Multi-system investigation of the mechanisms for raltegravir association with intestinal tissue after oral administration

Abstract: Recent data have suggested that concentrations of raltegravir (RAL) in gut tissue are very high (160-fold higher in ileum tissue than blood plasma) after oral administration to humans, with implications for treatment and prevention. We have used in silico, in vitro, ex vivo and in vivo models to further investigate the accumulation of RAL in comparison to lopinavir (LPV) in gut tissue.

**INTRODUCTION**

• Recent data have suggested that concentrations of raltegravir (RAL) in gut tissue are very high (160-fold higher in ileum tissue than blood plasma) after oral administration to humans, with implications for treatment and prevention.

• We have used in silico, in vitro, ex vivo and in vivo models to further investigate the accumulation of RAL in comparison to lopinavir (LPV) in gut tissue.

**METHODS**

• Gut-to-plasma ratio was predicted in silico for RAL and LPV using the Poulin Theil method. Values for lipophilicity (log P), free drug in plasma (fu) and pKa were obtained from literature and combined with data on gut tissue contents (fraction of neutral lipids, phospholipids, extracellular space and water) to estimate the affinity of the drug for gut tissue over plasma.

• Cellular accumulations of RAL and LPV (both 1 µM) were determined at pH 5, 6, 7 and 8 in Caco-2 gut epithelial cells (15x10⁶ cells, 37°C, 10 min, n = 3).

• Gut-associated concentrations were determined ex vivo after incubation of 100 g rat small intestine (SI) or large intestine (LI) with human plasma (3 mL plasma, 37°C, 4 hours, 60 rpm shaker, n = 4) containing RAL and LPV (both 50 µM).

• RAL concentrations in plasma, SI, LI, SI gut contents and LI gut contents were determined in vivo following oral dosing (8 mg/kg dose in phosphate buffered saline, n = 3) to male Wistar rats with 1 and 4 hour post-dose blood/tissue sampling.

• In vivo tissue samples were homogenised on ice and analysed using LC-MS/MS and other samples were analysed using scintillation counting (³H RAL and ¹⁴H LPV).

• Statistics were performed using SPSS 20. Normality was determined using the Shapiro-Wilk test. An independent t-test was used for normally distributed data and a Mann-Whitney test was used for non-normally distributed data. A p < 0.05 was deemed significant.

**RESULTS**

<table>
<thead>
<tr>
<th>Drug</th>
<th>log P</th>
<th>fu</th>
<th>pKa</th>
<th>Gut:plasma ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAL</td>
<td>0.4</td>
<td>0.17</td>
<td>6.7 (acid)</td>
<td>0.53</td>
</tr>
<tr>
<td>LPV</td>
<td>3.9</td>
<td>0.02</td>
<td>2.8 (base)</td>
<td>6.38</td>
</tr>
</tbody>
</table>

Table 1) In silico estimations of gut:plasma ratios for RAL and LPV. Factors used to determine the gut:plasma ratio (log P, fu and pKa) are also given.

**CONCLUSIONS**

• Gut-to-plasma affinity ratios for RAL and LPV were estimated at 0.53 and 6.38 in silico, respectively (Table 1).

• Intracellular RAL concentrations in Caco-2 cells were 1.41 µM (pH 5), 0.94 µM (pH 6), 0.72 µM (pH 7) and 0.42 µM (pH 8) (Figure 1A). Intracellular LPV concentrations were 24.8 µM (pH 5), 25.4 µM (pH 6), 25.8 µM (pH 7), 27.5 µM (pH 8) (Figure 1B).

• In ex vivo studies, RAL accumulated less than LPV in both SI (29.6 versus 65.7 µM, p < 0.05, Figure 2A) and LI (34.9 versus 53.5 µM, p < 0.05, Figure 2B).

• After oral administration to rats, RAL concentrations decreased between the 1-hour and 4-hour sampling periods in plasma (0.63 ± 0.33 µM to 0.05 ± 0.03 µM, p < 0.05) and in SI (1.4 ± 0.26 µM to 0.47 ± 0.27 µM, p < 0.05) but did not change in SI contents (5.2 ± 3.8 µM to 4.0 ± 3.2 µM, p = 0.72). Conversely, increases in LI (0.38 ± 0.15 µM to 1.36 ± 0.50 µM, p < 0.05) and LI contents (0.15 ± 0.09 µM to 40.6 ± 3.3 µM, p < 0.05) were observed (Figure 3).

**REFERENCES**

1. Patterson et al. AIDS 2013; 27(9):1413-9