Cell disposition of raltegravir and newer antiretrovirals in HIV-infected patients: high inter-individual variability in raltegravir cellular penetration

Aurélie Fayet Mello1, Thierry Buclin1, Claudia Franc2, Sara Colombo3, Sandra Cruchon1, Nicole Guignard1, Jérôme Biollaz1, Amalio Telenti3, Laurent A. Decosterd1*† and Matthias Cavassini2†

1Division of Clinical Pharmacology and Toxicology, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland; 2Service of Infectious Diseases, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland; 3Institute of Microbiology, Centre Hospitalier Universitaire Vaudois and University of Lausanne, Lausanne, Switzerland

*Corresponding author. Laboratory of Clinical Pharmacology, BH 18-218, University Hospital CHUV, 1011 Lausanne, Switzerland. Tel: +41-21-314-42-72; Fax: +41-21-314-80-98; E-mail: laurentarthur.decosterd@chuv.ch
†Both authors contributed equally to the study.

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Objectives: The site of pharmacological activity of raltegravir is intracellular. Our aim was to determine the extent of raltegravir cellular penetration and whether raltegravir total plasma concentration ($C_{\text{tot}}$) predicts cellular concentration ($C_{\text{cell}}$).

Methods: Open-label, prospective, pharmacokinetic study on HIV-infected patients on a stable raltegravir-containing regimen. Plasma and peripheral blood mononuclear cells were simultaneously collected during a 12 h dosing interval after drug intake. $C_{\text{tot}}$ and $C_{\text{cell}}$ of raltegravir, darunavir, etravirine, maraviroc and ritonavir were measured by liquid chromatography coupled to tandem mass spectrometry after protein precipitation. Longitudinal mixed effects analysis was applied to the $C_{\text{cell}}/C_{\text{tot}}$ ratio.

Results: Ten HIV-infected patients were included. The geometric mean (GM) raltegravir total plasma maximum concentration ($C_{\text{max}}$), minimum concentration ($C_{\text{min}}$) and area under the time–concentration curve from 0–12 h (AUC$_{0-12}$) were 1068 ng/mL, 51.1 ng/mL and 4171 ng·h/mL, respectively. GM raltegravir cellular $C_{\text{max}}$, $C_{\text{min}}$ and AUC$_{0-12}$ were 27.5 ng/mL, 2.9 ng/mL and 165 ng·h/mL, respectively. Raltegravir $C_{\text{cell}}$ corresponded to 5.3% of $C_{\text{tot}}$ measured simultaneously. Both concentrations fluctuate in parallel, with $C_{\text{cell}}/C_{\text{tot}}$ ratios remaining fairly constant for each patient without a significant time-related trend over the dosing interval. The AUC$_{\text{cell}}$/AUC$_{\text{tot}}$ GM ratios for raltegravir, darunavir and etravirine were 0.039, 0.14 and 1.55, respectively.

Conclusions: Raltegravir $C_{\text{cell}}$ correlated with $C_{\text{tot}}$ ($r=0.86$). Raltegravir penetration into cells is low overall (~5% of plasma levels), with distinct raltegravir cellular penetration varying by as much as 15-fold between patients. The importance of this finding in the context of development of resistance to integrase inhibitors needs to be further investigated.

Keywords: integrase inhibitor, cellular concentration, etravirine, maraviroc, darunavir, pharmacokinetics

Introduction

Raltegravir has demonstrated potent antiviral activity with so far a favourable safety profile.1–6 It is associated with a much more rapid reduction in viral load than other antiretroviral drugs (ARVs) in treatment-naïve patients,7 and has performed much better than anticipated, even in patients with limited options who have subtherapeutic raltegravir plasma concentrations.8 Raltegravir is characterized by large intra-individual (122%) and inter-individual (212%) pharmacokinetic variability,9 which may be related to food composition and intake,10 pH-dependent solubility (with higher solubility at increasing pH),11 and polymorphism of the UDP-glucuronosyl-transferases (UGTs).12 and, as a substrate of P-glycoprotein (P-gp), may possibly be influenced by P-gp expression level.13,14 Given the high pharmacokinetic variability and favourable safety profile at the recommended dose of 400 mg twice daily, relationships between raltegravir plasma exposure and virological response or toxicity have been so far difficult to establish. No consistent pharmacokinetic/pharmacodynamic relationships have been evidenced so far using raltegravir.

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concentration in plasma, suggesting that other markers of raltegravir exposure may better predict its pharmacological activity in patients. Raltegravir exerts its antiviral activity within the infected cells. However, there is at present very limited information on raltegravir penetration into cells, the extent of raltegravir cellular exposure and the relationships between raltegravir concentrations measured in vivo in plasma and those in peripheral blood mononuclear cells (PBMCs) from HIV patients.

We therefore initiated a pilot, prospective, pharmacokinetic study to determine over one dosing interval (12 h) raltegravir concentrations simultaneously in plasma and PBMCs in HIV-infected patients on stable raltegravir-containing regimens. Our aims were to assess whether raltegravir plasma concentrations reflect cellular levels, and to determine the overall raltegravir cellular disposition, its intra- and inter-patient variability and its correlation with plasma concentrations. Moreover, cellular disposition of etravirine, maraviroc, darunavir and ritonavir were similarly determined in cells and in plasma from patients receiving those drugs in combination with raltegravir.

Methods

Study design

This was an open-label, prospective, pharmacokinetic study on HIV-infected patients on a stable salvage raltegravir-containing regimen. The study was performed in accordance with the Declaration of Helsinki and its amendments, and in compliance with guidelines of good clinical practice.

Patients were admitted to the hospital at 7.15 a.m., 45 min before the time of their morning dose of raltegravir. They received a standardized breakfast at 7.45 a.m. before drug intake, and standardized lunch and dinner at 12 a.m. and 7 p.m., respectively. For C_{tot} determination, six blood samples (5 mL; Monovettes® with EDTA-K; Sarstedt, Nümbrecht, Germany) were collected starting at pre-dose (time = 0 h) and 1, 3, 6, 8 and 12 h after oral administration of the drugs (time 0 h). Additional blood samples (8 mL; Vacutainer® CPTTM cell preparation tubes with sodium citrate; Becton Dickinson, Franklin Lakes, NJ, USA) were collected at 0.5 h and 3, 8 and 12 h post-dose for C_{cell} determination. Patients were also invited to report the exact date and time of the last three raltegravir doses, the composition of the accompanying meal and the exact date and time of the last dose of all other drugs. The following laboratory measurements were performed at pre-dose: viral load; CD4 cell count; serum alanine and aspartate aminotransferases; γ-glutamyltransferase; amylases; alkaline phosphatase; total and direct bilirubin; cholesterol; high- and low-density lipoprotein; triglycerides; total magnesium; total calcium; albumin; creatine kinase; creatinine; full blood cell count; and pregnancy test.

Patients

The study protocol and informed consent form were approved by the local Ethics Committee of the University Hospital. Written informed consent was obtained from all patients. HIV-infected adults were eligible for inclusion if they had been on a stable raltegravir-containing antiretroviral regimen for at least 3 weeks. Pregnant or breastfeeding women were not eligible.

Materials

The solvents used for chromatography and all other chemicals were of analytical grade and used as received. Ultrapure water was obtained from a Milli-Q® UF-Plus apparatus (Millipore Corp., Burlington, MA, USA).

PBS solution was obtained from Sigma–Aldrich (Schnelldorf, Germany). RPMI 1640/25 mM L-glutamine medium and heat-inactivated fetal bovine serum (FBS) was used for the isolation of PBMCs from depleucyoylation filters for the preparation of matrix (see below) were obtained from Invitrogen (Basel, Switzerland). Ficoll Separating Solution for the separation of blank PBMCs from other cells was obtained from Biochrom AG (Berlin, Germany).

Total plasma and cellular concentrations

Raltegravir, darunavir, maraviroc, etravirine and ritonavir C_{tot} were measured by HPLC coupled to tandem mass spectrometry (LC-MS/MS; TSQ Quantum Ion Max; Thermo Fisher Scientific, Waltham, MA, USA) using our validated method. Limits of quantification for C_{tot} in plasma were 12.5 ng/mL for raltegravir, 25 ng/mL for darunavir, 10 ng/mL for etravirine, 2.5 ng/mL for maraviroc and 5 ng/mL for ritonavir, which correspond to the lowest levels that could be confidently measured with a bias and CV% below ±20% (where CV stands for coefficient of variation). Cell isolation from patient whole blood was performed in a Class II biohazard hood, using gloves and long sleeves, according to our previously published method. In brief, PBMCs were isolated by density gradient centrifugation in an 8 mL CPT tube. After three successive washings of cells at +4 °C with PBS containing 5% FBS to eliminate any residual plasma absorbed onto the cell surface, the total PBMCs contained in the pellet were counted in an aliquot using a Coulter instrument (Cell-dyn® 3500R; Abbott AG, Baar, Switzerland). Of note, the addition of FBS to PBS washing solution was based on the recent guidelines promulgated by the T-Cell Workshop Committee, reporting that the addition of serum proteins up to 10% to washing buffer increases the PBMC collection yield. The washed cell pellets were stored at −20 °C until drug extraction (MeOH/H_2O 50:50). All CPT tubes were processed within 5 min after blood withdrawal, and the total time between blood sampling and washed cell freezing was 2 h. All sample processing was carried out under ice-cold conditions to prevent drug loss. C_{tot} was determined using an adaptation of our LC-MS/MS method according to the methodology previously described by our group. Briefly, calibration samples for cellular determination were prepared using matrix-matched samples containing white blood cells (10^6 cells) isolated from depleucyoylation filters obtained from the Hospital Transfusion Unit. On the day of the analysis, white blood cells were spiked with ARV solutions containing internal standard darunavir-d9 in MeOH/H_2O 50:50 to obtain calibration ranges of 0.025–80 ng/mL for each drug. A 200 μL volume of extracting solution containing darunavir-d9 in MeOH/H_2O 50:50 was added to each isolated patient PBMC pellet. The PBMC lysates were vortexed, sonicated for 30 min for cell lysis and extracted for 30 min on a planar vortexing–vibrating mixer. The extracts were then centrifuged at 14,000 rpm (20,000 g) for 10 min at 20 °C. A 200 μL volume of supernatant was introduced into a microvial and a 20 μL volume was injected into the LC-MS/MS apparatus for drug quantification. C_{cell} was expressed in ng/mL, according to PBMC count, and assuming a 0.4 μL cell volume.

Statistical analysis

The values of pharmacokinetic parameters of raltegravir, darunavir, etravirine, maraviroc, and ritonavir were calculated by non-compartmental pharmacokinetic methods. The maximum concentration observed in plasma (C_{max}), the time to the maximum concentration (T_{max}), the minimum concentration observed in plasma (C_{min}) and the trough (pre-dose) concentration (C_{trough}) were read from the plasma concentration–time curves. The AUC from 0–12 h (AUC_{0–12}) was calculated over one dosing interval using the linear trapezoid method for C_{tot} and C_{cell} data for each patient. The apparent plasma clearance (CL/F, where F is the bioavailability) was calculated by dividing the administered dose by the AUC. Additional pharmacokinetic parameters were also
## Table 1. Total and cellular pharmacokinetic parameters for raltegravir, darunavir, etravirine, maraviroc and ritonavir

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Continued
subject pharmacokinetic variability of ARVs. Significant for other drugs, consistent with the known inter-

Figure 1. Both concentrations fluctuate in parallel. CVs of pharmacokinetic parameters determined from observed C\text{tot}–time curve. Geometrical means (GMs) and CVs were calculated from the average and standard deviation of log values. Cellular penetration was expressed as the \( C_{\text{cell}}/C_{\text{tot}} \) ratio, based on the measurements at each available time-point where both \( C_{\text{cell}} \) and \( C_{\text{tot}} \) were simultaneously determined. Changes in cellular penetration over the dosing interval were investigated using ratio values calculated at different times. The correlation between \( C_{\text{tot}} \) and \( C_{\text{cell}} \) was assessed by longitudinal mixed effects analysis.

Results

Patients

Ten patients were enrolled, all of whom had been on stable raltegravir-containing regimens (400 mg twice daily) for at least 3 weeks. Seven patients were male (70%) and all were Caucasian. Median age was 51 years (range 41–67 years) and median CD4 cell count was 368 cells/mm\(^3\) (range 239–839 cells/mm\(^3\)). Most (9/10) patients had an undetectable viral load (<40 copies/mL) while in one patient the viral load was 68 copies/mL. Co-administered ARVs included darunavir/ritonavir 600/100 mg twice daily (n=6), etravirine 200 mg twice daily (n=4), maraviroc 150 mg twice daily (n=2), efavirenz 600 mg once daily (n=1), atazanavir 400 mg once daily (n=1), plus emtricitabine/tenofovir (n=5), lamivudine (n=4) or abacavir (n=1).

Pharmacokinetic characteristics

Raltegravir, darunavir, etravirine, maraviroc and ritonavir pharmacokinetic parameters determined from observed \( C_{\text{tot}} \) and \( C_{\text{cell}} \) values are summarized in Table 1, and the GM plasma and cellular concentrations versus time profiles are shown in Figure 1. Both concentrations fluctuate in parallel. CVs of pharmacokinetic parameters were considerable for raltegravir, and significant for other drugs, consistent with the known inter-subject pharmacokinetic variability of ARVs.

Correlation between cellular and plasma concentrations

Figure 2 shows the log–log linear correlations between \( C_{\text{tot}} \) and \( C_{\text{cell}} \) for each drug. Good correlations were obtained for raltegravir (r=0.86) and maraviroc (r=0.96) with a slope of the \( C_{\text{cell}}/C_{\text{tot}} \) plot of 0.94 and 0.78, respectively. Correlations were moderate for darunavir (r=0.69) and ritonavir (r=0.44), and poor for etravirine (r=0.26) with a corresponding slope of 1.69, 0.45 and 0.34, respectively.

Cellular penetration

Raltegravir \( C_{\text{cell}} \) (GM) corresponds to 5.3% of \( C_{\text{tot}} \) measured simultaneously, with large inter-patient variability (range 1.3%–19.6%). Figure 3 shows the \( C_{\text{cell}}/C_{\text{tot}} \) ratios measured for raltegravir for each patient at different times after dose administration. \( C_{\text{cell}}/C_{\text{tot}} \) ratios varied significantly within each patient (CV intra-patient=83%); however, without a significant time-related trend over the dosing interval. \( C_{\text{cell}}/C_{\text{tot}} \) ratios varied to a larger extent between patients (CV inter-patient=96%; one-way ANOVA on log; P<0.001, \( r^2=0.62 \), highlighting the distinct cellular penetration of raltegravir in each patient. Raltegravir \( C_{\text{cell}}/C_{\text{tot}} \) ratios were not influenced by the presence of other ARVs; 0.044 without versus 0.059 with co-medication with darunavir/ritonavir (P=0.57), 0.051 without versus 0.055 with co-medication with etravirine (P=0.89) and 0.050 without versus 0.065 with co-medication with maraviroc (P=0.66). However, due to the limited number of patients, and the high amount of variability, this investigation would have been powered only to detect an effect size corresponding to a factor of 3.

AUC\text{cell} corresponds to 3.9% (range: 0.7%–17.7%), 14% (7%–38%), 155% (82%–552%), 274% (260%–280%) and 182% (85%–534%) of AUC\text{tot} for raltegravir, darunavir, etravirine, maraviroc and ritonavir, respectively (Table 1).

Discussion

Intracellular concentrations of ARVs are most likely a result of passive transport and active uptake and efflux from cells. To date, there has been limited information on the extent of raltegravir \( C_{\text{cell}} \), the impact of co-medications and whether raltegravir \( C_{\text{tot}} \) predicts \( C_{\text{cell}} \). The first study published in that field reported no measurable concentrations of raltegravir in cells (i.e. below the limit of quantification of 1 ng/mL of their assay), an unlikely finding for a drug expected to act intracellularly. Conversely, a \( C_{\text{cell}}/C_{\text{tot}} \) ratio for raltegravir of ~10%, also with large variability, was recently published in a small group of patients (n=5), using a once-a-day raltegravir regimen. In the present study, we confirmed results from Moltó et al., in a patient group of double the size. Further, we expanded our study by examining the importance of inter-individual variability in cellular penetration of raltegravir and other new ARVs.
We observed that plasma measurements are adequate predictors ($r=0.86$) of the cellular levels of raltegravir, without noticeable influence of co-administered ARVs. Drug interaction studies with darunavir, etravirine and maraviroc have shown a modest, not clinically significant, decrease of $\sim 30\%$ in raltegravir plasma exposure. This should not affect cell/plasma ratio values because the slope close to unity for raltegravir indicates that variations in plasma concentrations tend to translate into similar changes in cellular levels. For darunavir, the slope was 1.69 and the correlation was less precise ($r=0.69, n=22$). Etravirine $C_{\text{tot}}$ only moderately reflects the highly variable cellular levels, suggesting that alternative factors (uptake transporters or efflux transporters other than MDR1, etravirine being not a P-gp substrate$^{24}$ may modulate etravirine cellular penetration. As maraviroc is bound into transmembrane helices of its target CCR5,$^{25}$ the membrane-bound drug is analysed during measurement in PBMC lysates. We found an excellent correlation ($r=0.96$) between plasma and cellular concentrations of maraviroc, although the number of maraviroc data in our study were limited.

The GM cellular penetration ratios obtained for darunavir and etravirine were 0.09 and 1.29, respectively, using concentration ratios, and are therefore not in agreement with $C_{\text{cell}}/C_{\text{tot}}$ values of 1.32 and 12.9 previously published for darunavir and raltegravir.

![Figure 1. Total and cellular GM concentrations. RAL, raltegravir; DRV, darunavir; ETV, etravirine; MVC, maraviroc; RTV, ritonavir. Filled and open circles represent total and cellular GM concentrations (geometric SD), respectively.](image)
eteravirine, respectively. In the latter study by ter Heine et al., the plasma used for the determination of total drug concentrations was collected directly from Vacutainer® CPT tubes. These Vacutainer® CPT tubes contain liquid components that dilute the plasma phase, which in turn will result in lower total plasma concentrations, and hence spuriously high \( C_{\text{cell}}/C_{\text{tot}} \) values. This issue has been verified in our laboratory in a separate set of patients’ analyses; raltegravir total plasma concentrations (as well as those of other ARVs) measured in plasma collected from Vacutainer® CPT tubes were 20%–30% lower than those determined in parallel directly in EDTA tubes (data not shown). In fact, total plasma levels (and \( C_{\text{max}} \) values) reported in the ter Heine et al. study were low overall, and were consistently below those previously published for raltegravir,1,3,9 darunavir,27–30 etravirine24,29,31 and ritonavir.30,32 In our study, the penetration ratio values determined in parallel for ritonavir in the same cell pellets (i.e. 1.80, range 0.48–7.91) are in good agreement with known values previously published for this drug.17,33,34 Overall, the limited concordance for some cellular concentration values may be explained to some extent by differences in the methodologies applied for the isolation/washing of the PBMCs from blood (ter Heine et al.,20 Moltó et al.14 and the present study). The harmonization of cell isolation methods prior to intracellular drug measurement would be welcome and ideally would be based on recently published guidance.18

Our study shows that raltegravir cellular penetration is generally low (≏5% of plasma levels), and that each patient exhibited a distinct cellular penetration for raltegravir with \( C_{\text{cell}}/C_{\text{tot}} \) ratios ranging from 0.013 to 0.196, a 15-fold difference. This variability

\[ r = 0.86 \quad P < 0.0001 \quad n = 37 \]

\[ r = 0.69 \quad P = 0.0004 \quad n = 22 \]

\[ r = 0.26 \quad P = 0.34 \quad n = 15 \]

\[ r = 0.96 \quad P = 0.0001 \quad n = 8 \]

\[ r = 0.69 \quad P = 0.0004 \quad n = 22 \]

\[ r = 0.44 \quad P = 0.04 \quad n = 22 \]

\[ r = 0.26 \quad P = 0.34 \quad n = 15 \]

\[ r = 0.96 \quad P = 0.0001 \quad n = 8 \]

\[ r = 0.44 \quad P = 0.04 \quad n = 22 \]

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\[ r = 0.44 \quad P = 0.04 \quad n = 22 \]
Raltegravir cell penetration

Figure 3. Individual raltegravir C_{cell}/C_{tot} ratios measured in the 10 patients at different times after dose intake.

cannot be accounted for by a limitation of analytical performance (precision of 9.3% at the lowest calibrator of 0.025 ng/mL raltegravir in PBMC lysates) or by other confounding factors (notably cell isolation procedures) likely to affect measurements; darunavir, ritonavir and etravirine, simultaneously determined in the same cell pellets, had cellular penetration ratio variability differing by only 4-fold (n=6 patients), 5-fold (n=6) and 3.7-fold (n=4), respectively. The 15-fold inter-individual variability in raltegravir C_{cell}/C_{tot} ratios suggests therefore that alternative factors (transporters or intracellular metabolism) modulate cellular levels of raltegravir.

So far, because of the small number of patients exhibiting virological failure in clinical trials on raltegravir, pharmacokinetic/pharmacodynamic studies have had limited success in finding a clear relationship between clinical response and raltegravir exposure determined in plasma. Among the few patients who exhibited incomplete viral suppression on an integrase inhibitor-based regimen, most had no genotypic or phenotypic resistance to integrase inhibitor during early virological failure. Resistance emerged only in patients who remained on an integrase inhibitor despite detectable viraemia. In those failing patients, raltegravir concentrations in plasma were either not measured or, in another study, were found not to be predictive of virological failure, even though most failure cases occurred in patients with low raltegravir plasma concentrations.

In fact, the recent report published by Merck, on the initial results of their Phase III study of Isentress investigational once-daily dosing in treatment-naive HIV-infected adults, has shown that raltegravir 800 mg once daily is less effective (~5.7%) than the approved 400 mg twice-daily dose, and the difference in treatment response is primarily observed in patients with high viral load and lower drug levels (in the once-daily arm). For the first time, raltegravir pharmacokinetics was found to influence virological response. This should therefore stimulate further clinical pharmacokinetic/pharmacodynamic studies on raltegravir and the latest ARVs.

In our study, although limited to virologically controlled HIV patients (9 out of 10 patients), cellular penetration of raltegravir was found to be low and characterized by significant inter-patient variability. Raltegravir cellular penetration in the single patient for whom viral load was not fully suppressed (68 copies/mL, patient 8) did not differ from that in the other patients studied. It is not known at present whether in failing patients diminished raltegravir exposure at the expected site of antiviral action may have direct implications regarding incomplete viral suppression and long-term treatment effectiveness. However, chronic, suboptimal cellular exposure to raltegravir may in theory permit continuing viral evolution and the progressive emergence of raltegravir resistance. This is certainly a relevant issue given the almost 10-fold variability in the effective concentration inhibiting 95% of viral replication (EC_{95}; i.e. 2.7–22.2 ng/ml; 6–50 nM) of raltegravir found in vitro for clinical isolates from HIV-1 patients’ PBMCs.

In vitro studies have shown that the binding of raltegravir to the preintegration complex (PIC) is essentially irreversible, because the ‘off rate’ (the rate at which raltegravir dissociates from the PIC) is longer than the half-life of the complex. Once binding to the PIC occurs, removing the remaining raltegravir from culture does not diminish efficacy in vitro. It has been claimed therefore that raltegravir concentrations measured in patient plasma may be irrelevant as long as all intracellular PICs are bound. However, in studies on raltegravir effects on viral dynamics in patients, the unexpected second-phase HIV decay by an integrase inhibitor—not supposed to influence viral production from infected long-lived cells—was explained by the effect of raltegravir on both long-lived infected cells and latently infected cells with unintegrated virus. Since total HIV DNA exceeds integrated HIV DNA in resting CD4 T cells by 100-fold, it is therefore critical that raltegravir concentration in cells remains sufficiently therapeutic to effectively block new productive infection upon activation of long-lived unintegrated HIV DNA.

Thus, our study suggests that further investigations of raltegravir cellular disposition are needed in the poorly defined subset of patients in whom raltegravir fails to fully suppress viral replication down to <400 copies/mL, to investigate the relationships between raltegravir cellular penetration in cells and to study the possible constraints that may restrict raltegravir availability to its cellular target and its impact on the levels of both integrated and unintegrated HIV DNA in resting CD4 cells. More generally, because of the original action of raltegravir, further investigations into not only cellular but also tissue distribution of raltegravir in various body compartments are warranted, especially in the context of the recent trials of raltegravir intensification to reduce low-level HIV replication in plasma and gut. So far, these attempts have been of limited success, suggesting that residual viraemia is primarily due to HIV release from stable reservoirs (latently infected resting CD4 memory cells and other long-lived cells), but may possibly also arise from some cellular or tissue compartments (i.e. ileum) with ongoing low-level replication, for which raltegravir would have limited—and variable—penetration.

The present study is among the first to provide data on cellular concentrations of raltegravir and maraviroc, but may have some limitations: firstly, the limitation of the pilot study size; and secondly, the measurements in total PBMCs may only grossly reflect drug penetration in specific target cell populations, such as CD4 T lymphocytes where HIV replicates. Third, the cell volume used for C_{cell} calculation in our study (0.4 pL) may be somewhat overestimated, as recently reported by Simiele et al., who found a lower and more variable volume for PBMCs of between 0.23 and 0.34 pL. C_{cell} values reported in our study are therefore conservative; if these recent data on cell
volume are confirmed, higher \( C_{cell} \) values would be expected. Finally, raltegravir may be variably embedded in membrane lipid bilayers, complexed to cytoplasmic proteins or sequestered through intracellular protein binding, as for HIV protease inhibitors, so that only a small fraction of the measured cellular concentrations may remain available to exert the antiviral activity. Nevertheless, this cell-associated concentration remains the best marker of viral target exposure available at the cellular level. Obviously, drug penetration within cells is just one of the multiple factors that influence antiviral activity besides drug characteristics (intrinsic potency, affinity for intracellular components and pharmacological target), overall tissue distribution, virus characteristics (susceptibility and genotype) and host factors (genetic background).

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Author contributions

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