In Vitro Permeability and Solubility Study of Mefloquine Hydrochloride According to the Biopharmaceutics Classification System (BCS) Guidelines

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Purpose
This study intends to classify mefloquine, an anti-malarial drug, according to the Biopharmaceutics Classification System (BCS) guidelines. To date, studies of mefloquine have not conducted a thorough investigation of its BCS classification, and conflicting results suggest class I, II, or IV, partially due to inconsistent measurements of in vitro behavior and lack of adequate in vivo data. We aim to classify mefloquine based on its solubility profile and its permeability in our fully validated Caco-2 monolayer system, which utilizes a novel high Permeability Internal Standard (HPIS), minoxidil.

Methods
All samples were assayed by LC-MS/MS on an API 3000 or API 4000 Mass Spectrometer. Solubility Methods: Aqueous solubility was assessed using the shake-flask method. Solubility samples were prepared at a nominal concentration of approximately 2 mg/mL of base equivalent, above the high solubility threshold of 2 mg/mL (equivalent to 250 µg/mL). All solubility experiments had a 5 min pre-incubation with the dosing solution, unless otherwise noted. Unidirectional (apical→basolateral, A→B) experiments were conducted at n=4, and mefloquine was co-dosed with two internal permeability standards, 10 µM minoxidil (high-permeability reference compound) and 100 µM atenolol (monolayer integrity marker compound). Post-experiment, all four monolayers were lysed to establish mass balance. For bidirectional permeability (A→B and B→A), mefloquine was dosed alone (n=6) and was the only analyte quantified. The permeability experiment, three of the monolayers were lyzed with 500 µl of 1% Triton X-100 and analyzed for cellular accumulation of mefloquine (C_<sub>r</sub>). The other three monolayers were used for a post-experiment (PE) test of monolayer integrity with minoxidil and atenolol (30 min incubation).

Permeability Methods: Dosing solutions of mefloquine were prepared in permeability assay buffer (HBSSg, pH 7.4) at concentrations of 24, 240, and 400 µM. All permeability experiments have a 5 min pre-incubation with the dosing solution, unless otherwise noted. Unidirectional (apical→basolateral, A→B) experiments were conducted at n=4, and mefloquine was co-dosed with two internal permeability standards, 10 µM minoxidil (high-permeability reference compound) and 100 µM atenolol (monolayer integrity marker compound). Post-experiment, all four monolayers were lysed to establish mass balance. For bidirectional permeability (A→B and B→A), mefloquine was dosed alone (n=6) and was the only analyte quantified. After the permeability experiment, three of the monolayers were lyzed with 500 µl of 1% Triton X-100 and analyzed for cellular accumulation of mefloquine (C_<sub>r</sub>). The other three monolayers were used for a post-experiment (PE) test of monolayer integrity with minoxidil and atenolol (30 min incubation).

Results

Permeability
As shown in Table 1, the permeability rank order of the three compounds was mefloquine (at all three concentrations tested) > minoxidil > atenolol. Moreover, mefloquine should have greater than 90% absorption in humans since its permeability was higher than that of minoxidil, and minoxidil is at least 90% absorbed in humans. Thus, mefloquine can be classified as a highly permeable compound. When mefloquine was dosed alone in the bidirectional experiment (Table 2), the measured A→B P<sub>app</sub> values were similar to the values obtained when it was co-dosed at 24 µM with minoxidil and atenolol (Table 1). This result indicated that mefloquine’s solubility in the reference compounds, minoxidil and atenolol, did not interfere with the mefloquine permeability measurement. The efflux ratio for mefloquine was 1.43, suggesting no significant asymmetrical flux base on our validated system. These results show a lack of directional dependence and provide evidence that mefloquine permeates Caco-2 monolayers primarily by passive diffusion.

The A→B bidirectional recovery results were also similar to the unidirectional A→B recovery results. B→A recovery was higher than A→B, explaining the slight increase in B→A P<sub>app</sub> over A→B. Non-specific binding experiments (Table 2) showed that mefloquine did not stick to the side of the apparatus. Thus, it’s recovery was likely due to cellular accumulation. Recovery results in Table 1 showed a dose-dependent increase in P<sub>app</sub> values, likely due to saturation of intracellular drug concentration at high dose levels. Indeed, mass balance values, calculated by incorporation of antipyrine and metoprolol, indicated that the reference compounds, minoxidil and atenolol, did not interfere with the mefloquine permeability measurement. The result was possibly due to the formation of a complex with mefloquine or the presence of a pH or concentration dependent mechanism. However, mass balance values were not calculated with mefloquine concentrations. Therefore, we cannot conclude that these compounds did not interfere with the mefloquine permeability measurement.

Solubility
The measured solubility concentrations at all pH levels were lower than the concentration equivalent to the highest proposed dose strength dissolved in 250 mL (1 mg/mL) (Table 3). At pH 3.0 and pH 5.0, solubility was lost at pH 1.0 and pH 7.5 in phosphate buffer. This result was most likely an artifact of the buffer composition since mefloquine has a single pKa value, 8.6, and the result was possibly due to the formation of a much less soluble phosphate salt at pH 3.0 and pH 5.0. Therefore, an alternative buffer (phosphate buffer) was used at pH 3.0 and 5.0, revealing that the solubility in phosphate buffer at pH 3.0 exceeded the target concentration. Despite this, all other pH and buffer conditions did not achieve target concentrations, and therefore mefloquine cannot be classified as highly soluble according to the BCS criteria. The pH-solubility profile is shown in Figure 4.

Conclusions
- The aqueous solubility of mefloquine was lower than 1 mg/mL (equivalent to lower than 250 mg of mefloquine hydrochloride in 250 mL) at all four pH levels tested between 1.0 and 7.5 with USP buffers, with evidence of buffer interaction. Solubility was increased at pH 3.0 in phosphate buffer; however, solubility still remained below the target at all other pH levels. Thus, according to the BCS criteria, mefloquine cannot be classified as highly soluble.
- Mefloquine is highly permeable, primarily by passive diffusion, with apparent permeability at all three test concentrations greater than that of co-dosed minoxidil in a validated Caco-2 cell monolayer test system. As minoxidil is a marker for >90% fraction absorbed in humans, the results of this study predict that mefloquine will be well absorbed (at least 90%) in vivo.
- In an effort to address conflicting literature that has not been able to establish the BCS classification of mefloquine, our study clearly demonstrates that mefloquine is a BCS Class II compound.

Table 1. Unidirectional (A→B) Permeability of Mefloquine and Reference Compounds Across Caco-2 Monolayers (n = 4)

<table>
<thead>
<tr>
<th>Compound</th>
<th>P&lt;sub&gt;app&lt;/sub&gt; (x10&lt;sup&gt;-6&lt;/sup&gt; cm/sec)</th>
<th>Recovery</th>
<th>Mefloquine**</th>
<th>Minoxidil</th>
<th>Atenolol</th>
</tr>
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<tr>
<td>Mefloquine</td>
<td>8.33±0.72</td>
<td>11.86±0.78</td>
<td>51.5±15.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minoxidil</td>
<td>4.95±0.15**</td>
<td>4.81±0.62</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atenolol</td>
<td>0.44±0.07**</td>
<td>0.44±0.02</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
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Table 2. Bidirectional (A→B and B→A) Permeability of Mefloquine at 24 µM (n = 4)

<table>
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<tr>
<th>Compound</th>
<th>P&lt;sub&gt;app&lt;/sub&gt; (x10&lt;sup&gt;-6&lt;/sup&gt; cm/sec)</th>
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Figure 1. Caco-2 P<sub>app</sub> is for 61 Compounds

Figure 2. Minoxidil is an ideal high-permeability internal standard because of its proximity to the low/high permeability class boundary

Figure 3. Cumulative concentration in the receiver compartment normalized to dosing concentration for each compound.

Figure 4. pH-Solubility profile of mefloquine hydrochloride. At every pH tested with USP buffers, mefloquine shows lower solubility than dose strength (1 mg/mL). Phosphate buffer at pH 3.0 was the only concentration to give high solubility.