An assessment of the effect of mefloquine on artesunate pharmacokinetics

in healthy male volunteers

Timothy M. E. Davis¹*

Michelle England¹

Anne-Marie Dunlop¹

Madhu Page-Sharp¹

Nathalie Cambon²

Thomas G. Keller²

János L. Heidecker²

Kenneth F. Ilett¹,³

¹. School of Medicine and Pharmacology, University of Western Australia, Crawley, Western Australia, Australia.
². Mepha Ltd, Pharmaceutical Research Development and Manufacture, Dornacherstrasse 114, 4147 Aesch, Switzerland
³. Clinical Pharmacology and Toxicology Laboratory, PathWest Laboratory Medicine, Nedlands, Western Australia, Australia.

*Correspondence and reprints: Professor T.M.E. Davis, University of Western Australia, School of Medicine and Pharmacology, Fremantle Hospital, P.O. Box 480, Fremantle, Western Australia, 6959. Telephone: (618) 9431 3229; fax: (618) 9431 2977; e-mail: tdavis@cyllene.uwa.edu.au

Running head: Artesunate-mefloquine interactions

Word count: Abstract 50, main text 1000, 1 table, 1 figure
Abstract

The effect of mefloquine on artesunate pharmacokinetics was assessed in 20 volunteers given artesunate for 3 days followed ≥21 days later by combination therapy for 3 days. The AUC$_{0-\infty}$ for dihydroartemisinin, the active metabolite of artesunate, was similar on Day 3 of the two dosing periods ($P$=0.12), implying no interaction.

Key words: artesunate, dihydroartemisinin, mefloquine, pharmacokinetics, healthy volunteers
Mefloquine-artesunate was one of the first artemisinin combination therapies (ACTs) used clinically and it remains effective treatment for uncomplicated malaria (2,9). While the influence of artesunate on the pharmacokinetics of mefloquine has been investigated (12,16) with seemingly inconsistent results (11), the effect of mefloquine on artesunate disposition has not. The active metabolite of artesunate, dihydroartemisinin (DHA), is itself an antimalarial drug that has been partnered with mefloquine as a form of ACT (2,9). Mefloquine does not influence DHA pharmacokinetics in patients with falciparum malaria (13-15), but extrapolation of this finding to artesunate-mefloquine may be invalid. Artesunate and DHA have different chemical and pharmacologic properties (5), and between-subject variability and changes in drug disposition and metabolism during recovery from malaria complicate assessment of previously-published parallel-group DHA-mefloquine patient studies. We have, therefore, evaluated the effects of mefloquine on artesunate/DHA pharmacokinetics in healthy males using a crossover study design.

The study was approved by the South Metropolitan Health Service Human Research Ethics Committee, Western Australia and all subjects provided informed consent. Twenty of the 25 volunteers recruited met eligibility criteria and provided complete valid data for analysis. Their mean age was 28.9 (range 19.0-57.1) years and their mean body weight 77 (48-130) kg. Each subject received artesunate 200 mg (Mepha Ltd, Switzerland) by mouth after an overnight fast on three consecutive mornings (Period 1). After a wash-out phase of ≥21 days, this schedule was repeated but mefloquine (Mepha) 250 mg daily was given at the same time as artesunate (Period 2). On Days 1 and 3 of each period, blood samples were drawn for drug assay under a predetermined schedule from immediately before (0 h) to 8 h post-dose. Additional samples were taken for mefloquine assay on the mornings of Days 2, 4, 5 and 6 during and after Period 2.
Drug assays were by high performance liquid chromatography (HPLC). For mefloquine, extracted plasma (with clomipramine as internal standard) was injected onto a RP Select B column (E. Merck, Darmstadt, Germany) run on a 1100 HPLC (Agilent Technologies, Waldbronn, Germany) using a mobile phase of 40% v/v acetonitrile in 45 mM KH$_2$PO$_4$ (pH 3) at 1.3 mL/min with UV detection at 225 nm. Within- and between-day relative standard deviations (RSD) over 50-2000 µg/liter were ≤9.4% and ≤8.8%, respectively. The lower limit of quantitation (LLOQ) was 10 µg/liter. For artesunate/DHA, extracted plasma (with artemisinin as internal standard) was chromatographed on an Intersil ODS2 C18 column (MZ-Analysentechnik GmbH, Mainz, Germany) using a mobile phase of CH$_3$CN:H$_2$O:30 mM ammonium formate buffer (pH 4.3):CH$_3$COOH (700:266:33:1) at 0.35 milliliter/min, with detection at m/z 402/267 (artesunate) and m/z 302/267 (DHA) using an API 2000 triple quadrupole mass spectrometer (Applied Biosystems Inc, Foster City, US). Within- and between-day RSDs at 5-500 µg/liter for artesunate and 10-1000 µg/liter for DHA were ≤7.4% and ≤8.2%, and LLOQs were 5 µg/liter and 10 µg/liter, respectively. Pharmacokinetic analysis was by non-compartmental methods (18). Non-normally distributed variables were log-transformed before statistical analysis which was by general linear modelling for repeated measures.

As found previously (5), artesunate was measurable transiently and in low concentrations relative to DHA (see Figure) and we restricted artesunate pharmacokinetic analysis to estimation of the maximum plasma concentration ($C_{\text{max}}$) and time to $C_{\text{max}}$ ($T_{\text{max}}$) as a result (see Table). There was no difference between mean values of log-transformed values of these parameters by time of study. For DHA, there were similarly no differences between ln($C_{\text{max}}$) and ln($T_{\text{max}}$), and also elimination half-life ($t_{1/2\beta}$), and volume of distribution and clearance relative to bioavailability ($V_z/F$ and CL/F) (see Table). Since artesunate is
metabolized stoichiometrically to DHA (8), we selected the logarithm of the area under the DHA concentration-time curve from 0 h to ∞ (ln(DHA AUC\text{0-∞})) as the primary outcome variable. There was no significant difference between the mean values (see Table). With the DHA AUC\text{0-∞} for Day 3, Period 1 as Reference (R) and that of Day 3, Period 2 as Test (T), the mean percentage T/R was 102% (90% confidence interval 87-117%). This interval lies within the accepted 80-125% boundaries for bioequivalence (19).

Although we did not sample for long enough to characterize the pharmacokinetics of mefloquine, the C\text{max} following a total mean dose of 9.7 mg/kg during Period 2 was 1058±383 µg/liter. Since, in two previous healthy-volunteer studies, single-dose mefloquine given as means of 19.6 (10) and 27.5 (6) mg/kg produced proportionately lower C\text{max} values of 1220±360 and 1440±740 µg/liter, respectively, we assume that there is dose-dependent bioavailability. Consistent with this hypothesis, dividing the dose in patients with falciparum malaria increases bioavailability (1,17).

None of the 25 recruits withdrew from the study because of drug-related adverse events and there were no significant changes in routine hematologic and biochemical tests in any subject over the 4-week study period. Artesunate was well tolerated, with no changes in supine and erect blood pressure, rate-corrected electrocardiographic QT interval (QT\text{c}) or plasma glucose during Period 1 (data not shown). Three subjects (12%) experienced mild neurological symptoms which lasted a day in each case and required no medical intervention. There were similarly no changes in postural blood pressure, QT\text{c} or glycemia during Period 2 (data not shown). However, 9 subjects (43%) experienced neurological symptoms (mainly insomnia, dizziness or vivid dreams) which started after the second dose of artesunate-mefloquine and lasted a median of 3 days. None of these events led to
withdrawal from the study or medical intervention. A further 6 subjects (30%) reported mild self-limited gastrointestinal symptoms during Period 2. Because of i) these data, ii) previous volunteer studies involving single mefloquine treatment doses with relatively high rates of adverse events (6), and iii) the present and other studies in volunteers (6,10) and patients (1,17) indicating that dividing the dose increases plasma concentrations, care should be taken in designing dose regimens in future mefloquine volunteer studies.

We conclude that mefloquine does not alter the pharmacokinetics of artesunate when the drugs are co-administered. In contrast to artemisinin (3,4), and as would be predicted from the short-term artesunate/DHA exposure in our study and from the literature on autoinduction by artemisinin drugs (7), we observed no significant time-dependent changes in artesunate-DHA pharmacokinetics.

Acknowledgements

We are grateful to Mepha Pharmaceuticals for financial support. We thank A. Prestel and H. Bozler from BiochemA GmbH, Germany for artesunate/DHA assays, N. Kamber for valuable assistance with clinical procedures, and W. Davis for help with statistical analysis.
References


Table: Pharmacokinetic parameters for artesunate and dihydroartemisinin on Days 1 and 3 of Periods 1 and 2. Values are geometric mean (SD range) or mean [95% confidence interval]. The P-values are derived from general linear modelling for repeated measures.

<table>
<thead>
<tr>
<th>Time of study</th>
<th>Period 1, Day 1</th>
<th>Period 1, Day 3</th>
<th>Period 2, Day 1</th>
<th>Period 2, Day 3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artesunate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/liter)</td>
<td>135 (58-316)</td>
<td>113 (44-290)</td>
<td>91 (44-189)</td>
<td>109 (39-304)</td>
<td>0.42</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>0.6 (0.4-0.9)</td>
<td>0.6 (0.4-1.1)</td>
<td>0.5 (0.3-0.7)</td>
<td>0.6 (0.5-1.0)</td>
<td>0.19</td>
</tr>
<tr>
<td>Dihydroartemisinin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (µg/liter)</td>
<td>675 (522-873)</td>
<td>643 (450-917)</td>
<td>508 (345-748)</td>
<td>591 (357-978)</td>
<td>0.11</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>1.0 (0.6-1.8)</td>
<td>0.8 (0.4-1.5)</td>
<td>1.3 (0.7-2.3)</td>
<td>0.9 (0.5-1.6)</td>
<td>0.06</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (µg*h/liter)</td>
<td>1443 (1082-1924)</td>
<td>1365 (1038-1795)</td>
<td>1217 (850-1742)</td>
<td>1307 (903-1892)</td>
<td>0.12</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2β&lt;/sub&gt; (h)</td>
<td>1.14 [0.98-1.31]</td>
<td>1.14 [0.98-1.30]</td>
<td>1.02 [0.90-1.94]</td>
<td>1.09 [0.92-1.26]</td>
<td>0.93</td>
</tr>
<tr>
<td>V&lt;sub&gt;d&lt;/sub&gt;/F (liter)</td>
<td>174 [143-205]</td>
<td>184 [148-221]</td>
<td>201 [160-243]</td>
<td>186 [150-222]</td>
<td>0.75</td>
</tr>
</tbody>
</table>
Figure caption

Mean plasma concentrations of dihydroarteminin (upper panel) and artesunate (lower panel) during Period 1 (Days 1 and 3) and Period 2 (Days 1 and 3) in healthy male volunteers.