
Interventions: Oseltamivir carboxylate adsorption to the haemofilter and circuit was determined by circulating a blood-crystalloid mixture containing oseltamivir carboxylate through a haemofilter circuit and returning the ultrafiltrate to the mixing chamber. Ultrafiltrate was then removed and replaced with a bicarbonate based fluid to enable
calculation of the sieving coefficient. The study was repeated 5 times using two types of haemofilter. Studies to determine the stability of oseltamivir carboxylate in solution were also undertaken. All blood samples collected were assayed according by HPLC-MS/MS for oseltamivir carboxylate.

Results: The mean (S.D.) oseltamivir carboxylate adsorption at 80 mins was 58.18 ±17.84ng for the PAN filter and 75.22± 36.88ng for the polyamide filter. There was no statistical difference in adsorption between the two haemofilters. Sixteen percent of the initial drug concentration was adsorbed by the haemofilter and circuit. Initial drug concentration was a significant predictor of adsorption (R²=0.734). The mean (S.D.) sieving coefficient for oseltamivir carboxylate for PAN filters was 1.06 (0.04) and polyamide filters was 1.03 (0.06) with no statistical difference between the two haemofilter types. Stability tests showed that the drug was stable in solution for 90 mins.

Conclusions: Adsorption of oseltamivir carboxylate occurs to the haemofilter and circuit and is dependent on the initial drug concentration. The sieving coefficient of oseltamivir carboxylate is 1 therefore clearance is primarily dependent on ultrafiltration rate.

Descriptor:
Key words: Renal replacement therapy; Adsorption; Kidney failure; Oseltamivir; Pharmacokinetics

Introduction
Oseltamivir is the drug of choice for treatment of avian influenza A/H5N1 infection. A review of the published cases reveals that 24% of patients who require advanced life support develop acute renal failure (1-7). It is therefore important to know the elimination of oseltamivir carboxylate (the active metabolite of oseltamivir) by haemofiltration should an avian flu epidemic occur. Given its small molecular size and low protein binding (3%) oseltamivir carboxylate should have a sieving coefficient close to 1 and clearance should approximate the ultrafiltration rate. However sieving coefficients can be affected by the charge on the haemofilter membrane and may vary between haemofilters (8). Additionally, some elimination may occur as a result of adsorption of drug to the haemofilter membrane and circuit (9).

As it is currently uncommon for patients to require haemofiltration and treatment with oseltamivir the opportunity to study oseltamivir carboxylate elimination by continuous venovenous haemofiltration (CVVH) in vivo is limited. We therefore carried out an in vitro study to determine the adsorption and sieving coefficient of oseltamivir carboxylate using two different haemofilters.

Methods
A previously described in vitro one compartment model of CVVH was used (9). In phase 1 adsorption of oseltamivir carboxylate to haemofilter and circuit was studied, in phase 2 the sieving coefficient, and in phase 3 the stability of the drug in solution.

Phase 1
During this phase a closed circuit was used (figure 1). The circuit was primed with heparinized normal saline (5000 U heparin/L) and the mixing chamber was filled with a blood-crystalloid mixture, consisting of one unit of expired whole blood, made up to a total volume of 1000 mL with heparinized lactated Ringer’s solution (5000 U heparin/L). Sodium bicarbonate 8.4% was added to bring the pH of the blood-crystalloid mixture to 7.3 and the temperature of the solution was maintained at 35-38°C using a hotplate and magnetic stirrer. Oseltamivir carboxylate was introduced into the mixing chamber after a 10 minute equilibration period in variable doses designed to produce clinically relevant concentrations. After a further 10 mins, a blood sample (5 mls) was taken from the mixing chamber to measure baseline oseltamivir carboxylate and haemoglobin concentrations. Following baseline sampling circulation through the circuit was started using a blood flow of 200 ml/min and ultrafiltrate rate of 2000 ml/h. The ultrafiltrate was returned to the mixing chamber and no replacement fluid was infused. Heparin (1000 U/ml) was continuously infused at 5 ml/h. As a result of the closed circuit elimination could only occur by spontaneous degradation, metabolism by blood cells or adsorption to the haemofilter and circuit. Blood samples (5 mls) were taken at 30, 60 and 90 mins and oseltamivir carboxylate concentrations measured to determine adsorption.

Drug adsorption was calculated as follows:

\[
\text{Adsorption} = (\text{Concentration of drug at baseline} \times \text{Volume of blood-crystalloid mixture in mixing chamber}) - (\text{Concentration of drug at end of time 30, 60 or 90 mins} \times \text{Volume of blood-crystalloid mixture in the mixing chamber and circuit})
\]

Phase 2

The second phase was a continuation of the first except that ultrafiltrate was removed and replaced with bicarbonate based replacement fluid (Haemosol BO, Hospal, Lyon, France) infused at 2000mls/hr to simulate haemofiltration in a patient. Four pairs of blood samples (arterial and venous limbs) and 4 ultrafiltrate samples were taken at 3 minute intervals.

The sieving coefficient (SC) was calculated as follows:

\[
\text{SC} = \frac{\text{Ultrafiltrate concentration}}{\text{Blood concentration}}
\]

where blood concentration is the mean of the concentration in the arterial and venous limbs of the haemofilter circuit

The study was carried out 5 times with 0.6m² polyamide filters (Haemofilter 6S, Hospal, Lyon, France) and 5 times with 0.6m² polyacrylonitrile (PAN) filters (Multiflow 60, Hospal, Lyon, France).

Phase 3

To determine the stability of oseltamivir carboxylate in solution and exclude the possibility of metabolism by blood, oseltamivir carboxylate was added to the blood-crystalloid mixture (temperature maintained at 35-38°C and pH kept at 7.3) without
circulation through the circuit. Blood samples (5 mls) were taken 10, 30, 60 and 90 mins. This phase was repeated 5 times.

All blood samples were collected in heparinised tubes and centrifuged at 3000rpm for 15 mins. Plasma was separated and stored at -80 °C. Ultrafiltrate samples were collected and stored at -80 °C. Plasma and ultrafiltrate samples were assayed by high performance chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) for oseltamivir carboxylate (BAS Analytics, Kenilworth, UK) (10). Samples were measured against calibration standards that had been prepared in human serum, along with quality control samples in either human plasma or urine to ensure the integrity of the method in another biological fluid. The assay range was from 10 to 10000ng/ml.

Statistical Analysis and power calculation
Sieving coefficients were compared using Student’s t-tests and adsorption values were compared using repeated measures analysis of variants (ANOVA). P-values less than 0.05 were considered statistically significant. Repeating the study 5 times with each filter gave a power of 80% based on an alpha value of 0.05 and effect size of 2 standard deviations.

Results
The mean (standard deviation (S.D.)) haemoglobin concentrations were 6.2 (0.711)g/dl. The mean (S.D.) initial oseltamivir carboxylate concentrations were 411 (154). Oseltamivir carboxylate adsorption at 80 mins was 58.18±17.84ng for the PAN filter and 75.22±36.88ng for the polyamide filter. Time course for adsorption is shown in figure 2. There was no difference in adsorption between the two haemofilter types. Sixteen percent of the initial drug concentration was adsorbed by the haemofilter and circuit. Initial drug concentration appeared to be a significant predictor of adsorption (figure 2). There was no difference in mean ±SD sieving coefficient between PAN filters (1.06 ±0.04) and polyamide filters (1.03 ±0.06). Oseltamivir carboxylate remained stable in solution and did not undergo spontaneous degradation or metabolism by blood (mean % change from baseline at 30 mins:+3.97%, 60 mins:+1.91%, 90mins:+2.36%).

Discussion
This study demonstrates that the sieving coefficient of oseltamivir carboxylate is close to 1. Drug adsorption by the haemofilter and circuit appears to be concentration dependent but at clinically relevant concentrations the absolute adsorption is low. Neither sieving coefficient nor adsorption was affected by the type of filter membrane (polyamide or PAN).

Oseltamivir is a readily absorbed prodrug that is extensively converted to the active metabolite oseltamivir carboxylate. The bioavailability of oseltamivir in the form of the carboxylate is 75-80%. Oseltamivir carboxylate is renally eliminated by glomerular filtration (approximately 50%) and tubular secretion (approximately 50%) (11,12,13). There are no specific dosage recommendations for patients receiving haemofiltration. Dosage reductions are recommended for patients with a creatinine clearance less than 30ml/min (14,15,16,17) but this recommendation cannot be directly applied to anuric
patients receiving haemofiltration because of the absence of excretion by tubular secretion in these patients. As the sieving coefficient of oseltamivir carboxylate is very close to 1 clearance by haemofiltration should approximate the ultrafiltration rate. Maintenance doses of drugs can be calculated from the formula:

\[
\text{Required dose: } \frac{\text{Cp} \times I \times \text{CL}}{f}
\]

where \(\text{Cp}\) = desired plasma concentration (mg/ml), \(I\) = dosage interval (min), \(\text{CL}\) = clearance (ml/min), \(f\) = bioavailability (19).

At the dose recommended for treatment of H5N1 influenza (150 mg twice daily) the mean maximum oseltamivir carboxylate concentration is 705 ng/ml and the mean minimum 288 ng/ml in patients with normal renal function (12,13). Based on a bioavailability of 80%, a daily maintenance dose between 0.52 to 1.27 times the ultrafiltration rates should maintain steady state concentrations between these values in anuric patients receiving haemofiltration. Note that this dose is only an estimate which should, ideally, be confirmed by clinical studies. However we believe that it may provide an acceptable guide to dosing should an avian flu epidemic precede such studies, particularly as the therapeutic index for oseltamivir is high.

Absolute adsorption of oseltamivir carboxylate was low and can probably be disregarded. Total adsorption was <1% of even the reduced maintenance doses that we propose for anuric patients on hemofiltration.

The main weakness of this study is that it was an in vitro study. Although the blood-crystalloid mixture used in our model had a low haemoglobin concentration, to simulate critically ill patients, the pH was normal and clinically relevant concentrations of oseltamivir carboxylate were used, there may be other factors that affect sieving coefficients or adsorption in vivo. However, data to determine the pharmacokinetics of oseltamivir carboxylate in patients on haemofiltration are likely to be difficult to obtain. In addition in vivo measurement of adsorption is problematic (9). Only one brand of PAN filter and one brand of polyamide filter were used in this study and it is possible, though unlikely that the findings are specific to the brand of filter used. The effect of other filter materials (eg. polysulphone) was not determined in this study.

In summary, adsorption and sieving coefficient appear to be independent from the type of haemofilter membrane however adsorption is dependent on the initial drug concentration. The sieving coefficient of oseltamivir carboxylate is close to 1 and therefore clearance of oseltamivir carboxylate can be estimated from the ultrafiltration rate.

Reference List


Figure 2 (a) Oseltamivir carboxylate adsorption

Mean adsorption (ng) by PAN and Polyamide haemofilters

<table>
<thead>
<tr>
<th>Haemofilter</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAN (n=4)</td>
<td>55.14 ± 20.34</td>
<td>50.20 ± 28.78</td>
<td>58.18 ± 17.84</td>
<td>54.51 ± 20.91</td>
</tr>
<tr>
<td>Polyamide (n=5)</td>
<td>68.70 ± 20.51</td>
<td>71.47 ± 31.11</td>
<td>75.22 ± 36.88</td>
<td>71.80 ± 28.16</td>
</tr>
</tbody>
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Figure 2 (b) Relationship between initial dose and adsorption. Using linear regression, the following equation was derived: adsorption = 0.152 (initial dose) + 1.543. The square of regression coefficient, $R^2=0.734$. 
Figure 1 Circuit arrangement during adsorption phase of the experiment. Arrows indicate direction of flow.