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

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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[1].

• 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[2]. 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 (15x10^6 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 (^{3}H RAL and ^{3}H 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

Drug	log P	fu	pKa	Gut:plasma ratio
RAL	0.4	0.17	6.7 (acid)	0.53
LPV	3.9	0.02	2.8 (base)	6.38

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.

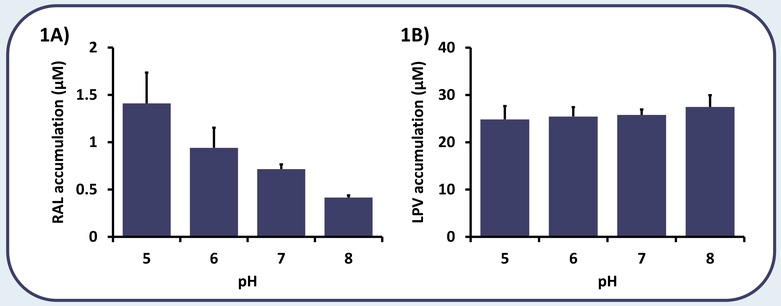


Figure 1A) Accumulation of RAL in Caco-2 cells (µM ± SD, 1 µM initial drug incubation, 15x10^6 cells, 37°C, 30 min, n = 3) using transport buffer at pH 5, 6, 7 or 8. **Figure 1B)** Accumulation of LPV in Caco-2 cells (µM ± SD, 1 µM initial drug incubation, 15x10^6 cells, 37°C, 30 min, n = 3) using transport buffer at pH 5, 6, 7 or 8.

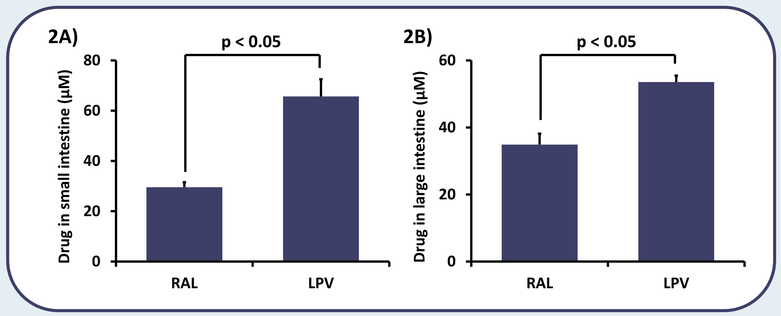


Figure 2A) *Ex vivo* accumulation of RAL and LPV in rat small intestine (µM ± SD, 50 µM initial drug incubation, 100 g tissue in 3 mL human plasma, 37°C, 4 hours, 60 rpm shaker, n = 4). **Figure 2B)** *Ex vivo* accumulation of RAL and LPV in rat large intestine (µM ± SD, 50 µM initial drug incubation, 100 g tissue in 3 mL human plasma, 37°C, 4 hours, 60 rpm shaker, n = 4)

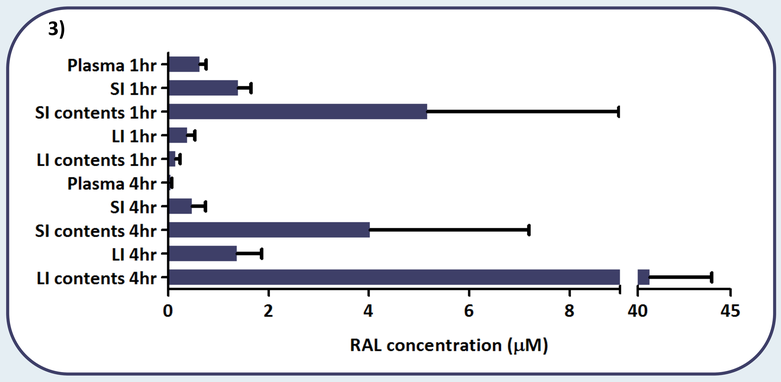


Figure 3) Concentrations of RAL 1-hour and 4-hour post dose in Wistar rat plasma, SI, LI, SI gut contents and LI gut contents (µM ± SD, 8 mg/kg RAL dose in phosphate buffered saline, n = 3).

• 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 ± 0.13 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).

CONCLUSIONS

• *In silico*, *in vitro* and *ex vivo* data all suggest that RAL has less propensity than LPV to accumulate in cellular gut tissue.

• In contrast, *in vivo* data suggest RAL concentrations in LI can remain high even when RAL plasma concentrations are reduced.

• However, very high RAL concentrations in LI contents make it likely that they are driving LI RAL exposure. Intracellular penetration is a prerequisite for RAL activity and further study is required to preclude artefacts that may arise from contamination with gut contents.

• The gut is a relevant site for both pre-exposure prophylaxis (PrEP) and HIV eradication, and the presented methodology may be useful in selecting drugs with preferential accumulation in gut tissue.

REFERENCES

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