Intracellular accumulation of ritonavir combined with different protease inhibitors and correlations between concentrations in plasma and peripheral blood mononuclear cells.

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Intracellular accumulation of ritonavir combined with different protease inhibitors and correlations between concentrations in plasma and peripheral blood mononuclear cells

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Abstract

Objectives Ritonavir, used at low doses as a boosting agent of other protease inhibitors (PIs), is known to be associated with metabolic complications and gastrointestinal disturbances. The rate of accumulation of ritonavir within cells is still debated due to scarce data and methodological limitations. Therefore, our aim was to evaluate intracellular ritonavir penetration when used with different boosted PIs in the clinical setting.

Methods Patients administered with atazanavir/ritonavir (300/100 mg, once daily), darunavir/ritonavir [600/100 mg, twice daily (darunavir-600) and 800/100 mg, once daily (darunavir-800)], lopinavir/ritonavir (400/100 mg, twice daily) and tipranavir/ritonavir (500/200 mg, twice daily) were considered. Blood sampling at the end of the dosing interval (Ctrough) was performed. Peripheral blood mononuclear cell (PBMC)-associated and plasma ritonavir and PI concentrations were measured by validated HPLC methods. PBMC count and individual mean cell volume (MCV) were measured using a Coulter Counter instrument.

Results One hundred patients were enrolled. Frequencies of ritonavir-boosted PIs were atazanavir, 37%; darunavir-600, 23%; lopinavir, 19%; tipranavir, 13%; and darunavir-800, 8%. The median intracellular and plasma concentrations of ritonavir were 1279 ng/mL (IQR 727–2087) and 170 ng/mL (IQR 82–384), respectively, accounting for a cellular accumulation ratio of 7.69 (5.7–10.9). Significant differences in ritonavir intracellular concentrations emerged among different PIs ($P < 0.001$): specifically between darunavir-600 and atazanavir ($P < 0.001$), between darunavir-600 and tipranavir ($P = 0.009$), between atazanavir and lopinavir ($P < 0.001$) and between lopinavir and tipranavir ($P = 0.027$).

Conclusions Our study showed a higher rate of ritonavir intracellular accumulation than previously reported, possibly due to the more accurate calculation of intracellular concentrations by MCV. The ratio varied according to concomitantly administered PIs, suggesting their influence on the rate of ritonavir intracellular penetration.

Introduction

The advent of combination antiretroviral therapy revolutionized the care of HIV-infected patients, with profound impact on morbidity and mortality (1,2). Since the early randomized clinical trials of combination antiretroviral therapy just over a decade ago, over 20 agents have been approved to treat HIV-infected patients. Although treatment guidelines have evolved significantly over this time period, the use of protease inhibitors (PIs), and in particular the use of ritonavir-boosted PIs, has remained a mainstay of therapy (3,4).

Ritonavir boosting of PIs decreases the pill burden and frequency of dosing. Boosted PIs are recommended for first-line therapy and play a key role in the management of treatment-experienced patients. Potential disadvantages associated with ritonavir-boosted PIs include metabolic abnormalities (e.g. dyslipidaemia), increased cardiovascular risk and drug–drug interactions (5).

Moreover, treatment failure is multifactorial and includes viral resistance, poor adherence and pharmacological and host factors. Much interest has been generated by potential pharmacological mechanisms of failure. HIV replicates within cells; therefore, drugs must reach intracellular concentrations sufficient to fully inhibit viral replication. Failure to obtain such exposure may result in the establishment of sanctuary sites, where virus may evolve in the absence of selective pressure (6) or where subtherapeutic levels select drug-resistant viruses with subsequent 'seeding' into the plasma compartment. Viruses from sanctuary sites, such as the CNS and seminal fluid, can exhibit genotypic resistance profiles that differ from peripheral blood isolates (7,8). Pharmacological studies that examine the cellular and tissue penetration of HIV drugs are crucial to understand sanctuary sites and the subsequent evolution of drug resistance and the failure of antiretroviral therapy. This understanding may be useful for designing strategies to maximize drug potency and minimize toxicity. Specifically, the role of cellular efflux transporters (such as P-glycoprotein) in limiting the intracellular penetration of drugs and the potential for ritonavir to inhibit such mechanisms deserves further investigation (9).

Peripheral blood mononuclear cells (PBMCs) may be adequate substrates to measure the intracellular penetration of drugs and to investigate the impact of the intracellular accumulation ratio on drug efficacy or toxicity. Recent scientific evidences have shown a large variability in intracellular drug measurement; critical methodological factors have emerged such as the PBMC isolation method, the correct assessment of cell numbers and the correct use of mean cell volume (MCV) (10–12).

There are no currently available data comparing the intracellular accumulation of different PIs and ritonavir using the same laboratory methods.

In this report, we describe the intracellular concentrations reached by atazanavir, tipranavir, lopinavir, darunavir (both once and twice daily) and coadministered ritonavir using standardized methods in HIV-positive patients. Moreover, we investigated the intracellular/plasma concentration ratio and correlations.

Methods

Patients

HIV-positive patients treated with PI-based antiretroviral therapy at the University Hospital 'Amedeo di Savoia' in Turin, Italy, were enrolled. Sampling was performed after obtaining written informed consent in accordance with local Ethics Committee indications. The patients included were treated with darunavir/ritonavir [800/100 mg, once daily (darunavir-800)], darunavir/ritonavir [600/100 mg, twice daily (darunavir-600)], atazanavir/ritonavir (300/100 mg, once daily), lopinavir/ritonavir (400/100 mg, twice daily) and tipranavir/ritonavir (500/200 mg, twice daily).

The main inclusion criteria were no concomitant interacting drugs and self-reported adherence >95%. Patients with significant liver or renal impairment and other disorders were excluded from this study.

Measurement of plasma and PBMC antiretroviral concentrations

Blood samples were collected 12 h (±2), for twice-daily drugs, and 24 h (±2) after drug intake obtaining a trough concentration (Ctrough). Samples were collected in lithium heparin tubes (7 mL) and plasma, obtained after centrifugation at 1400 g for 10 min at +4°C, was stored at −20°C until analysis.

PBMCs were isolated and the cell number and MCV were determined as described previously.9–15

PBMC-associated and plasma ritonavir and PI concentrations were measured by validated HPLC-Mass spectrometry (11) and HPLC–photodiode array methods (16), respectively. The median value of individual measurements was considered.

Statistical analysis

For descriptive statistics, continuous variables were summarized as the median (IQR). Categorical variables were described as the frequency and percentage. All data were assessed for normality using a Shapiro–Wilk test and categorical data were compared using a Mann–Whitney or Kruskal–Wallis statistical test. To investigate continuous data, a Spearman rank correlation test was utilized. Statistical analyses were conducted using SPSS software package version 18.0 (Chicago, IL, USA).

Results

One hundred patients met the inclusion criteria and were included in this analysis. The frequencies of ritonavir-boosted PIs were atazanavir, 37%; darunavir-600, 23%; lopinavir, 19%; tipranavir, 13%; and darunavir-800, 8%. Patients were mainly male (69%); their median age and weight were 46 years (IQR 39–53) and 70 kg (IQR 59–78), respectively.

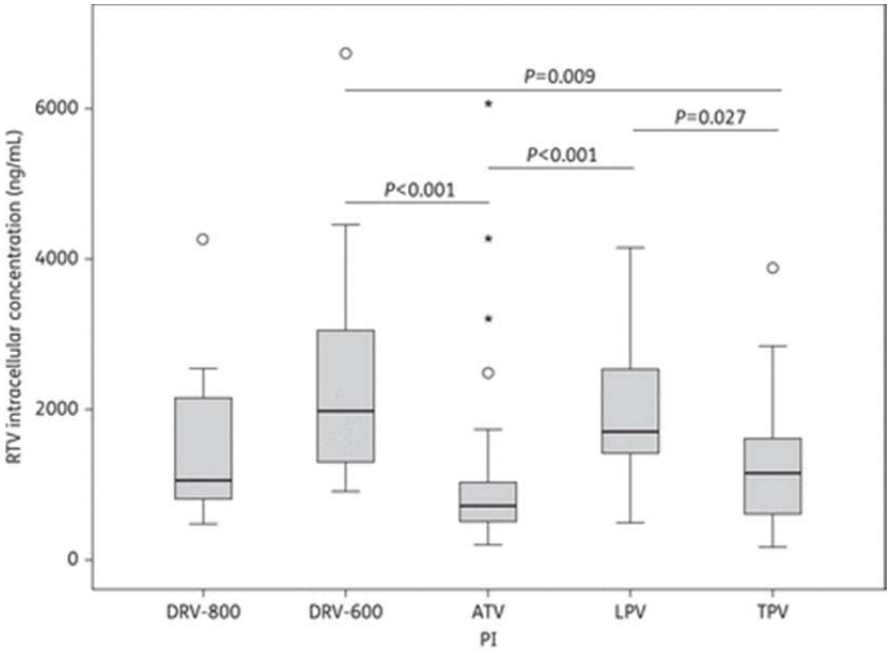
Intracellular and plasma ritonavir concentrations were 1279 ng/mL (IQR 727–2087) and 170 ng/mL (IQR 82–384), respectively. The ritonavir cellular accumulation ratio was 7.69 (IQR 5.7–10.9). As shown in Table 1, significant differences in the overall cellular accumulation ratios among patients treated with different PIs were observed ($P < 0.001$).

Table 1. Ritonavir, according to concomitant PIs, and PI plasma and intracellular concentrations and cellular accumulation ratios (IQR)

	Plasma (ng/mL)	Intracellular (ng/mL)	Cellular accumulation ratio
Ritonavir			
darunavir 800 mg ($n = 8$)	123 (97–182)	1044 (800–2341)	9.6 (7.5–12.1)
darunavir 600 mg ($n = 23$)	297 (153–457)	1974 (1279–3220)	7.6 (6.3–8.9)
atazanavir ($n = 37$)	75 (45–172)	716 (495–1153)	9.2 (6.0–12.7)
lopinavir ($n = 19$)	314 (156–439)	1698 (1397–2620)	6.1 (5.1–9.5)
tipranavir ($n = 13$)	244 (130–453)	1152 (618–1605)	5.0 (3.2–6.3)
PI			
darunavir 800 mg ($n = 8$)	3121 (1587–8804)	414 (140–2504)	0.22 (0.05–0.3)
darunavir 600 mg ($n = 23$)	3077 (2668–4663)	402 (235–654)	0.12 (0.06–0.3)
atazanavir ($n = 37$)	645 (454–1029)	1844 (949–3498)	2.44 (1.7–5.1)
lopinavir ($n = 19$)	7779 (5615–8658)	2340 (1301–4891)	0.35 (0.16–0.93)
tipranavir ($n = 13$)	37 862 (17 485–48 530)	5740 (2014–9839)	0.14 (0.13–0.18)

Significant differences were also observed in intracellular concentrations of ritonavir ($P < 0.001$) among the different PIs (Figure 1); subgroup analysis revealed significant differences in the cellular accumulation ratios between darunavir-600 and atazanavir ($P < 0.001$), darunavir-600 and tipranavir ($P = 0.009$), atazanavir and lopinavir ($P < 0.001$) and lopinavir and tipranavir ($P = 0.027$). Patients treated with atazanavir generally showed the lowest ritonavir intracellular concentrations [716 ng/mL (495–1153)].

Figure 1.



Ritonavir intracellular concentrations (ng/mL) according to concomitant PIs. Mild outliers (>1.5 × IQR from other values) are shown by open circles. Extreme outliers (>3 × IQR from other values) are shown by asterisks. Ritonavir, RTV; darunavir, DRV; atazanavir, ATV; lopinavir, LPV; tipranavir, TPV.

A correlation between the plasma and intracellular concentrations of ritonavir could be observed across the different groups ($\rho = 0.843$, $P < 0.001$). However, it should be noted that no correlation between plasma and intracellular concentrations emerged in patients receiving once-daily darunavir-800.

As summarized in Table 1, intracellular concentrations of the active PI varied between different groups ($P < 0.001$) and atazanavir was the only PI with a cellular accumulation ratio >1. Plasma and intracellular concentrations of the active PI were correlated for patients treated with darunavir-800 ($\rho = 0.738$, $P = 0.037$), atazanavir ($\rho = 0.544$, $P = 0.001$) and tipranavir ($\rho = 0.929$, $P < 0.001$). No correlations could be observed for patients treated with darunavir-600 or lopinavir.

Discussion

To date, intracellular drug levels have been poorly studied in vivo, due to methodological difficulties and the relatively large volumes of blood required. The study of the intracellular pharmacokinetics of HIV drugs is a key element to investigate the putative sanctuary sites where HIV may replicate with little selective pressure and it is crucial for eradication studies. However, stringent methodological procedures need to be applied and there is no standard technique for measuring intracellular exposure.

Our group recently proposed a method aimed at properly quantifying intracellular drugs using an MCV individualized for each patient (11,12). This method allowed us to accurately calculate the PBMC concentrations in ng/mL (instead of concentrations per PBMC number) and to compare them with plasma concentrations.

Hence, our aim was to describe the intracellular concentrations of atazanavir, tipranavir, lopinavir, darunavir (once and twice daily) and the related low-dose ritonavir with the same method (11,12).

If we exclude atazanavir, which seems to accumulate intracellularly, other PIs had significantly lower intracellular concentrations than plasma concentrations (3–10-fold). Atazanavir, tipranavir and darunavir-800 intracellular concentrations correlated with their plasma concentrations, supporting the measurement of plasma exposure as a surrogate for exposure at the site of action.

The relationship between plasma and intracellular concentrations is statistically significant for ritonavir in all groups considered (excluding the darunavir-800 group, probably due to the small number of patients). Furthermore, ritonavir tends to accumulate within PBMCs, with high intracellular/plasma ratios (ranging from 5.0 to 9.6). This accumulation phenomenon is interesting and it is high for all patients considered.

Ritonavir intracellular concentrations in patients concomitantly treated with atazanavir were the lowest observed (716 ng/mL, IQR 495–1153). The highest intracellular concentrations were observed in the darunavir-600 (1974 ng/mL) and lopinavir groups (1698 ng/mL). Ritonavir intracellular penetration was low in patients coadministered with tipranavir, even if a 200 mg twice-daily dose was administered (1152 ng/mL, IQR 618–1605).

The reasons for the observed differences in the intracellular concentrations require further mechanistic investigation. One could hypothesize that intracellular ritonavir concentrations are dependent upon transport proteins in PBMCs and variability may be related to competition between ritonavir and concomitant medications.

Since ritonavir is associated with lipid disorders and intestinal problems, it is possible that intracellular concentrations may impact toxicity and efficacy. In the past, ritonavir, administered full dose as an active PI, was an extremely potent drug against HIV. Therefore, in our opinion, a question to be explored is if ritonavir, as a booster, could display direct antiviral activity against HIV (perhaps in synergy with the PI). The efficacy of PIs combined with new boosters that lack antiviral activity will probably suggest an answer to this question.

In conclusion, our study shows a higher ritonavir intracellular accumulation rate than previously reported (9,13,15); this is probably explained by the accurate calculation of intracellular concentrations by using a personalized MCV. This intracellular ratio seems to vary according to the concomitantly administered PI, suggesting their influence on the rate of ritonavir intracellular penetration (darunavir-800 > atazanavir > darunavir-600 > lopinavir > tipranavir). Atazanavir was the drug with the highest intracellular accumulation ratio (2.4, IQR 1.7–5.1), while other PIs showed values <1. Further clinical studies are warranted in order to elucidate interindividual differences and the clinical implications of ritonavir intracellular exposure.

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