Intracellular and plasma pharmacokinetics of 400 mg of etravirine once daily versus 200 mg of etravirine twice daily in HIV-infected patients

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Objectives: To compare intracellular and plasma etravirine concentrations when etravirine was given at 200 mg/12 h versus 400 mg/24 h and to evaluate whether the results would support once-daily dosing.

Methods: This was an open-label sequential study in which eight patients on protease inhibitor (PI)-sparing regimens containing etravirine were included. Full pharmacokinetic profiles were performed while on 200 mg of etravirine/12 h and after switching to 400 mg of etravirine/24 h. Intracellular and plasma levels were determined by liquid chromatography coupled with mass spectrometry. Pharmacokinetic parameters were calculated by non-compartmental analysis and compared by geometric mean ratios (GMRs) using 200 mg of etravirine/12 h as the reference group. Trial registration: ClinicalTrials.gov NCT01121809.

Results: The geometric mean (GM) for etravirine AUC0–t (5602 versus 5076 ng.h/mL, GMR 0.91), Cmax (403 versus 495 ng/mL, GMR 1.23) and Cmin (139 versus 102 ng/mL, GMR 0.74) were similar with both dosing schedules at the intracellular level. In plasma, the GMRs for AUC0–t, Cmax and Cmin were 1.31, 1.76 and 0.99, respectively. The mean intracellular penetration, evaluated as intracellular and plasma AUC0–t ratios, was 81% when etravirine was dosed twice daily and 56% with once-daily dosing.

Conclusions: Our results show that intracellular etravirine levels were similar with both dosing regimens in patients with PI-sparing regimens, while etravirine plasma AUC0–t and Cmax ratios were 30% and 76% higher with the once-daily regimen, respectively. Thus, a once-daily dosing regimen is supported not only by plasma etravirine pharmacokinetic profiles but also by intracellular levels.

Keywords: antiretroviral treatment, non-nucleoside reverse transcriptase inhibitors, liquid chromatography, mass spectrometry

Introduction

Etravirine is a non-nucleoside reverse transcriptase inhibitor (NNRTI) incorporated into the antiretroviral arsenal for salvage therapy in experienced patients with a recommended dose of 200 mg twice daily.1–3 Although the long terminal elimination half-life of etravirine made it suitable for once-daily dosing, the high pill burden needed with the initial formulations hindered this regimen. Administering etravirine once daily would improve compliance and efficacy as there is an inverse relationship between adherence and the number of daily doses prescribed.4,5 Although several studies have evaluated the plasma pharmacokinetics of etravirine administered at 400 mg once daily,5–8 there are no available data on intracellular etravirine levels with this dose. The aim of our study was therefore to compare the intracellular and plasma etravirine concentrations when given at 400 mg/24 h versus 200 mg/12 h.

Methods

Study design

This was an open-label, sequential study in which eight adult HIV-infected subjects with stable antiretroviral treatment containing 200 mg of etravirine/12 h for at least 3 months and HIV RNA <50 copies/mL were enrolled after providing written consent. Exclusion criteria were pregnancy, the presence of diarrhea and concomitant use of drugs or non-prescription traditional or herbal medications with potential interactions with etravirine pharmacokinetics.6 Patients were admitted to the hospital in the morning, and blood samples were obtained before and at 1, 2, 3, 4, 6, 8, 10 and 12 h after...
the supervised etravirine intake in the fasting state. The etravirine regimen was then changed to 400 mg once daily and full 24 h pharmacokinetic profiles were determined a week later with samples drawn before and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 h after etravirine intake. The study was conducted according to the Declaration of Helsinki guidelines, approved by the Spanish Agency for Medicines and Healthcare Products and registered at ClinicalTrials.gov (NCT01121809).

**Analytical method**

Blood samples were collected into cell preparation tubes (CPTs; Becton Dickinson Vacutainer®) and EDTA tubes. Within 1 h after collection, the tubes were centrifuged at 1500 g for 20 min at room temperature. Plasma was transferred to cryotubes and stored at −80°C until analysis. The cell layer from the CPT was transferred to 15 mL Falcon tubes, diluted with 0.9% NaCl to a total volume of 10 mL and centrifuged at 1500 g for 10 min at 4°C. The cell pellet was transferred to Eppendorf tubes and washed with 1.5 mL of 0.9% NaCl. Once centrifuged, the supernatant was aspirated and the cell pellet was weighed and stored at −80°C until analysis. For etravirine intracellular concentrations, peripheral blood mononuclear cell aliquots were weighed and their volume was calculated as volume = weight/density. Since the density of mononuclear cells is 1.077 and that of plasma 1.030, the weight of the aliquots was equalized with their volume. Plasma and intracellular concentrations were determined after drug extraction by liquid chromatography coupled with mass spectrometry using adaptations of methods previously reported. The accuracy and precision were 100 ± 15% and <10%, respectively. Standard curves were highly linear over the range 10–10000 ng/mL for plasma and intracellular levels.

**Pharmacokinetic and statistical analysis**

The pharmacokinetic parameters for each individual were calculated by non-compartmental analysis (WinNonlin software; Pharsight, Mountain View, CA, USA). These parameters were summarized as geometric means (GMs) and were compared between days 0 and 7 as geometric mean ratios (GMRs) and their 90% CIs using 200 mg of etravirine/12 h as the reference group. The differences in pharmacokinetic parameters between the regimens were considered significant when the interval between the low and high 90% CI did not include the value 1.0. Inter-individual variability in etravirine concentrations was assessed by measuring the coefficient of variation. The correlations between plasma and intracellular concentrations were assessed as Spearman’s correlation coefficients. Statistical calculations were performed with the Statistical Product and Service Solutions for Windows (version 15.0, SPSS, Chicago, IL, USA).

**Results**

The trial enrolled eight HIV-1-infected patients (four males and four females) with a median age and body mass index of 44 years (range 28–62) and 24.5 kg/m² (range 19.5–30.4), respectively. All patients had an undetectable viral load, a CD4 cell count of 598 cells/mm³ (range 177–993), a median CD4 cell count of 598 cells/mm³ (range 177–993), a normal renal function and no clinical signs of liver impairment, with only one of them suffering from chronic hepatitis C genotype 1. Concomitant drugs were tenofovir and emtricitabine (n=5), tenofovir and zidovudine (n=2) and raltegravir (n=1). Both dosing regimens were well tolerated and pharmacokinetic profiles were obtained for both regimens for all patients (Figure 1).

At the intracellular level, the GMs for etravirine \( AUC_{0-\text{\textmin}} \), \( C_{\text{\textmax}} \), and \( C_{\text{\textmin}} \) were similar with both dosing schedules (Table 1).

However, the individual values for \( AUC_{0-\text{\textmax}} \), \( C_{\text{\textmax}} \), and \( C_{\text{\textmin}} \) were lower in four, three and five patients, respectively, with the 400 mg once-daily regimen. The mean intracellular penetration, evaluated as intracellular and plasma \( AUC_{0-\text{\textmax}} \) ratios, was 81% (90% CI 0.65–1.06) with etravirine dosed every 12 h and 56% (90% CI 0.46–0.67) with the once-daily dosing regimen. Regarding plasma etravirine pharmacokinetic parameters, the 400 mg/24 h regimen gave rise to a 30% higher \( AUC_{0-\text{\textmax}} \) and a 76% higher \( C_{\text{\textmax}} \) when compared with the 200 mg/12 h dose. In contrast, \( C_{\text{\textmin}} \) values were similar in both dosing regimens, although lower \( C_{\text{\textmin}} \) was observed in three out of eight patients with etravirine dosed at 400 mg/24 h (Table 1). Furthermore, the elimination half-lives from both plasma and intracellular compartments were higher with the 400 mg/24 h regimen.

There were highly linear relationships between plasma etravirine \( AUC_{0-\text{\textmax}} \) and both \( C_{\text{\textmax}} \) and \( C_{\text{\textmin}} \) (r=0.985; P=0.000) with the 200 mg/12 h dose. These correlations were lower with the once-daily regimen due to higher inter-individual variability in plasma levels (\( AUC_{0-\text{\textmax}} \) versus \( C_{\text{\textmax}} \): r=0.850; P=0.007; \( AUC_{0-\text{\textmax}} \) versus \( C_{\text{\textmin}} \): r=0.752; P=0.031), but a high correlation was observed between \( C_{\text{\textmax}} \) and \( C_{\text{\textmin}} \) (r=0.974; P=0.000). Moreover, there were linear relationships between plasma and intracellular \( AUC_{0-\text{\textmax}} \), and \( C_{\text{\textmax}} \) (r=0.799; P=0.017, and r=0.860; P=0.006), respectively, with the once-daily dose, but a significant correlation between plasma and intracellular \( C_{\text{\textmin}} \) (r=0.713; P=0.047) was observed only with the twice-daily regimen.

**Discussion**

Our results show that the main differences between the two regimens were in plasma pharmacokinetics as the once-daily dose provided a 76% higher \( C_{\text{\textmax}} \) and a slightly higher \( AUC_{0-\text{\textmax}} \), while \( C_{\text{\textmin}} \) values were similar for the two regimens. Although the potential safety implications of these results may be worrisome, both in our study and in previous reports the 400 mg/24 h regimen was well tolerated, at least in the short term. At the intracellular penetration level the average etravirine concentrations were 81% and 56% of plasma levels for the twice-daily regimen and the once-daily regimen, respectively, but intracellular levels were similar in both cases. The intracellular levels observed are compatible with passive transcellular diffusion of etravirine, as demonstrated in in vitro studies. These
findings disagree with those in the only study reported until now on intracellular etravirine levels, in which an intracellular accumulation ratio of 12.9 was observed in nine patients who were given etravirine at 200 mg/12 h, suggesting active transmembrane transport, which is still unknown for NNRTIs.¹⁰

A relationship between etravirine levels and efficacy has not been observed yet, including the pivotal clinical trials DUET-1 and 2. This could be due to confounding factors, such as the high viral resistance level and the complex regimens used in these patients. Perhaps simpler regimens in less experienced patients would be a more appropriate setting to demonstrate this relationship, and would indicate whether the lower plasma and intracellular $C_{\text{min}}$ levels observed in some patients with the once-daily regimen have clinical consequences. The high correlations observed between AUC₀–₁₂ and $C_{\text{max}}$ and $C_{\text{min}}$ when etravirine was given at 200 mg/12 h will facilitate future pharmacodynamic studies using only $C_{\text{min}}$ levels to estimate total drug exposure. However, these correlations are not as high with the 400 mg/24 h regimen, but, instead, $C_{12}$ could be used as an indirect measure to estimate the AUC₀–₂₄.

Some limitations of our study are that our patients were on protease inhibitor (PI)-sparing regimens and etravirine is currently registered only for use in combination with a boosted PI, which could significantly change the pharmacokinetics of etravirine. However, the mean etravirine plasma levels with the once-daily regimen were similar with and without 800/100 mg of darunavir/ritonavir.⁸ Moreover, etravirine is frequently used in non-PI-based regimens in clinical practice, and in the future it may be recommended as part of PI-sparing regimens.

Furthermore, etravirine was administered in the fasting state, although it is currently recommended to be administered following a meal to increase its absorption. Likewise, the use of tenofovir was associated with a 26% decrease in etravirine AUC₀–₁₂ in Phase I drug–drug interaction studies¹¹ and most of our patients were receiving this drug concomitantly, although this mirrors better the clinical practice. In conclusion, our results show that etravirine intracellular levels are similar when given at 200 mg/12 h and at 400 mg/24 h. Thus, a once-daily dosing regimen is supported not only by plasma etravirine pharmacokinetics but also by intracellular etravirine levels.

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