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A multisystem investigation of raltegravir association with intestinal tissue: implications for pre-exposure prophylaxis and eradication

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Objectives: Recent clinical data have suggested high raltegravir concentrations in gut tissue after oral administration, 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 raltegravir in gut tissue.

Methods: Affinity of raltegravir for gut tissue was assessed in silico (Paulin–Theil method), in vitro (Caco-2 accumulation) and ex vivo (rat intestine) and compared with the lipophilic drug lopinavir. Finally, raltegravir concentrations in plasma, gut contents, small intestine and large intestine were determined after oral dosing to Wistar rats 1 and 4 h post-dose. Samples were analysed using LC-MS/MS and scintillation counting.

Results: Gut tissue accumulation of raltegravir was less than for lopinavir in silico, in vitro and ex vivo (P<0.05). After oral administration to rats, raltegravir concentrations 4 h post-dose were lower in plasma (0.05 μM) compared with small intestine (0.47 μM, P=0.06) and large intestine (1.36 μM, P<0.05). However, raltegravir concentrations in the contents of both small intestine (4.0 μM) and large intestine (40.6 μM) were also high.

Conclusions: In silico, in vitro and ex vivo data suggest low raltegravir accumulation in intestinal tissue. In contrast, in vivo animal data suggest raltegravir concentrates in intestinal tissue even when plasma concentrations are minimal. However, high raltegravir concentrations in gut contents are the likely driving factor, rather than blood-to-tissue. The methods described can be combined with clinical investigations to provide a complete strategy for selection of drugs with high gut accumulation.

Keywords: PreP, HIV, tissue drug concentrations

Introduction

ART has been extremely effective in improving mortality and morbidity in HIV infection. However, despite successful treatment, patients still experience re-emergence of virus following cessation of ART.1 Current ART is not capable of eradicating HIV from infected individuals, which is due to the existence of latently infected cells and the continuing replication of HIV in sanctuary sites, where drug concentrations are insufficient to halt viral replication.2–5 Antiretrovirals are also being investigated for pre-exposure prophylaxis (PreP), where drugs are administered to individuals at high risk of infection.5

The gut-associated lymphoid tissue (GALT) is important in the context of PreP and eradication. The GALT harbours 80% of total lymphocytes in humans, which are the primary cell type infected by HIV, and consists of mesenteric lymph nodes, Peyer’s patches in the small intestine and follicular aggregates in the large intestine and caecum.6 From an eradication perspective, the GALT can produce new virus in patients with undetectable viral loads in peripheral blood and this virus is capable of subsequent migration to other sites.7,8 The GALT shows incomplete immunological recovery following initiation of ART, which could be associated with inadequate drug exposure.9,10 From a PreP perspective, the GALT is a central site for establishment of primary HIV infection, where up to 60% of lymphocytes in the lamina propria are lost as early as 2 weeks after infection.9 Therefore, sufficient and sustained concentrations of antiretrovirals are required in the GALT to fully block continuing viral replication at this site (eradication) and to prevent the initial establishment of GALT-associated infection following exposure (PreP).

Clinical trials have previously attempted to quantify antiretroviral concentrations in human intestinal tissue, where tissue is sampled, processed and analysed for drug content. When administered as a single oral dose to HIV-negative men, the relative exposures of darunavir, ritonavir and etravirine in rectal tissue compared with blood following initiation of ART, which could be associated with inadequate drug exposure.9,10 From a PreP perspective, the GALT is a central site for establishment of primary HIV infection, where up to 60% of lymphocytes in the lamina propria are lost as early as 2 weeks after infection.9 Therefore, sufficient and sustained concentrations of antiretrovirals are required in the GALT to fully block continuing viral replication at this site (eradication) and to prevent the initial establishment of GALT-associated infection following exposure (PreP).

Clinical trials have previously attempted to quantify antiretroviral concentrations in human intestinal tissue, where tissue is sampled, processed and analysed for drug content. When administered as a single oral dose to HIV-negative men, the relative exposures of darunavir, ritonavir and etravirine in rectal tissue compared with blood plasma were 1.26, 5.77 and 15.7, respectively.11 A similar study
gave the relative exposure of maraviroc in rectal tissue compared with blood plasma as 7.48 following single dosing, increasing to 26.2 following multiple dosing. When administered to a healthy cohort of men and women, relative exposures of tenofovir and emtricitabine in rectal tissue compared with blood plasma were 32.8 and 3.2, respectively. Interestingly, the active forms of these drugs, tenofovir diphosphate and emtricitabine triphosphate, showed extremely high exposures in rectal tissue even compared with parent drug.

The integrase inhibitor raltegravir has shown by far the highest relative exposure in gut tissue of all antiretrovirals tested. Following a single 400 mg standard raltegravir dose given to HIV-negative men, a relative exposure compared with blood plasma of 39, 68 and 160 was reported in rectal tissue, splenic flexure and terminal ileum, respectively. Exposure was even higher following 7 days of twice-daily dosing, with a relative exposure of 231, 659 and 156 in rectal tissue, splenic flexure and terminal ileum, respectively. In contrast, dolutegravir, another integrase inhibitor, showed lower concentrations in rectal tissue compared with blood plasma, with a relative exposure of 0.17 following 8 days of once-daily dosing.

Knowledge of drug concentrations in the GALT is crucial to understand the factors dictating exposure and for appropriate selection of drugs for PreP. Intestinal tissue sampling may help in clarification but there are complications when attempting to distinguish between intracellular and extracellular drug measurements. Specifically, there is a possibility of artefacts arising through contamination of tissue with unabsorbed drug in the intestinal contents. Also, drug concentrations in whole intestinal tissue may not necessarily represent exposure of drug in the GALT. It is acknowledged that there is currently a paucity of information regarding the relationship between drug concentrations in the GALT and the use of whole tissue as a surrogate. The purpose of this study was to use several separate methodological approaches (in silico, in vitro, ex vivo and in vivo) to investigate the affinity of raltegravir for intestinal cells and tissue and to further understand the apparent high tissue-associated raltegravir exposure observed in patients.

**Materials and methods**

**Materials**

Caco-2 cells were purchased from the European Collection of Cell Cultures (Salisbury, UK). Raltegravir potassium salt was purchased from Selleckchem (Munich, Germany) and [3H]raltegravir was a gift from Merck (NJ, USA). Lopinavir was a gift from Abbott (IL, USA). [3H]Lopinavir was purchased from Moravek Biochemicals (CA, USA). Ultima Gold scintillation fluid and OptiSolv were purchased from Perkin Elmer (Boston, MA, USA). Male Wistar rats (ordered at 100–125 g, 33–35 days old) were purchased from Charles River (Kent, UK). All other drugs and reagents were obtained from Sigma (Poole, UK).

**In silico prediction of intestinal tissue affinity**

Intestine-to-plasma affinity ratios were predicted in silico for raltegravir and lopinavir using the Poulin–Theil method. Values for drug lipophilicity (log P), free fraction of drug in plasma (fu) and pka were combined with data on plasma and intestinal tissue constituents (fraction of neutral lipids, phospholipids, extracellular space and water) to estimate the affinity of the drug for intestinal tissue over plasma. The log P and pka of raltegravir were previously determined by our group. The fu of raltegravir was obtained from the literature. The log P of lopinavir was predicted using the ALOGP method. The fu and pka of lopinavir were obtained from the literature.

**In vitro accumulation using Caco-2 cells**

Caco-2 cells were maintained in cell culture (37°C, 5% CO2) by passing at 70% confluence using cell culture medium (DMEM/15% (v/v) FCS). The passage number of the cells used in this study was between 30 and 35.

For the experiment, Caco-2 cells were seeded (5 × 10⁶ cells/mL) into 6-well plates and cultured for 5 days to allow plate surface coverage (DMEM/15% (v/v) FCS, 37°C, 5% CO2). Medium was removed and cells were washed with warm Hanks balanced salt solution (HBSS) and replaced with the appropriate pH-buffered incubation solution and allowed to equilibrate (37°C, 5% CO2, 15 min). A range of pH conditions were used to simulate the varied pH found in the gastrointestinal system. Incubation solutions were adjusted using hydrochloric acid and sodium hydroxide and consisted of HBSS containing 10 mM MOPS (used for pH 5 and pH 6) or HBSS containing 25 mM HEPES (used for pH 7 and pH 8). Raltegravir (1 μM) was included in the wells and plates were incubated (2 mL, 37°C, 5% CO2, 10 min, n = 3 replicates). Parallel experiments were also performed to assess the accumulation of lopinavir (2 mL, 0.4 μCi/mL, 1 μM, 37°C, 5% CO2, 10 min, n = 3 replicates) as a comparator. Following incubation of either raltegravir or lopinavir, 100 μL extracellular samples were taken for analysis, wells were washed three times with ice-cold HBSS and 500 μL of tap water was added to each empty well to lyse cells. Plates were kept at −20°C overnight to facilitate the removal of cells. Plates were thawed and lopinavir samples were analysed by liquid scintillation counting (Beckman TRI-CARB®). For raltegravir samples, 500 μL of acetonitrile was added to each well to release drug from protein. The well contents were transferred to separate 1.5 mL tubes for centrifugation (10 min, 3000 g, 22°C) and supernatant was collected. Supernatant was then vacuum dried and reconstituted in 150 μL of HPLC-grade water for analysis using a previously validated LC-MS/MS method.

**Accumulation experiments using ex vivo rat intestinal tissue**

Four male Wistar rats were sacrificed using Schedule 1 procedures, blood samples were taken by cardiac puncture and stored in heparin tubes on ice for future analysis. Intestinal tissue was then harvested. Specifically, the section of tissue immediately following the duodenum (the jejunum) was used for the ‘small intestine’ and the section of tissue immediately following the caecum (the colon) was used for the ‘large intestine’. Tissue was cut open and rinsed with ice-cold PBS solution to remove intestinal contents. Approximately 100 mg of small intestine tissue was incubated with human plasma (3 mL of plasma, 37°C, 4 h, 60 rpm shaker, n = 4) containing either raltegravir or lopinavir (both at 1 μCi/mL, 50 μM). A parallel incubation was also performed using ~100 mg of large intestine. Following incubation, a 100 μL extracellular sample was taken from wells for determination of extracellular drug concentrations. Tissue was removed and washed thoroughly using ice-cold PBS. Tissue solubilization was performed using the following protocol. Each tissue segment was processed as a comparator. Following incubation of either raltegravir or lopinavir, 100 μL extracellular samples were taken for analysis. Concentrations of raltegravir and lopinavir were measured using liquid scintillation counting (Beckman TRI-CARB®). A parallel incubation was also performed using ~100 mg of large intestine. Following incubation, a 100 μL extracellular sample was taken from wells for determination of intracellular drug concentrations. Tissue was removed and washed thoroughly using ice-cold PBS. Tissue solubilization was performed using the following protocol. Each tissue segment was processed as a comparator.
Association of raltegravir with gut tissue

**Determination of raltegravir concentrations in vivo in plasma and intestinal tissue after oral administration to rats**

All in vivo experiments were undertaken following institutional and national standards for animal care and experimentation. The protocol was approved for use by the Research Ethics Committee at the University of Liverpool. Raltegravir concentrations in blood plasma, small intestine tissue, large intestine tissue, small intestine contents and large intestine contents were determined in vivo following oral dosing directly into the stomach using an oral gavage (8 mg/kg, 5 mL/kg, dosed using PBS, n=3) to male Wistar rats with 1 h post-dose sampling of blood and tissue. A parallel experiment was also performed where sampling of plasma and tissue was taken 4 h post-dose. At the point of sampling, rats were sacrificed using Schedule 1 procedures and blood samples were taken by cardiac puncture and stored in heparin tubes on ice for future analysis. Intestinal tissue was harvested as described above. Tissues were cut open and the contents removed and stored on ice for analysis. Tissue was then rinsed with ice-cold PBS solution to remove the remaining intestinal contents and was stored on ice. Blood samples were centrifuged (10 min, 3000 g, 22°C) and supernatant plasma was removed. Plasma was added to solvent (100 µL of plasma into 300 µL of methanol containing 100 nM ritonavir as internal standard), vortexed for 5 min and centrifuged (10 min, 3000 g, 22°C). Intestinal content was added to solvent (100 µL of intestinal content into 300 µL of methanol containing 100 nM ritonavir as internal standard), vortexed for 5 min and centrifuged (10 min, 3000 g, 22°C). Intestinal tissue was mechanically homogenized on ice until having a liquid consistency, added to solvent (100 µL of homogenate into 300 µL of methanol containing 100 nM ritonavir as internal standard), vortexed for 5 min and centrifuged (10 min, 3000 g, 22°C). The supernatants from the plasma, intestinal content and intestinal tissue samples were all carefully removed and 200 µL of each supernatant was vacuum dried and reconstituted in 100 µL of HPLC-grade water for analysis using a previously validated LC-MS/MS method. To account for the existence of a matrix effect, calibration curves were created using the different matrices (plasma, intestinal tissue and intestinal contents). Quality control (QC) samples (high QC 2000 ng/mL, medium QC 200 ng/mL and low QC 100 ng/mL) were included in analyses for confirmation, where a >20% deviation from the standard curve was considered a failed analysis. When calculating concentrations of raltegravir in tissue, the previously published conversion factor was used where 1.04 g of intestinal tissue is equivalent to 1 mL.15

**Statistical analysis**

Data were analysed using SPSS 20 for Windows. All data were tested for normality using the Shapiro–Wilk test. An independent t-test was used to determine significance of normally distributed data. The Mann–Whitney U-test was used for all other data. A two-tailed P value of <0.05 was accepted as being statistically significant. A two-tailed P value of ≤0.1 was accepted as showing a trend.

**Results**

**In silico prediction of intestinal tissue affinity**

The log P, fu and pKa of raltegravir were 0.4, 0.17 and 6.7 (acid), respectively. The log P and fu of lopinavir was 3.9 and 0.02, respectively. The pka of lopinavir was not within the range of physiological pH. The intestine-to-plasma affinity ratios of raltegravir and lopinavir were predicted as 0.53 and 6.38, respectively.

**In vitro accumulation using Caco-2 cells**

When Caco-2 cells were incubated with 1 µM raltegravir for 10 min, intracellular raltegravir concentrations were 1.41 ± 0.33 µM (pH 5), 0.94 ± 0.21 µM (pH 6), 0.72 ± 0.05 µM (pH 7) and 0.42 ± 0.02 µM (pH 8) (Figure 1a). Intracellular raltegravir concentrations were not significantly different from incubation concentrations when pH 5 (P=0.09) and pH 6 (P=0.68) buffers were used, but were significantly lower than incubation concentrations when pH 7 (P=0.01) and pH 8 (P<0.01) buffers were used. Extracellular concentrations of raltegravir following the 10 min incubations using pH 5, pH 6, pH 7 and pH 8 buffers were 0.95 ± 0.10, 1.02 ± 0.08, 1.05 ± 0.11 and 0.97 ± 0.15 µM, respectively. When Caco-2 cells were incubated with 1 µM lopinavir for 10 min, intracellular lopinavir concentrations were 24.8 ± 2.8 µM (pH 5), 25.4 ± 2.0 µM (pH 6), 25.8 ± 1.2 µM (pH 7) and 27.5 ± 2.5 µM (pH 8) (Figure 1b). Intracellular lopinavir concentrations were significantly higher than incubation concentrations for all pH buffers used (P<0.01) and did not differ across the pH range (P>0.05 for all comparisons). Extracellular concentrations of lopinavir following the 10 min incubations using pH 5, pH 6, pH 7 and pH 8 buffers were 0.83 ± 0.07, 0.80 ± 0.08, 0.80 ± 0.14 and 0.78 ± 0.03 µM, respectively.

**Accumulation experiments using ex vivo rat intestinal tissue**

In ex vivo studies when incubating 50 µM drug with Wistar rat intestinal tissue, raltegravir accumulated less than lopinavir in both the small intestine tissue (29.6 ± 2.0 versus 65.7 ± 6.8 µM, pH 5, 6, 7 or 8).

![Figure 1](http://example.com/figure1.png) (a) Accumulation of raltegravir in Caco-2 cells (µM±SD, 1 µM initial drug incubation, 15 × 10⁶ cells, 37°C, 10 min, n=3) using transport buffer at pH 5, 6, 7 or 8. (b) Accumulation of lopinavir in Caco-2 cells (µM±SD, 1 µM initial drug incubation, 15 × 10⁶ cells, 37°C, 10 min, n=3) using transport buffer at pH 5, 6, 7 or 8. RAL, raltegravir; LPV, lopinavir.
drug concentrations in plasma showed a trend to being lower than in the small intestine (P<0.01). Lopinavir showed significantly higher concentrations in the small intestine tissue compared with the plasma (P=0.03), whereas only a trend was observed for higher concentrations in the large intestine tissue compared with the plasma (P=0.051). Concentrations of raltegravir in plasma at the 4 h timepoint in the small intestine and large intestine incubations were 46.1±1.5 and 50.5±1.0 μM, respectively. Concentrations of lopinavir in plasma at the 4 h timepoint in the small intestine and large intestine incubations were 47.5±2.5 and 47.0±3.0 μM, respectively.

Discussion

The in silico, in vitro and ex vivo data presented here all suggest that raltegravir has less propensity than lopinavir to accumulate in intestinal cell lines and intestinal tissue. In contrast, in vivo animal data suggest raltegravir concentrations in the intestinal tissue can remain high even when raltegravir plasma concentrations are reduced. At the 4 h sampling point, the concentration of raltegravir is 26-fold higher in the large intestine tissue than in blood plasma. However, at the 4 h sampling point, the concentration of raltegravir in the large intestine contents is much higher still, showing a 29-fold higher concentration than in the tissue itself. Therefore, it seems likely that the high raltegravir concentrations detected in tissue are driven primarily by the local distribution of drug from the adjacent intestinal contents, rather than blood-to-tissue.

Previously published raltegravir pharmacokinetic profiles in rats show the C_{max} to be ~1 h post-dose and a large decrease in concentrations at after 4 h, which support our data.\textsuperscript{23} The mean gastric emptying time in Wistar rats is 1.7 h, so the 1 h timepoint represents when raltegravir has begun to empty from the

\[ \text{Drug in small intestine (μM)} \]

\[ \text{Drug in large intestine (μM)} \]

\[ P<0.05; \text{Figure 2a) and the large intestine tissue (34.9±3.3 versus 53.5±1.9 μM, P}<0.05; \text{Figure 2b). Tissue-associated raltegravir concentrations did not match the levels added to the plasma (P}<0.01). Lopinavir showed significantly higher concentrations in the small intestine tissue compared with the plasma (P}=0.03), whereas only a trend was observed for higher concentrations in the large intestine tissue compared with the plasma (P}=0.051). Concentrations of raltegravir in plasma at the 4 h timepoint in the small intestine and large intestine incubations were 46.1±1.5 and 50.5±1.0 μM, respectively. Concentrations of lopinavir in plasma at the 4 h timepoint in the small intestine and large intestine incubations were 47.5±2.5 and 47.0±3.0 μM, respectively.\]

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stomach and be absorbed by the small intestine, with very little yet reaching the large intestine. The mean transit time to the large intestine in Wistar rats is 3.4 h, so the 4 h timepoint represents when unabsorbed raltegravir has mostly entered the large intestine, explaining the large increase in the large intestine contents seen between the 1 and 4 h timepoints. Despite thoroughly washing the contents from tissue following sampling, it is possible that not all raltegravir was removed from the surface of the tissues. For context, at the 4 h timepoint, if only 5 µL of the large intestine contents remained for every 100 mg of tissue, then this would equate to a 2 µM contamination for the tissue data. It is also important to note that ex vivo experiments measured total radioactivity (raltegravir parent, metabolites etc.) whereas the in vivo experiments measured just raltegravir in the parent form. As the in vivo samples were analysed using MS, it can be assumed that all detected drug is in the active form. The amount of raltegravir bound to gut contents was not determined, but it can be predicted that it would not be particularly high as raltegravir is not particularly lipophilic (log P of 0.59 at pH 7), is a weak acid with a pKa of 6.7 and does not have a restrictively high plasma protein binding (83% bound).

Humans and rats can differ in characteristics known to affect drug metabolism, distribution and elimination and it is important to consider this when interpreting results in this study. The free fraction of raltegravir is 17% in human plasma and is similarly mild at 26% in rat plasma. Therefore, this factor is unlikely to substantially alter raltegravir pharmacokinetics between the species. Raltegravir is metabolized to an inactive form by UGT1A1 in humans and the rat ugt1a1 enzyme is believed to play a similar role (although this has not been empirically shown). Rats, as well as mice, dogs and even non-human primates, are generally poor predictors of drug bioavailability in humans, making it potentially difficult to utilize these animals for optimizing drug exposure in intestinal tissue and the GALT.

Raltegravir shows very high interpatient and intrapatient pharmacokinetic variability and absorption is influenced by pH-altering agents, metal-containing agents and food. When administered as a 400 mg oral tablet, the absorption of raltegravir is believed to be incomplete, although total bioavailability has not been determined in humans. Our group previously published a physiologically based pharmacokinetic model investigating the influence of gastrointestinal pH and metal-containing products on raltegravir exposure, where the fraction of drug absorbed was predicted to be incomplete in most simulated subjects, with some subjects showing as low as 13% absorption.

Considering that simulated subjects were given a single 400 mg of raltegravir tablet, this would potentially leave up to 354 mg of raltegravir unabsorbed in the intestinal contents. In the context of PreP, it is unknown whether this ‘reservoir’ of unabsorbed drug in the intestinal contents may provide any local protection of the rectal tissue from infection, in a similar way as in the use of vaginal and rectal microbiotics. It is also unknown whether drug concentrations are maintained in rectal tissue following enemas or defecation.

If both the percentage bioavailability of an antiretroviral and the dose size are taken into account, an estimate can be made of the amount of parent drug that was not bioavailable. Using published clinical data, a plot comparing this value with the relative exposure of each drug in rectal tissue is shown (Figure 4). The relative exposures of drugs in rectal tissue were taken from single-dose studies. Using bivariate analysis, a correlation is observed where a high amount of drug that is not bioavailable is associated with a high relative drug concentration in rectal tissue (P=0.042, R²=0.88). The bioavailability of dolutegravir is unknown, but the study that detected low dolutegravir concentrations in rectal tissue also found <15% drug in the rectal mucosal fluid compared with rectal tissue, suggesting that there would be minimal contamination issues. Knowing this, and considering the small dose size of dolutegravir (50 mg), the drug has been included in the plot using an estimated 85% bioavailability. Maraviroc was not included in the plot as its low bioavailability (25%) is known to be metabolism-related and is not due to poor absorption, evidenced by the low amount of radiolabelled parent drug collected in faeces following oral administration (25% of total administered drug). Both darunavir and maraviroc undergo high CYP3A4-mediated first-pass metabolism and this may explain why the bioavailability of these drugs is not a good indicator of rectal tissue exposure. For these highly metabolized drugs, it can be hypothesized that a factor that could give a better correlation to rectal tissue exposure would be the fraction of drug absorbed, which is the fraction leaving the luminal fluid and entering the epithelial cells of the intestine.

The concentration of raltegravir in tissues required to prevent initial infection in vivo is not known. This value is likely to differ depending on multiple factors, such as the site of infection and the initial amount of HIV introduced. Therefore, it is difficult to decide on a potential target concentration for raltegravir and other antiretrovirals for use in PreP. However, the IC₉₀ of raltegravir in 50% human serum ex vivo is 15 ng/mL and this is often used as a surrogate concentration target for PreP studies in the absence of...
clinical data. Furthermore, studies in a humanized mouse model have investigated the use of raltegravir to prevent initial infection via the rectal route and this study could be combined with a study linking raltegravir concentrations in the intestine with PreP success in rodents.18

Despite the apparent association seen in Figure 4, it should be acknowledged that drug association with rectal tissue is likely to occur via multiple factors. Tenofovir has been shown to be an effective drug for PreP, probably due to the mechanism of drug action where the active form of the drug is phosphorylated and trapped inside tissues, including the intestine.19 It should also be acknowledged that the investigations in this study have not directly measured raltegravir concentrations in the GALT and have instead used complete intestinal tissue concentrations as a surrogate. This has also been the approach in previous antiretroviral intestinal tissue concentration investigations undertaken in humans. There is not a complete understanding of the relationship between concentrations of antiretrovirals in the GALT and whole intestinal tissue and this is an area that requires further investigation if drug concentrations in whole intestine are to be used to determine sufficient drug exposure in the GALT.

Considering the importance of the GALT for both virus eradication and for PreP, there is a current need for a rational, methodological approach for the selection and design of antiretrovirals able to protect the GALT from infection. The methods described here could be combined with clinical investigations to provide a complete strategy for this selection. These data underscore the importance of washing tissue from clinical studies to limit the contamination with intestinal contents, especially for poorly absorbed drugs such as raltegravir.

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**Transparency declaration**

None to declare.

**References**


