Saquinavir, nelfinavir and M8 pharmacokinetics following combined saquinavir, ritonavir and nelfinavir administration

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Objectives: This study evaluated the steady-state pharmacokinetic interaction between ritonavir-boosted saquinavir and nelfinavir.

Methods: Open label, multiple-dose, two parallel-groups, single crossover study conducted in 24 HIV-infected patients (12 in each group). Patients in the nelfinavir group added saquinavir/ritonavir, 1000/100 mg twice daily to their ongoing stable treatment regimen consisting of nelfinavir, 1250 mg twice daily and two nucleoside reverse transcriptase inhibitors (NRTIs). Patients in the saquinavir group added nelfinavir, 1250 mg twice daily to their ongoing stable treatment regimen consisting of saquinavir/ritonavir, 1000/100 mg twice daily and two NRTIs. Pharmacokinetic assessments were performed before and 7 days after the start of combined treatment with nelfinavir/saquinavir/ritonavir. Blood samples were collected before and 1, 2, 3, 4, 6, 8, 10 and 12 h after dosing for measurement of nelfinavir, the nelfinavir metabolite M8 and saquinavir using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Results: The addition of saquinavir/ritonavir to the nelfinavir-containing regimen resulted in significant increases in the M8 pharmacokinetic parameters AUC0–12, Cmax and C12; geometric mean ratios (90% confidence intervals) of 2.25 ng·h/mL (1.47–3.44), 1.74 ng/mL (1.25–2.40) and 4.21 ng/mL (2.10–8.47), respectively. The intra-individual changes in nelfinavir and saquinavir concentrations were highly variable. Statistical analysis could not discard a relevant interaction but includes the possibility that some parameters may be halved, others more than doubled. At the same time the analysis failed to show any directed change.

Conclusions: The co-administration of nelfinavir and saquinavir/ritonavir leads to unpredictable changes in concentrations of both drugs. It is unclear whether the increased concentrations of M8 are associated with a clinical benefit.

Keywords: HIV, AIDS, double boosting, drug interactions, cytochromes

Introduction

Antiretroviral treatment with two ritonavir-boosted protease inhibitors (PIs) is an experimental strategy, which may be beneficial for selected HIV-infected patients. Resistance to both nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTIs and NNRTIs) and sometimes triple class resistance may be successfully managed with a double boosted PI regimen.1

Although the combined effects of nelfinavir and saquinavir have not been studied in vitro, nelfinavir and unboosted
Pharmacokinetic interaction between nelfinavir and ritonavir-boosted saquinavir

Saqinavir have been co-administered in uncontrolled trials involving both therapy-naive and -experienced patients. A small pharmacokinetic study has shown that nelfinavir increases saquinavir concentrations. However, little is known about the pharmacokinetics of this combination when given with ritonavir.

The addition of ritonavir results in a dramatic increase in saquinavir exposure. In contrast, the boosting effect of ritonavir on the concentrations of nelfinavir and its active metabolite nelfinavir hydroxy-t-butylamide (M8) is less pronounced. Since nelfinavir, saquinavir and ritonavir can modify cytochrome P450 activity, their co-administration may result in relevant changes of drug concentrations. This study was designed to assess the steady state pharmacokinetic interaction between nelfinavir and ritonavir-boosted saquinavir.

Patients and methods

Patients

Twenty-four HIV-1-infected adult patients who had been taking two NRTIs in combination with either nelfinavir, 1250 mg twice daily (nelfinavir group, n = 12), or the ritonavir-boosted hard gel formulation (HGC) of saquinavir, 1000/100 mg twice daily (saquinavir group, n = 12), for a minimum of 2 weeks prior to enrolment were included. Exclusion criteria were any clinically significant abnormality on physical examination, abnormal laboratory findings, regular alcohol consumption and concomitant medication or substances known to interfere with hepatic drug metabolism. The study adhered to the European Guidelines for Good Clinical Practice. The local ethics committee had approved the protocol.

Study design

This open label, multiple-dose, two parallel-group, single crossover study was conducted in two phases: a pre-study phase (day −13 to day 0) and an experimental study phase (day 1 to day 8). On days 1 and 8 patients were admitted to the study unit in a fasted state. The time of the previous three doses of study drugs was recorded according to the patients’ study diary. Sampling started 30 min after a standardized breakfast (two slices of bread, two slices of cheese, butter, jelly and one cup of coffee or tea) and 12 h ± 30 min after the last dose. Following the collection of the first blood sample the morning dose was ingested with 200 mL of water. Further samples were collected 1, 2, 3, 4, 6, 8, 10 and 12 h post-dose. In both groups patients started taking combined treatment comprising nelfinavir, saquinavir (HGC) and ritonavir with NRTIs immediately after the collection of the 12 h sample on day 1 and stayed on this regimen until the morning of day 8. A follow-up visit was conducted within 2 weeks after day 8.

Analytical methods

Serum concentrations of saquinavir, nelfinavir and M8 were determined by LC-MS/MS. Following drug extraction into acetonitrile which contained the internal standards [deuterium labelled saquinavir for saquinavir and nelfinavir and 6-di(trideuteromethyl)amino-4,4-diphenyl-1-trideuteromethyl-3-heptanone for M8], the supernatants were injected onto a Eurospher C18 (5µ; 4.6 × 30 mm) column (Knauer, Berlin, Germany). HPLC separation was achieved with mobile phase gradient elution. An API 365 mass spectrometer (Applied Biosystems, Ontario, Canada) equipped with an electrospray ionization source was used for detection. The analytical ranges for saquinavir (39–10000 ng/mL) nelfinavir (31–7980 ng/mL) and M8 (30–3840 ng/mL) were defined by a set of nine calibration standards run in duplicate in every batch. Assay performance was controlled by inclusion of quality control samples at three different concentrations. The mean (range) inter-assay precision (C.V.) of the low, medium and high quality control samples was 8.0% (6.9–9.3) (nelfinavir), 10.8% (6.4–12.1) (M8) and 5.9% (4.1–8.2) (saquinavir). The corresponding overall mean accuracy was 103.7%, 93.4% and 98.8%, respectively.

Pharmacokinetic methods

Based on the plasma concentration data the following model-independent pharmacokinetic variables were determined. The areas under the plasma concentration-time curves from time 0 to 12 h post-dose (AUC0−12) were calculated using the log/linear trapezoidal rule. The apparent maximum concentration (Cmax) and time to reach Cmax (Tmax) values were estimated by visual inspection of the plasma drug concentration–time data.

Statistical methods

The primary hypothesis was that of bioequivalence of combined treatments and separate treatments [i.e. that the geometric mean ratios and the corresponding 90% confidence intervals (CI) of the primary pharmacokinetic parameters AUC0−12, Cmax, Tmax and C12 were within the standard bioequivalence interval of 0.80 and 1.25]. Assuming that the true ratios lie between 0.95 and 1.05 and that the intra-individual variability for all metrics is 15% a sample size of 12 subjects per group would be sufficient to provide 80% power to conclude bioequivalence.

Two patients in the nelfinavir group had M8 concentrations below the limit of detection for hours 0 to 12 on both pharmacokinetic days. In this instance the value of 30 ng/mL (lower limit of quantification) was imputed. The Tmax mean values and 90% CI were calculated from data of the remaining 10 subjects.

Results

Patient demographics

Twenty-four HIV-infected, male Caucasians with a median (range) age of 41 years (29–62), a median (range) body weight of 77 kg (56–100) and a median (range) body mass index of 24 (19–29) were enrolled. All patients had HIV RNA <50 copies/mL while receiving two of the following NRTIs along with their PI: lamivudine (n = 20), stavudine (n = 19), tenofovir (n = 7) and didanosine (n = 2).

Pharmacokinetics

The concentration–time profiles and box plots showing the distribution of the intra-individual ratios for the parameters
AUC_{0-12}, C_{\text{max}} and C_{12} are displayed in Figure 1. Table 1 summarizes the pharmacokinetic data and presents geometric mean ratios and 90% confidence intervals. The AUC_{0-12}, C_{\text{max}} and C_{12} of M8 were significantly higher when nelfinavir was combined with saquinavir/ritonavir. Of note, two subjects in the nelfinavir group had undetectable M8 concentrations during both separate and combined treatment.

The intra-individual changes in nelfinavir and saquinavir concentrations were highly variable. Statistical analysis could not discard a relevant interaction but includes the possibility that some parameters may be halved while others more than doubled. At the same time the analysis failed to show any directed change.

**Safety and tolerability**

Treatment with nelfinavir/saquinavir/ritonavir was well tolerated. The most frequently reported adverse event was mild to moderate diarrhoea. There were no serious adverse events and no discontinuations due to adverse events. Fasting serum triglycerides in the saquinavir group increased by a median (IQR) of 24 mg/dL (4.3–62); $P = 0.026$ (Wilcoxon signed ranks test).

**Discussion**

Selected patients may benefit from combination therapy with two ritonavir-boosted PIs. Additive or synergistic effects probably result from pharmacokinetic interactions leading to higher drug levels. However, unfavourable interactions have also been reported. This study investigated the pharmacokinetic interaction of ritonavir-boosted saquinavir and nelfinavir. The addition of saquinavir/ritonavir to nelfinavir led to an increase in the exposure, maximum and trough concentration of M8 in nine patients while two patients had undetectable M8 during separate and combined treatment. The cytochrome mainly responsible for M8-formation is the genetically polymorphic CYP2C19 whose
activity is only minimally inhibited by saquinavir and ritonavir. Two to five percent of Caucasians are homozygotes for the polymorphism CYP2C19 681G→A which generates a poor metabolizer phenotype and results in diminished M8-formation. The patients with undetectable M8 probably have this polymorphism. M8 itself is biotransformed by cytochromes inhibited by ritonavir and saquinavir with CYP3A4 being the most likely isoform. The changes in M8 pharmacokinetics found in this study are comparable to those seen after the addition of ritonavir to nelfinavir suggesting that the effect of saquinavir/ritonavir is mainly attributable to the action of ritonavir. This is consistent with the finding that ritonavir strongly inhibits CYP3A4 in vitro while the effect of saquinavir is much weaker.

Although the geometric mean ratios for saquinavir and nelfinavir pharmacokinetic parameters were within the bioequivalence interval (except for nelfinavir C12 and Tmax) the absence of relevant interactions cannot be concluded as the corresponding confidence limits were outside this range. The enormous variability of intra-individual changes may be due to an insufficient sample size or unexpectedly large variability. It may also indicate that the interaction results in higher variability rather than in some uniform increase or decrease. In any case, it seems impossible to predict drug concentrations therefore therapeutic drug monitoring is recommended when using this combination.

In conclusion, this study demonstrated that combination therapy with nelfinavir/saquinavir/ritonavir results in unpredictable changes of the pharmacokinetics of nelfinavir and saquinavir. In those patients capable of forming M8 the concentrations of this metabolite are increased. This may be clinically important since M8 is approximately as active against HIV as the parent drug. However, this combination should not be routinely used until truly comparative clinical trials have demonstrated its efficacy and safety.

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Table 1. Summary of pharmacokinetic parameters

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<th>NFV w/o SQV/RTV</th>
<th>NFV with SQV/RTV</th>
<th>GMR (90% CI)</th>
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<tr>
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<td>GM (95% CI)</td>
<td>GM (95% CI)</td>
<td>GMR (90% CI)</td>
</tr>
<tr>
<td>AUC0−12 (ng⋅h/mL)</td>
<td>31906 (22763−44722)</td>
<td>29936 (18482−48489)</td>
<td>0.94 (0.72−1.22)</td>
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<td>Cmax (ng/mL)</td>
<td>4701 (3459−6389)</td>
<td>4448 (3038−6512)</td>
<td>0.95 (0.77−1.16)</td>
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<tr>
<td>C12 (ng/mL)</td>
<td>746 (391−1422)</td>
<td>1095 (547−2194)</td>
<td>1.47 (0.92−2.35)</td>
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<tr>
<td>Tmax (h)</td>
<td>3.1 (2.6−3.7)</td>
<td>2.3 (1.7−3.2)</td>
<td>0.74 (0.56−0.98)</td>
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<table>
<thead>
<tr>
<th></th>
<th>M8 w/o SQV/RTV</th>
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<tr>
<td></td>
<td>GM (95% CI)</td>
<td>GM (95% CI)</td>
<td>GMR (90% CI)</td>
</tr>
<tr>
<td>AUC0−12 (ng⋅h/mL)</td>
<td>4154 (1815−9510)</td>
<td>9352 (3457−25298)</td>
<td>2.25 (1.47−3.44)*</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>686 (254−1850)</td>
<td>1190 (393−3605)</td>
<td>1.74 (1.25−2.40)*</td>
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<tr>
<td>C12 (ng/mL)</td>
<td>96 (44−209)</td>
<td>406 (173−950)</td>
<td>4.21 (2.10−8.47)*</td>
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<td>Tmax (h)</td>
<td>3.4 (2.8−4.2)</td>
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<td>1.00 (0.81−1.24)</td>
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<table>
<thead>
<tr>
<th></th>
<th>SQV w/o NFV</th>
<th>SQV with NFV</th>
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<td>GM (95% CI)</td>
<td>GM (95% CI)</td>
<td>GMR (90% CI)</td>
</tr>
<tr>
<td>AUC0−12 (ng⋅h/mL)</td>
<td>13467 (8079−22446)</td>
<td>15212 (10550−21935)</td>
<td>1.13 (0.73−1.74)</td>
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<tr>
<td>Cmax (ng/mL)</td>
<td>2297 (1415−3726)</td>
<td>2492 (1762−3524)</td>
<td>1.09 (0.73−1.61)</td>
</tr>
<tr>
<td>C12 (ng/mL)</td>
<td>404 (220−743)</td>
<td>355 (234−537)</td>
<td>0.88 (0.51−1.50)</td>
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<tr>
<td>Tmax (h)</td>
<td>3.7 (2.7−5.2)</td>
<td>3.1 (2.4−3.9)</td>
<td>0.82 (0.61−1.11)</td>
</tr>
</tbody>
</table>

SQV, saquinavir; RTV, ritonavir; NFV, nelfinavir; AUC, area under the concentration-time curve; Cmax, maximum concentration; C12, concentration 12 h after drug administration; Tmax, time to reach maximum concentration; GM, geometric mean; GMR, geometric mean ratio; CI, confidence interval; w/o, without.

*aThe value of 30 ng/mL was imputed for every undetectable M8 concentration in two patients; n was adjusted to 10 for the analysis of Tmax.

*aStatistical significance with P < 0.05 (Wilcoxon signed ranks test).
Transparency declarations

Hartmut Stocker has received funds for speaking at symposia organized on behalf of Hoffmann-La Roche, Bristol-Myers Squibb, Abbott and GlaxoSmithKline. Andrew Hill has received consultancy payments from F. Hoffmann-La Roche. Jutta Steinmüller is an employee of Hoffmann-La Roche, Germany. Mark Becker has received consultant fees from Hoffman-La Roche and Pfizer, Inc. Keikawus Arastéh and Michael Kurowski have received funds for speaking at symposia organized on behalf of Hoffmann-La Roche, Bristol-Myers Squibb, Abbott Laboratories, Boehringer Ingelheim, Gilead Sciences and GlaxoSmithKline. They have received funds for research from Hoffmann-La Roche, Boehringer Ingelheim, BMS, GSK and Abbott Laboratories. In addition Michael Kurowski has received consultancy payments from Tibotec/Johnson & Johnson. Guido Kruse has received funds for speaking at symposia organized on behalf of Gilead Sciences and Boehringer Ingelheim. He has received funds for research from Bristol-Myers Squibb and GlaxoSmithKline. Antje Breske: none to declare. Hubert Schulbin: none to declare. Marcel Berger: none to declare. Christian Herzmann: none to declare.

References


