Background: We investigated the pharmacokinetics of atazanavir/ritonavir once daily and lopinavir/ritonavir twice and once daily over 72 h following drug intake cessation.

Methods: This was an open-label, three-session, pharmacokinetic trial. Healthy volunteers received atazanavir/ritonavir 300/100 mg once daily and lopinavir/ritonavir 400/100 mg twice daily and 800/200 mg once daily separately for 10 days. Pharmacokinetic profiles were assessed for each phase on day 10 over 72 h. Pharmacokinetic parameters were determined over 12 or 24 h and to the last measurable concentration by non-compartmental methods.

Results: Sixteen participants completed the study. Geometric mean terminal elimination half-life to 72 h of atazanavir was 8.35 h and not different from the 0–24 h half-life (9.91 h). Terminal elimination half-life of lopinavir was 2.33 h (twice daily) and 2.44 h (once daily). These values were lower compared with the half-life over the respective dosing intervals (7.15 and 4.88 h for 0–12 and 0–24 h, respectively). No participant on atazanavir had concentrations below the minimum effective concentration (MEC) of 150 ng/ml at 24 h. In total, 44% of the participants on lopinavir once daily had concentrations below the MEC of 1,000 ng/ml at 24 h. At 16 h and 20 h, 13% and 63% of participants were below target for twice daily lopinavir, respectively. At 36 h, all participants on lopinavir and 31% on atazanavir were below target. Ritonavir area under the plasma concentration–time curve was 30% lower and 26% higher when dosed at 100 mg or 200 mg with lopinavir versus atazanavir.

Conclusions: This study investigated the pharmacokinetic forgiveness of two boosted protease inhibitors. Whereas the decline in lopinavir concentrations occurred rapidly as the boosting effect of ritonavir diminished, the rate of decline of atazanavir remained constant to 72 h.

Introduction

Combination antiretroviral therapy has transformed the management of HIV infection and dramatically reduced HIV-related morbidity and mortality [1]. Despite these benefits, taking antiretroviral therapy can be challenging. Many of the combination regimens are complex, have important adverse effects, can be difficult for patients to adhere to and could lead to antiretroviral drug resistance [1]. These concerns continue to limit the success of antiretroviral therapy and complicate the management of HIV-infected patients.

The combination of a protease inhibitor and two nucleoside or nucleotide reverse transcriptase inhibitors (N(t)RTIs) has been shown to achieve durable viral suppression in most antiretroviral-naive and -experienced patients [1]. Importantly, most protease inhibitors must be coadministered with low dose ritonavir (100 or 200 mg daily) in order to enhance patient exposure to the full-dose protease inhibitor, thus preventing or overcoming resistance and allowing less frequent dosing. The advantages offered by ritonavir boosting are primarily attributable to its pharmacokinetic properties. Ritonavir reduces the metabolism of concomitantly administered protease inhibitors and changes their pharmacokinetic parameters by inhibiting cytochrome P450 (mainly the isoform 3A4 [CYP3A4]) [2].

Boosted protease inhibitors differ in terms of dosing schedule. Some are approved for once daily use; others are not, but in clinical practice are administered
once daily to antiretroviral-naive patients in order to simplify the daily dosing schedule and reduce the likelihood of missed doses [1].

Atazanavir is a potent HIV protease inhibitor that shows efficacy in both antiretroviral-naive and experienced patients [3,4]. Atazanavir is a substrate of CYP3A4 and coadministration with ritonavir has been shown to significantly increase plasma atazanavir concentrations [4]. It has good oral bioavailability and pharmacokinetic properties that allow once daily dosing, either with or without ritonavir boosting, whereas in Europe it is only licensed for use in treatment-experienced patients [4]. In the United States, atazanavir has been approved for first-line use either with or without ritonavir boosting, whereas in Europe it is only licensed for use in treatment-experienced patients, boosted by ritonavir [1].

Coformulated lopinavir/ritonavir-based antiretroviral therapy (Kaletra®, Abbott Laboratories, North Chicago, IL, USA) has shown durable virological treatment efficacy in clinical trials in both treatment-naive and -experienced patients [5] and is generally well tolerated.

The rapid and extensive first-pass oxidative metabolism of lopinavir in the liver is mediated primarily by CYP3A4/5 isoenzymes; ritonavir inhibits the activity of CYP3A4 in a concentration-dependent manner in human liver microsomes resulting in increased plasma lopinavir concentrations [5].

Lopinavir/ritonavir has been approved for twice daily use (400/100 mg) in Europe and for twice (400/100 mg) and once (800/200 mg) daily use in the United States. Different clinical trials have shown similar virological responses when comparing twice with once daily administration (in combination with two N[t]RTIs) in patients with baseline viral loads <100,000 copies/ml. However, when comparing outcomes between these treatments for patients with plasma HIV RNA levels >100,000 copies/ml, a significant difference favouring twice daily dosing was observed [6,7].

Interestingly, protease inhibitors that are boosted by ritonavir could themselves affect ritonavir pharmacokinetics. An analysis of several crossover pharmacokinetic trials and clinical datasets showed that ritonavir concentrations were different in the presence of different full-dose protease inhibitors [8]. Whereas slightly higher than expected concentrations have been observed following the administration of 100 mg of ritonavir with atazanavir, significantly lower concentrations have been observed in the presence of lopinavir, fosamprenavir and tipranavir [8]. This might be explained by the different effects that the protease inhibitors have on CYP3A4 activity, as this isoenzyme is also responsible for ritonavir metabolism itself.

Adherence to antiretroviral therapy is key for successful treatment of HIV-infected persons [1]. Theoretically, antiretroviral agents should also be taken following any restrictions on food and at the right time of day, especially when their plasma concentrations decline rapidly because of short half-lives. Delaying drug dosing and allowing drug concentrations to fall to subtherapeutic levels might lead to poorer virological control and the emergence of resistance. However, information on how frequently doses must be delayed in order to facilitate the emergence of resistance is lacking.

Recently, a study by Cohen et al. [9] examined the feasibility of intermittent antiretroviral combination therapy taken 5 days a week, with two consecutive days off. The study found that doses might be missed in the case of regimens containing long half-life drugs, such as the non-NRTI efavirenz. However, the strategy appeared much riskier for patients on protease inhibitors. In fact, five of the six individuals on lopinavir/ritonavir or saquinavir/ritonavir had subtherapeutic drug concentrations at the end of the second day off therapy, suggesting that despite the presence of the ritonavir-boosting effect, lopinavir and saquinavir plasma concentrations decline rapidly.

In this study we assessed the pharmacokinetics of atazanavir/ritonavir once daily and lopinavir/ritonavir twice and once daily over 72 h following drug intake cessation.

Methods

Participants and ethics

The study protocol was approved by the Riverside Research Ethics Committee (London, UK) and by the Medicines and Healthcare products Regulatory Agency (London, UK) and was conducted according to the Declaration of Helsinki.

Written informed consent was obtained from healthy male and female volunteers aged between 18 and 65 years. Before entering the study, the volunteers were established as HIV-negative and in good health by medical history, physical examination, electrocardiogram and standard haematological and blood chemistry tests. None of the volunteers were receiving any concomitant medication, including oral contraceptives or natural products.

Study design

This was an open-label, three-session, pharmacokinetic trial carried out at the Pharmacokinetic Unit of the St Stephen’s Centre, Chelsea and Westminster Hospital, London, UK.

During session one, volunteers were administered atazanavir/ritonavir 300/100 mg once daily in the morning for 10 days. On study days 10–13, atazanavir and ritonavir plasma concentrations were assessed predose and 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, 24, 30, 36, 48, 60 and 72 h after dose administration.

After a washout period of 7 days, all participants were administered lopinavir/ritonavir 400/100 mg twice daily...
for 10 days, with the omission of the evening lopinavir/ritonavir dose on day 27. On study day 27, a witnessed dose of lopinavir/ritonavir 400/100 mg was administered in the morning and blood was drawn over 72 h at the same time points reported above. Following a second washout period of 7 days, lopinavir/ritonavir was administered at 800/200 mg once daily for 10 days and drug concentrations were measured on study days 44–47, over 72 h following the last dose. On the pharmacokinetic days, the study medication was taken with a standardized breakfast (626 Kcal) and 240 ml of water.

Safety assessment
The safety and tolerability of study medications were evaluated throughout the study on the basis of clinical adverse events (using the AIDS Clinical Trials Group toxicity grading scale to characterize abnormal findings), clinical laboratory tests (at screening, on days 1, 5, 22 and 39, and at follow-up), vital signs and physical examinations.

Analytical and pharmacokinetic methods
Concentrations of atazanavir, lopinavir and ritonavir in plasma were measured using validated HPLC–tandem mass spectrometry methods [10]. The lower limit of quantification was 26, 5 and 2 ng/ml for atazanavir, lopinavir and ritonavir, respectively. For concentrations below the assay limit of quantification, a value of one half of the quantification limit (13.0, 2.5 and 1.0 ng/ml, respectively) was used.

The calculated pharmacokinetic parameters for atazanavir, lopinavir and ritonavir were the trough plasma concentration (C_{trough}) defined as the 12 h (for twice daily administration) or 24 h (for once daily administration) concentration after the observed dose, the maximum observed plasma concentration (C_{max}) and the area under the plasma concentration–time curve (AUC) from 0–12 h (for twice daily administration), from 0–24 h (for once daily administration) and from 0–72 h (for all dosages). The half-life was determined from the elimination phase within the normal dosing interval of 0–12 h (for twice daily administration) or 0–24 h (for once daily administration) and as a terminal elimination half-life, to the last measurable concentration within 72 h. All pharmacokinetic parameters were calculated using actual blood sampling times and non-compartmental modelling techniques (WinNonlin Professional™ software version 5.0; Pharsight Corp., Mountain View, CA, USA).

Statistical analyses
Descriptive statistics, including geometric mean values and 90% confidence intervals (CIs), were calculated for atazanavir, lopinavir and ritonavir pharmacokinetic parameters.

Intraindividual changes in atazanavir and lopinavir terminal elimination half-life over 72 h relative to the half-life observed within the dosing interval were assessed using geometric mean ratios (GMRs) with 90% CIs. The CIs were determined using logarithms of the individual geometric mean values and the calculated values were then expressed as linear values. Differences between the half-lives were considered significant when the CI did not cross the value of one.

Intraindividual changes in ritonavir pharmacokinetic parameters were also evaluated by calculating GMRs and 90% CIs. The ritonavir concentrations measured in the presence of atazanavir were used as reference points.

Interindividual variability in drug pharmacokinetic parameters was expressed as a coefficient of variation ([sd/mean] × 100).

Linear regression analysis was used to assess any correlation between body mass index (BMI) and atazanavir or lopinavir AUC. Comparison of pharmacokinetic parameters between males and females was performed by analysis of covariance.

All data were analysed with the statistical programme SAS (version 9.1; SAS Institute Inc., Cary, NC, USA).

Results
Study population
A total of 18 volunteers were screened. Two withdrew for personal reasons before any pharmacokinetic assessment and 16 were enrolled and completed all phases of the study. Of the 16 evaluable participants, median age was 42 years (range 25–55) and median BMI was 26 kg/m² (range 20–32). Six (37.5%) were females, 11 were Caucasian, three were Hispanic and two were Black. No major protocol deviations were recorded during the study.

Pharmacokinetics
Geometric mean values and 90% CIs for the steady-state pharmacokinetic parameters measured over the scheduled dosing interval and over 72 h for atazanavir, lopinavir and ritonavir are summarized in Table 1. Plasma concentration–time curves for atazanavir, lopinavir and ritonavir are shown in Figure 1. The atazanavir geometric mean terminal half-life over 72 h was 8.35 h and was slightly, but significantly, reduced (∼16%) relative to the 0–24 h dosing interval half-life (9.91 h, GMR 0.84 [90% CI 0.76–0.94]).

Geometric mean terminal half-life to 72 h of lopinavir was 2.3 h following twice daily dosing and 2.44 h following once daily dosing. These values were significantly reduced (∼67% for twice daily dosing and ∼50% for once daily dosing) compared with the half-life measured over the respective dosing intervals: 7.15 and 4.88 h

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Table 1. Geometric mean values and 90% confidence intervals for the steady-state pharmacokinetic parameters measured over the scheduled dosing intervals*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Atazanavir/ritonavir 300/100 mg</th>
<th>Lopinavir/ritonavir 400/100 mg</th>
<th>Lopinavir/ritonavir 800/200 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC, ng·h/ml</td>
<td>34,556 (31,063–38,547)</td>
<td>43,248 (36,526–51,207)</td>
<td>7,268 (6,318–8,361)</td>
</tr>
<tr>
<td>Cmax, ng/ml</td>
<td>3,257 (2,996–3,829)</td>
<td>966 (878–1,166)</td>
<td>10,265 (9,515–11,075)</td>
</tr>
<tr>
<td>Ctrough, ng/ml</td>
<td>ND</td>
<td>ND</td>
<td>4,597 (3,790–5,575)</td>
</tr>
<tr>
<td>Half-life, h</td>
<td>9.91 (8.96–11.94)</td>
<td>8.35 (7.54–9.25)</td>
<td>5.34 (4.61–6.18)</td>
</tr>
</tbody>
</table>

All values shown are geometric mean values (95% confidence interval). *Scheduled dosing intervals were 24 h for atazanavir/ritonavir 300/100 mg and lopinavir/ritonavir 800/200 mg, 12 h for lopinavir/ritonavir 400/100 mg and 72 h for atazanavir, lopinavir and ritonavir. AUC, area under the plasma concentration–time curve; Cmax, maximum observed plasma concentration; Ctrough, trough plasma concentration; ND, not detectable.

Figure 1. Steady-state plasma concentrations and geometric mean concentration–time curves*

*Geometric mean concentration–time curves denoted by thick black lines. (A) Atazanavir/ritonavir 300/100 mg once daily. (B) Lopinavir/ritonavir 400/100 mg twice daily. (C) Lopinavir/ritonavir 800/200 mg once daily. LOQ, limit of quantification; MEC, minimum effective concentration.
for the 0–12 and 0–24 h dosing intervals, respectively (GMR [90% CI] 0.33 [0.25–0.43] for twice daily dosing and 0.50 [0.38–0.66] for once daily dosing).

When coadministered with atazanavir, the low-dose ritonavir geometric mean terminal elimination half-life was significantly longer (~28%) relative to the half-life observed over the 0–24 h dosing interval (GMR [90% CI] 1.28 [1.02–1.61]). Equally, in the presence of lopinavir, following twice daily and once daily dosing, the ritonavir terminal elimination half-life was significantly prolonged (43% and 13%) in comparison to the dosing interval half-life over the 0–12 and 0–24 h dosing intervals (GMR [90% CI] 1.43 [1.15–1.78] for twice daily dosing and 1.13 [1.02–1.26] for once daily dosing).

Table 2 reports the number of participants with concentrations below the proposed minimum effective concentration (MEC) for atazanavir and lopinavir at the time points studied over 72 h following drug intake cessation.

At the time of the next due dose (24 h for once daily dosing and 12 h for twice daily dosing), no participants had atazanavir concentrations below the MEC of 150 ng/ml [11] (geometric mean $C_{\text{trough}}$ 636 ng/ml). Although no participants receiving lopinavir twice daily had lopinavir concentrations below the MEC of 1,000 ng/ml [11] at 12 h (geometric mean $C_{\text{trough}}$ 4,597 ng/ml), 44% of participants on lopinavir once daily had concentrations <1,000 ng/ml at 24 h (geometric mean $C_{\text{trough}}$ 1,283 ng/ml), respectively.

At 48 h (once daily dosing) and 24 h (twice daily dosing), the times at which dosing would be delayed by an additional 24 h (once daily dosing) or 12 h (twice daily dosing) in the case of a participant omitting a single dose, 69% of participants on atazanavir, 100% on lopinavir once daily and 81% on lopinavir twice daily were below the proposed MEC.

The interindividual variability in $C_{\text{trough}}$ of atazanavir, lopinavir, and ritonavir administered twice and once daily was 63%, 48% and 86%, respectively.

Interestingly, when comparing ritonavir pharmacokinetic parameters, significantly lower concentrations were observed when 100 mg ritonavir was administered with 400 mg lopinavir than with atazanavir (GMR [90% CI] AUC$_{0–24}$ 0.70 [0.58–0.83], $C_{\text{max}}$ 0.81 [0.67–0.97] and half-life over 72 h 0.72 [0.60–0.86]). Moreover, when comparing these parameters during 200 mg ritonavir administration with 800 mg lopinavir and 100 mg with atazanavir, ritonavir $C_{\text{max}}$ and AUC$_{0–24}$ were only 43% and 26% higher despite the double dose and its half-life was significantly lower (GMR [90% CI] 0.76 [0.66–0.88] over 24 h and 0.67 [0.56–0.80] over 72 h).

A significant relationship between lopinavir AUC (only when dosed twice daily) and BMI was observed ($P=0.038$), whereas no differences in lopinavir or atazanavir pharmacokinetic parameters were seen between males and females.

**Safety and tolerability**

Treatment was generally well tolerated and no serious adverse events occurred during the study. The most common adverse events observed throughout the study were diarrhoea, scleral icterus (during the atazanavir/ritonavir session) and headache reported in 8, 5 and 3 of the volunteers, respectively. All adverse events were of grade 1 or 2 of severity. No clinically relevant changes in laboratory or cardiovascular variables were reported.

**Discussion**

We report here the steady-state plasma pharmacokinetics of atazanavir/ritonavir (300/100 mg once daily) administered...
and lopinavir/ritonavir (400/100 mg twice daily and 800/200 mg once daily) over 72 h following drug intake cessation, in 16 healthy male and female volunteers.

Whereas atazanavir elimination half-life over 72 h was only 16% lower compared with the 24 h half-life, the half-life measured for lopinavir over 72 h was ≥50% lower than during the scheduled dosing intervals of 12 and 24 h. This shows a remarkable difference between the two protease inhibitors; whereas lopinavir/ritonavir disappears rapidly from plasma if a dose was omitted at the end of the dosing interval, atazanavir concentrations decline at a slower rate. Moreover, most participants had atazanavir concentrations higher than the suggested MEC 36 h following drug intake while on atazanavir/ritonavir.

Atazanavir/ritonavir and lopinavir/ritonavir were well tolerated, with adverse events limited to a small number of study participants who reported grade 1 or 2 diarrhoea, scleral icterus and headache during the study period.

Antiretroviral therapy is lifelong and adherence to drug intake has been shown to be fundamental for treatment success [12,13]. Importantly, preliminary data from recent studies demonstrated that higher than anticipated levels of adherence are required in HIV therapeutics [12].

Antiretroviral combination regimens involve the administration of three to four different agents characterized by different half-lives. Therefore, the different components of the regimen might persist in body fluids and cells for different times. The precise effect of taking the drug at the right time on therapeutic outcome has not been fully measured. Although good adherence is crucial for virological success, a number of variables, such as time of intake, could modify the possibility of achieving such success.

Certain antiretroviral agents, when characterized by longer half-lives, might be more forgiving than others by allowing for delayed doses without the achievement of subtherapeutic concentrations before the following dose.

One of the limitations of this study was that the enrolled participants were healthy volunteers rather than HIV-infected patients. However, for safety reasons HIV-infected patients could not be studied because in these individuals drug intake should not be stopped unless clinically required [14,15]. The potential for differences in drug disposition between HIV-infected patients and HIV-negative patients is high and it is important to recognize that the pharmacokinetics of a drug might be altered in persons with HIV infection compared with healthy individuals [16]. There are data indicating that atazanavir concentrations are lower in patients with HIV infection than in HIV-negative healthy volunteers [17]. However, although the atazanavir concentrations measured in our study were obtained from healthy volunteers, they were similar to those observed in HIV-infected patients [18,19]. We measured mean atazanavir concentrations of 763, 455 and 262 ng/ml at 24, 30 and 36 h after dose administration, respectively. These values are higher than the suggested MEC for atazanavir.

Interestingly, we measured higher ritonavir concentrations during coadministration with atazanavir than with lopinavir. Ritonavir is not only an inhibitor but also a substrate of CYP3A4. Therefore, its plasma concentrations are increased in the presence of a moderate CYP3A4 inhibitor such as atazanavir [8]. The slightly longer plasma persistence of ritonavir might be one of the reasons behind the slightly longer persistence of atazanavir than lopinavir.

The clinical significance of the slower decay in atazanavir concentrations compared with lopinavir concentrations remains unclear. These data confirm the findings of the FOTO study, in which short half-life drugs also disappeared rapidly when doses were omitted over 48 h [9]. However, data on how many antiretroviral doses can be delayed in order to prevent virological failure associated with the development of resistance are lacking. Nevertheless, the fast decay observed for lopinavir/ritonavir when dosed once daily suggests there is the likelihood of the achievement of subtherapeutic concentrations that can explain the higher occurrence of virological failure and development of protease inhibitor-related mutations observed in some clinical trials in the past [6,7]. HIV-infected patients on once daily lopinavir/ritonavir should therefore observe drug intake time strictly, whereas this might not be as important for those taking a more forgiving drug such as atazanavir/ritonavir. By contrast, it has been speculated that detectable concentrations persisting below the MEC and within the hypothetical resistance selection window (or zone of selective pressure) might also lead to the development of mutations conferring drug resistance [20,21].

In conclusion, our study has explored the pharmacokinetic forgiveness of two boosted protease inhibitors. Whereas the decline in lopinavir concentrations occurs rapidly as the boosting effect of ritonavir diminishes, the rate of decline of atazanavir remains constant to 72 h, resulting in a delayed onset of subtherapeutic concentrations.

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The results of this study have been presented at the 11th European AIDS Conference, Madrid, Spain (24–27 October 2007).
Disclosure statement

The authors declare no competing interests.

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