Comparison of oseltamivir and oseltamivir carboxylate concentrations in venous plasma, venous blood, and capillary blood in healthy volunteers

Running title: Oseltamivir concentrations in different matrices

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Abstract

Oseltamivir and oseltamivir carboxylate concentrations were measured in venous plasma, venous blood, and capillary blood taken simultaneously from 24 healthy volunteers. Median (range) venous blood to plasma ratios were 1.42 (0.920-1.97) for oseltamivir and 0.673 (0.564-0.814) for oseltamivir carboxylate. Capillary blood/venous plasma ratios were 1.32 (0.737-3.16) for oseltamivir and 0.685 (0.502-1.34) for oseltamivir carboxylate. Oseltamivir concentrations in venous and capillary blood were
similar. Oseltamivir carboxylate showed a time-dependent distribution between venous and capillary blood.

**Keywords**: oseltamivir, oseltamivir carboxylate, capillary blood, pharmacokinetics, influenza, blood/plasma ratio

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Oseltamivir (OS) is an orally bioavailable neuraminidase inhibitor active against most strains of influenza viruses and licensed for the prevention and treatment of influenza infections (1). OS is rapidly absorbed after oral administration and is extensively converted by hepatic and plasma esterases to the bioactive metabolite, oseltamivir carboxylate (OC) (2). OS and OC exhibit dose linear pharmacokinetics (3) with peak plasma concentrations observed after approximately 1 and 4 hours, respectively. OS has an elimination half-life of between 1 to 1.5 hours while the OC half-life is longer, ranging from 5 to 8 hours (2, 3). The fraction of OC bound to plasma proteins is negligible (<3%) compared to OS (approximately 42%) (4).

OS and OC are usually quantified in venous plasma. Simpler sampling procedures using venous blood or capillary blood taken by finger-prick would be advantageous for field studies, particularly in young children and infants. We report a comparison of the concentrations of OS and OC in samples of venous plasma, venous blood, and capillary blood taken simultaneously from healthy volunteers.

This study was nested into pharmacokinetic investigations in 24 healthy volunteers conducted at the Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University, Bangkok, in 2010. It was approved by the ethics committee of the Faculty of Tropical Medicine. Venous blood samples (2.0 mL) taken through an indwelling catheter and capillary blood taken by finger-prick into a heparinized capillary tube were obtained from subjects at 1, 4, and 24 hours after dosing with either 75 or 150 mg of oseltamivir (F. Hoffmann-La Roche Ltd., Basel, Switzerland) and transferred immediately into fluoride oxalate tubes. A 50 µL whole blood aliquot was stored, and the remainder was centrifuged to obtain plasma. All
samples were stored at -80°C until analysis. Plasma OS and OC concentrations were quantified using liquid chromatography coupled to tandem mass-spectrometry (LC-MS/MS) (5). The lower limits of quantification (LLOQ) of OS and OC were set at 1 ng/mL and 10 ng/mL, respectively. The sample preparation and drug quantification in venous and capillary blood were identical to that described previously for plasma (5) except that the sample volume was set to 20 µL and the injection volume to 5 µL.

Comparisons were performed using the Wilcoxon matched-pairs signed rank test for paired observations and the Friedman test for groups. Pairs were excluded from analysis if the analyte concentrations in one or both samples were below the LLOQ. Agreement between matrices and potential concentration-dependent differences were also assessed using Bland-Altman plots. The matrices were considered bioanalytically equivalent if >67% of data pairs were within ±20% (6). Statistical analyses were performed using GraphPad Prism 5 (San Diego, USA).

The concentration measurements of OS and OC are summarized in Table 1 and Figure 1. All OS concentrations were below the LLOQ at 24 hours after dosing. OS concentrations in venous plasma were significantly lower than those in venous blood (median [range]: 27.5 [3.52-171] ng/mL vs 37.5 [5.18-250] ng/mL, N=96 pairs; p<0.0001). Bland-Altman plots showed that 14.6% (14/96) of data pairs were within the ±20% limit of agreement. OS venous plasma concentrations were also significantly lower than those in capillary blood (25.4 [3.52-171] vs 31.1 [4.41-258] ng/mL, N=89 pairs; p<0.0001). 33.7% (30/89) of data pairs were within the ±20% limit of agreement. OS concentrations in venous blood were slightly higher than those in capillary blood (33.6 [5.18-250] vs 31.1 [4.41-258] ng/mL, N=89 pairs; p=0.03) although 70.8% (63/89)
of data pairs were within the ±20% limit of agreement. OS did not show time dependent or concentration dependent differences between matrices (Table 1).

OC concentrations in venous plasma were significantly higher than those in venous blood (101 [16.0-888] vs 73.0 [10.4-562] ng/mL, N=137 pairs; \(p<0.0001\)). The venous blood to plasma ratios were similar at each time point (Table 1 and Figure 1). All data pairs were outside the ±20% limit of agreement. OC concentrations in venous plasma were also significantly higher than those in capillary blood (92.9 [9.85-888] vs 78.2 [10.3-556] ng/mL, N=130 pairs; \(p<0.0001\)). The OC venous plasma to capillary blood ratios at 1, at 4, and at 24 hours after dosing were significantly different (\(p<0.0001\)), indicating a time-dependent distribution between the two matrices (Table 1).

All data pairs at 4 and 24 hours after dosing were outside the limits of agreement (Figure 2). OC concentrations in venous blood were significantly lower than those in capillary blood (66.8 [10.4-562] vs 78.5 [10.3-556] ng/mL, N=129 pairs; \(p=0.0014\)). This was because venous blood OC concentrations were significantly lower at 1 hour (56.2 [13.6-335] vs 76.3 [16.4-329] ng/mL, \(p<0.001\)), whereas they were similar at 4 hours (209 [94.6-562] vs 216 [98.3-556] ng/mL, \(p=0.070\)), and were slightly higher at 24 hours (29.0 [10.4-92.5] vs 27.5 [10.3-92.1] ng/mL, \(p<0.001\)) after dosing. The OC venous blood to capillary blood ratios at 1, 4 and 24 hours after dosing were therefore significantly different, indicating a time-dependent distribution between venous and capillary blood (\(p<0.0001\)) (Table 1). 100% and 97.9% of data pairs were within the limits of agreement at 4 and 24 hours after dosing, respectively (Figure 3). There was no evidence of concentration-dependent distribution between the matrices.
Total inter-subject variability (%CV) measurements in venous plasma, venous
blood, and capillary blood were 100%, 100%, and 107% for OS and 105%, 114%, and
97%, respectively, for OC. There was no correlation between the hematocrit values
(median 38.8%; range 31.6% to 46.8%) and OS blood/plasma ratios \((p=0.071)\), but
there was a very weak negative correlation between hematocrits and the OC
blood/plasma ratios \((r^2=0.069; p=0.002)\).

In this study, OS concentrations in venous blood were approximately 42% higher
than those in venous plasma whereas concentrations of the active metabolite OC were
33% higher in plasma than in venous blood (Table 1). Similar results were found
comparing venous plasma and capillary blood concentrations. Compound lipophilicity is
an important determinant of distribution into cells (7). OC is a highly hydrophilic
compound with a log \(P\)-value of -2.1 (\(P\) is the octanol-water partition coefficient), which
is much more polar than OS (log-\(P\) 0.36) (8).

Blood taken from a finger-prick is often referred to as a capillary blood, although
it is a mixture of arterial, venous and capillary blood, and may also contain interstitial
and intracellular fluids (9). The greater pressure in arterioles and in the arterial limb of
capillaries results in a greater ratio of arterial to venous blood in finger-prick blood (9).
Significant arterio-venous concentration differences have been identified in a number of
drugs, and for these compounds venous-capillary differences would also be expected
(10). Venous-capillary differences in drug concentrations have been reported for
piperaquine (11), paracetamol (12) and artemisinin (13), while venous-capillary
concentrations of cyclosporine (9) and tobramycin (14) are reported to be similar.
In this study, there was a reasonable agreement between venous blood and capillary blood for OS measurements (Table 1). However, a time-dependent distribution of OC between the two matrices was evident. At 1 hour after dosing, venous blood OC concentrations were significantly lower than that in capillary blood, indicating incomplete equilibration. By 4 hours concentrations were similar (Figure 3).

The overall variability of OS and OC were comparable in the different matrices, even though venous and capillary blood sample volumes (20 µL) for OS and OC measurements were lower than that used for quantification in venous plasma (50 µL). Sampling capillary blood taken by finger-prick is simple and should facilitate pharmacokinetic sampling in field studies, especially in pediatric studies. The next step will be to determine whether accurate and reliable assays can be performed on dried blood spot samples taken onto filter paper.

In conclusion, measurements of OS and OC in venous plasma and venous blood are not readily interchangeable whereas venous blood and capillary blood sampling can be used interchangeably provided the time-dependent distribution of OC is taken into consideration.

Acknowledgement

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References


**Table and Figure legends**

**Table 1.** Measured oseltamivir and oseltamivir carboxylate concentration ratios in venous plasma, venous blood and capillary blood.

**Figure 1.** Oseltamivir (OS) concentrations measured in venous plasma, venous blood, and capillary blood at (A) 1 hour and (B) 4 hours and oseltamivir carboxylate (OC) concentrations measured at (C) 1 hour, (D) 4 hours and (E) 24 hours after dosing. Error bars indicate median and inter-quartile ranges.

**Figure 2.** Bland-Altman plots of oseltamivir carboxylate concentrations in venous plasma and capillary blood at (A) 1 hour, (B) 4 hours, and (C) 24 hours after dosing.
Percent (%) difference on the y-axis is the measured venous plasma OC concentration minus the capillary blood OC concentration divided by the mean of capillary blood and venous plasma concentrations. (D) Venous plasma to capillary blood oseltamivir carboxylate concentration ratios at 1 hour, 4 hours, and 24 hours after dosing, with error bars indicating median and inter-quartile ranges.

**Figure 3.** Bland-Altman plots of oseltamivir carboxylate concentrations in venous blood and capillary blood at (A) 1 hour, (B) 4 hours, and (C) 24 hours after dosing. Percent (%) difference on the y-axis is the measured venous blood OC concentration minus the capillary blood OC concentration divided by the mean of venous blood and capillary blood concentrations. (D) Venous blood to capillary blood oseltamivir carboxylate concentrations ratio at 1 hour, 4 hours, and 24 hours after dosing.
Table 1. Measured oseltamivir and oseltamivir carboxylate concentration ratios in venous plasma, 
venous blood and capillary blood

<table>
<thead>
<tr>
<th>Ratio</th>
<th>1 hour after dosing</th>
<th>4 hours after dosing</th>
<th>24 hours after dosing</th>
<th>Pooled</th>
<th>Statistic test</th>
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<tbody>
<tr>
<td></td>
<td>Median (range)</td>
<td>Median (range)</td>
<td>Median (range)</td>
<td>Median (range)</td>
<td>Wilcoxon&lt;sup&gt;a&lt;/sup&gt; Friedman&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Oseltamivir:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Venous blood/venous plasma</td>
<td>1.40 (0.925-1.69)</td>
<td>1.44 (1.04-1.97)</td>
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<td>1.42 (0.920-1.97)</td>
<td>p=0.162</td>
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<td>Venous blood/capillary blood</td>
<td>1.04 (0.456-1.67)</td>
<td>1.06 (0.760-1.68)</td>
<td>-</td>
<td>1.05 (0.460-1.68)</td>
<td>p=0.065</td>
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<td>Venous plasma/capillary blood</td>
<td>0.760 (0.320-1.36)</td>
<td>0.760 (0.450-1.21)</td>
<td>-</td>
<td>0.759 (0.317-1.36)</td>
<td>p=0.693</td>
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<tr>
<td>Oseltamivir carboxylate:</td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Venous blood/venous plasma</td>
<td>0.683 (0.564-0.814)</td>
<td>0.664 (0.575-0.755)</td>
<td>0.685 (0.578-0.794)</td>
<td>0.673 (0.564-0.814)</td>
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<td>Venous blood/capillary blood</td>
<td>0.760 (0.520-1.02)</td>
<td>0.985 (0.901-1.10)</td>
<td>1.07 (0.847-1.29)</td>
<td>0.992 (0.525-1.20)</td>
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<tr>
<td>Venous plasma/capillary blood</td>
<td>1.14 (0.746-1.68)</td>
<td>1.47 (1.31-1.81)</td>
<td>1.55 (1.30-1.99)</td>
<td>1.46 (0.746-1.99)</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>Oseltamivir measured in all matrices were all below the lower limit of quantification at 24 hours after dosing.

<sup>b</sup>Comparison of oseltamivir concentration ratios between matrices at 1 and 4 hour after dosing.

<sup>c</sup>Comparison of oseltamivir carboxylate concentration ratios between matrices at 1, 4, and 24 hours after dosing.

<sup>d</sup>Dunn’s multiple comparison analysis: the ratio at 1 vs 4 hours, at 1 vs 24 hours and at 4 vs 24 hours after dosing were all significantly different (p<0.05).

<sup>e</sup>Dunn’s multiple comparison analysis: the ratio at 1 vs 4 hours, and at 1 vs 24 hours were significantly different (p<0.05), the ratio at 4 vs 24 hours was not significantly different (p>0.05).
Average oseltamivir carboxylate concentration (ng/mL)

% Difference

Upper limit

Lower limit

Mean = 9.81%

21/36 (58.3%)

Mean = 39.6%

Mean = 44.2%

Time after dosing (hour)
(A) Mean = -27.2% 
+1.96 SD
-1.96 SD

(B) Mean = -1.4%
+1.96 SD
-1.96 SD

(C) Average oseltamivir carboxylate concentration (ng/mL)

(D) Average oseltamivir carboxylate concentration (ng/mL)