Background: A saquinavir/ritonavir-containing regimen is one option for the prevention of mother-to-child transmission of HIV during pregnancy. We evaluated the pharmacokinetics, efficacy and safety of saquinavir/ritonavir 1,000/100 mg twice daily plus nucleos(t)ide reverse transcriptase inhibitors in 13 women during late pregnancy and compared the results to those of 15 non-pregnant women.

Methods: Protease inhibitor plasma concentration profiles were assessed at 12 h using a standardized therapeutic drug monitoring procedure and measured by LC-MS/MS. Minimum and maximum concentrations (Cmin and Cmax), area under the plasma concentration–time curve (AUC0–12 h), and total clearance (CL total) were compared between the groups and correlated to demographic, physiological and clinical cofactors. Antiviral and immunological efficacy and safety were investigated.

Results: The geometric means (90% confidence interval [CI]) for saquinavir Cmin, Cmax and AUC0–12 h of pregnant versus non-pregnant women were 572 (437–717) versus 765 (485–1,052; P=0.064) ng/ml, 2,168 (1,594–2,807) versus 3,344 (2,429–4,350; P=0.045) ng/ml and 15,512 (11,657–19,943) versus 24,027 (17,454–31,548, P=0.029) ng•h/ml. The geometric means (90% CI) for ritonavir Cmin, Cmax and AUC0–12 h, were 190 (148–234) versus 310 (240–381, P=0.011) ng/ml, 781 (580–999) versus 1,552 (1,127–2,007, P=0.004) ng/ml and 5,576 (4,303–7,006) versus 10,528 (7,487–14,105, P=0.003) ng•h/ml. Age, weight, saquinavir dose per weight and body mass index differed significantly; saquinavir Cmin and AUC0–12 h were correlated with ritonavir Cmin and saquinavir dose per weight. After a mean of 11 weeks treatment, 12 of 13 pregnant women had a viral load <400 copies/ml, which was similar to the results of non-pregnant women.

Conclusions: Although saquinavir plasma concentrations were significantly lower in pregnant women compared with non-pregnant women, all pregnant women displayed a saquinavir AUC0–12 h >10,000 ng•h/ml, 92.3% had a viral load <400 copies/ml at birth. Saquinavir was well tolerated by the mothers and all newborn children were HIV type-1 negative at 18 months of age.

Introduction

The prevention of mother-to-child transmission (PMTCT) of HIV type-1 (HIV-1) is one of the major aims of highly active antiretroviral therapy in the developing world, as approximately one-third of infections occur at pregnancy or at birth of children. Preventive medical and surgical treatment including antiretroviral therapy (ART) during the third trimester of pregnancy, caesarean section and a strict denial of breastfeeding after birth can decrease the number of transplacental and intrapartum HIV-1 infections to 1–2% [1].

For a decade, treatment with nevirapine, a non-nucleos(t)ide reverse transcriptase inhibitor (NNRTI), plus two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) had been propagated as the therapy of choice for the PMTCT [2,3]. Nevirapine is widely used in resource-poor areas and is considered as part of generic (fixed) drug combinations [4]. Nevirapine crosses the placenta, resulting in a drug concentration ratio of 0.8 in cord blood compared with maternal blood, and is expected to provide at least a certain degree of safety to the child [5,6]. However, previously published data...
suggest a higher risk of severe hepatotoxicity in women with a low body mass index (BMI) and/or a CD4+ T-cell count of ≥250 cells/µl [7–11]. In addition, other patient characteristics can influence the pharmacokinetics of nevirapine, leading to either very high or suboptimal plasma concentrations, especially in women [5,6,12].

Therefore, alternative treatments for the PMTCT have been evaluated, including HIV protease inhibitors, which are currently part of international guidelines for the treatment of HIV during pregnancy. One protease inhibitor that is increasingly used in the PMTCT is saquinavir. However, data on the pharmacokinetics of saquinavir administered during late pregnancy at standard doses are scarce. Previous studies have evaluated saquinavir doses of 1,200 mg thrice daily without the addition of the pharmacoenhancer ritonavir [13,14] or saquinavir/ritonavir doses of 800/100 mg twice daily [15] and 1,200/100 mg once daily [16,17]. These dose regimens are no longer used in many countries because the manufacturer now produces a saquinavir formulation with 500 mg saquinavir per tablet [13,14,18,19]. Furthermore, pharmacokinetic data have been presented from studies evaluating the twice-daily intake of saquinavir 500 mg tablet together with low-dose 100 mg ritonavir and NRTIs during late pregnancy [20–22].

The present study evaluated the pharmacokinetics, pharmacodynamics and safety of a therapy regimen with saquinavir gel capsule formulations plus low-dose ritonavir at the twice-daily standard dose of 1,000/100 mg together with NRTIs during the third trimester of pregnancy. These results were compared with those of non-pregnant women who were assessed during the first 12 weeks on therapy in order to qualify potential pregnancy-related deviations in pharmacokinetics, efficacy and safety of this regimen.

Methods

Patients

A total of 13 pregnant and 15 non-pregnant women, who were infected with HIV-1, received a combination of saquinavir/ritonavir 1,000/100 mg twice daily plus ≥2 NRTIs.

There were no CD4+ T-cell count or viral load restrictions to be included in this study. Patients completed a 12 h pharmacokinetic assessment according to a standardized therapeutic drug monitoring (TDM) protocol [23]. Pharmacokinetic data were assessed from all women who started with a saquinavir/ritonavir therapy regimen and participated in routine TDM procedures between June 2003 and June 2006.

Patients with hepatic impairment defined by the Child-Pugh classification B or C and patients receiving comedication with CYP3A4/5 enzyme inhibitors or inducers as well as antacids were not included in the analyses; the same applied to women who took antibiotics or antifungals, such as azoles, which are frequently used to treat opportunistic HIV infections. An exception was made if 960 mg cotrimoxazole was taken thrice weekly as *Pneumocystis jirovecii* pneumonia prophylaxis, which is known to have no effect on protease inhibitor metabolism because of pharmacological interactions [24].

This pharmacokinetic study design was observational, no additional intervention was performed and ethics approval was not obtained according to the national laws and the previously obtained advice of the responsible institutional ethics committee. However, patients were individually informed and trained in the TDM procedure and agreed with the recording of their data, which were anonymous for analysis.

Therapeutic drug monitoring protocol

After ≥2 weeks on the regimen, patients underwent a pharmacokinetic assessment at steady-state conditions. The schedule of drug intake was documented by patients for 3 days prior to the pharmacokinetic assessment. In addition, concomitant drug intake had to be documented by the patient and physician, including daily intake of herbal agents or nutritive supplements. On the day of the pharmacokinetic assessment, patients were weighed, body height was measured and BMI calculated (BMI= [weight] kg/ [body height] m²). Fasting trough levels were obtained immediately before drug intake, followed by a standardized breakfast of approximately 2,500 kJ (25% from fat). Plasma samples were then collected at 1, 2, 4, 6, 9 and 12 h after drug intake. Blood was centrifuged for 10 min at 2,000 min⁻¹ within 20 min after sampling. Plasma was separated and stored at -80°C pending analysis.

Pharmacokinetic assay and evaluation

Saquinavir and ritonavir plasma concentrations were measured by validated and externally quality-controlled high-pressure liquid chromatography-tandem mass spectrometry methods [25] (Merck–Hitachi, Darmstadt, Germany; Applied Biosystems, Streetsville, ON, Canada; and Therapia GmbH, Berlin, Germany), which are described elsewhere [26]. The reliable lower limit of quantification (LLQ) was 20 ng/ml and linearity of the calibration curve for all tested compounds was proven up to 20,000 ng/ml [26,27].

Pharmacokinetic calculations were on the basis of plasma concentrations that exceeded the LLQ. The minimum concentration (*C*ₘᵢₙ) and maximum concentration (*C*ₘₐₓ) values of the non-compartmental analysis were read directly from the plasma concentration–time curves of saquinavir and ritonavir within the standard dosing interval (τ=0–12 h). The area
under the plasma concentration–time curve \( \text{AUC}_{\text{T}} \) at steady state, the elimination half-life \( (t_{\text{1/2}}) \) and the total clearance \( (\text{CL}_{\text{total}}) \) were obtained by using a non-compartmental analysis module. All pharmacokinetic analyses were performed using WinNonLin \( 5.2^\text{®} \) (Mountain View, CA, USA) [28].

Statistical methods

**Pharmacokinetics**

The pharmacokinetic variables measured were the \( \text{AUC}_{\text{T}} \) at steady state, \( C_{\text{max}} \), \( C_{\text{min}} \), \( \text{CL}_{\text{total}} \) and \( t_{\text{1/2}} \) of saquinavir and ritonavir. The statistical analyses used parametric methods for the comparison of the geometric means of these variables. Absence of a significant difference following saquinavir and ritonavir exposure between groups was indicated when no significant difference according to the Student’s \( t \)-test and a 90% confidence interval \( (\text{CI}) \), including 0, was determined.

The appropriateness of the pharmacokinetic assessment (seven samples over 12 h) under open conditions in an outpatient centre was demonstrated by comparing the robust non-compartment analysis with the fitting according to a predefined pharmacokinetic model in which accuracy depends on the closeness of sampling. According to the primary pharmacokinetic variables, as assessed by the non-compartmental analysis, with the results of the two-compartment model after curve fitting was proven by linear regression analysis. All statistical analyses were performed using SPSS 15.0 for Windows\( ^\text{®} \) (SPSS Inc., Chicago, IL, USA).

The previously described Student’s \( t \)-tests, Spearman’s rank regression analyses and Fisher’s exact tests identified significantly different variables between both groups. Subsequently, these were included into a linear regression analysis using the stepwise backward deletion of variables, testing for the effect on the saquinavir \( C_{\text{min}}, C_{\text{max}}, \text{AUC}_{\text{T}} \) and \( \text{CL}_{\text{total}} \).

**Pharmacodynamics**

**Efficacy**

The primary efficacy target parameter was sustained virological response to therapy at labour, expressed as HIV-1 RNA<50 copies/ml, in an intention-to-treat analysis. Response or non-response was dichotomized to 0 or 1, respectively, and submitted to binary logistic regression. Demographic (that is, age and sex), pharmacokinetic and virological parameters were included as regressors after univariable analysis at a significance level of \( P\leq 0.05 \). Specifically, the pharmacokinetic parameters tested were the saquinavir \( C_{\text{min}}, C_{\text{max}}, \text{AUC}_{\text{T}} \). In addition, baseline CD4\(^+\) T-cell count, HIV-1 RNA at baseline, the number of previously taken antiretrovirals and protease inhibitors and the accumulated duration of ART prior to onset of the actual therapy were included. Most interval-scaled variables were included after log transformation because of the log normal rather than normal distribution. Logistic regression was performed with stepwise deletion of variables, using minus 2\( \times \) the log likelihood as significance criteria and with \( \alpha \) levels for inclusion and deletion of 0.01 and 0.05, respectively. Subsequently, significant variables were selected by the stepwise deletion of variables during the logistic regression analysis, which removes covariates from the model if they are non-significant and not a confounder.

Secondly efficacy target parameters were reduction in viral load at week 12 as compared with baseline, the slope of regression of the viral load decrease through week 12 and increase in CD4\(^+\) T-cell count from baseline to week 12. These parameters were analysed by means of multiple linear regression analyses using the same regressors as in the logistic regression. Backward stepwise deletion was employed with an \( \alpha \) level of 0.01 to remain in the model and of 0.05 to be deleted.

**Safety and tolerability**

Adverse events were graded according to the Division of AIDS toxicity tables (National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA) [29]. Symptoms were reported if graded as 2 (moderate), 3 (severe) or 4 (life-threatening). The maternal laboratory adverse events included grade 2–4 events for glucose, aspartate transaminase (AST), alanine aminotransferase (ALT), lactic acid, proteinuria, anaemia, thrombopaenia and lymphopaenia.

**Results**

**Baseline characteristics**

Demographic data differed significantly between pregnant and non-pregnant women. The mean (95% CI) for age was 28.2 years (24.8–31.7) versus 36.3 years (32.8–39.8, \( P=0.001 \)), for weight was 67.8 kg (59.6–75.9) versus 55.7 kg (49.7–61.7, \( P=0.014 \)), for saquinavir dose per weight was 15.3 mg (13.4–17.3) versus 18.6 mg (16.7–20.5, \( P=0.016 \)), and for BMI was 24.4 (21.9–27.0) versus 21.1 (19.2–22.9, \( P=0.014 \)).

All women received ≥2 NRTIs as backbone therapy, without displaying significant differences between both groups (Table 1). A total of 6 pregnant and 10 non-pregnant women were ART-naive at therapy onset (Fisher’s exact \( P=0.239 \)), 3 pregnant women and 2 non-pregnant women took protease inhibitors as part of previous ART (\( \chi^2 P=0.746 \)). All pregnant women and 9 non-pregnant women took saquinavir hard-gel formulation.
Significant differences were detected in the protease inhibitor pharmacokinetics of pregnant versus non-pregnant women. The geometric means (90% CI) for saquinavir Cmin, Cmax and AUC(tau) were 572 ng/ml (437–717) versus 765 ng/ml (485–1,052, P=0.064), 2,168 ng/ml (1,594–2,807) versus 3,344 ng/ml (2,429–4,350, P=0.045) and 15,512 ng•h/ml (11,657–19,943) versus 24,027 ng•h/ml (17,454–31,548, P=0.029) in pregnant versus non-pregnant women, respectively. The geometric means (90% CI) for ritonavir Cmin, Cmax and AUC(tau) were 190 ng/ml (148–234) versus 310 ng/ml (240–381, P=0.011), 781 ng/ml (580–999) versus 1,552 ng/ml (1,127–2,007, P=0.004) and 5,576 ng•h/ml (4,303–7,006) versus 10,528 ng•h/ml (8,131–13,177, P=0.003; Figures 1A & 1B) in pregnant versus non-pregnant women, respectively. The t1/2 and CL total showed no significant differences between pregnant and non-pregnant patients. The geometric means (90% CI) for saquinavir t1/2 and CL total were 3.80 h (3.25–4.15) versus 4.10 h (3.51–4.46, P=0.386) and 1,074 ml/min (803–1,374) versus 694 ml/min (499–903, P=0.285), respectively.

Significant correlations (Spearman's ρ) were found for saquinavir Cmin with ritonavir Cmin (P=0.001), Cmax (P=0.016) and AUC(tau) (P=0.017) and saquinavir Cmax with ritonavir Cmin (P<0.001), Cmax (P=0.003), AUC(tau) (P=0.006) and body weight (P=0.017). The same correlations appeared for the saquinavir AUC(tau) with ritonavir Cmin (P<0.001), Cmax (P=0.005), AUC(tau) (P=0.004) and body weight (P=0.031). Ritonavir Cmin, Cmax and AUC(tau), respectively, also correlated with either saquinavir hard-gel or soft-gel intake (P=0.029, P=0.020 and P=0.006, respectively) or body weight/BMI (P=0.014, P=0.32 and P=0.046, respectively).

The parameters, which appeared to be significant in the univariate analyses, were selected for a linear regression analysis using the stepwise deletion of variables testing their effect on the saquinavir plasma concentrations included age, pregnancy, ritonavir Cmin, ritonavir Cmax, ritonavir AUC(tau), weight, dose per

Table 1. Baseline characteristics of pregnant and non-pregnant women treated with saquinavir/ritonavir twice daily plus nucleos(t)ide reverse transcriptase inhibitors

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pregnant, n=13</th>
<th>Non-pregnant, n=15</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years*</td>
<td>28.2 (24.8–31.7)</td>
<td>36.3 (32.8–39.8)</td>
<td>0.002</td>
</tr>
<tr>
<td>Weight, kg*</td>
<td>67.8 (59.6–75.9)</td>
<td>55.7 (49.7–61.7)</td>
<td>0.014</td>
</tr>
<tr>
<td>Dose per weight, mg*</td>
<td>15.3 (13.4–17.3)</td>
<td>18.6 (16.7–20.5)</td>
<td>0.016</td>
</tr>
<tr>
<td>BMI, kg/m²*</td>
<td>24.4 (21.9–27.0)</td>
<td>21.1 (19.2–22.9)</td>
<td>0.029</td>
</tr>
<tr>
<td>CD4+ T-cell count, cells/µl*</td>
<td>305 (190–420)</td>
<td>171 (67–276)</td>
<td>0.074</td>
</tr>
<tr>
<td>HIV RNA, log10 copies/ml*</td>
<td>3.95 (3.39–4.50)</td>
<td>4.76 (4.00–5.52)</td>
<td>0.081</td>
</tr>
<tr>
<td>PK, week*</td>
<td>4.5 (2.9–5.5)</td>
<td>7.3 (5.8–8.8)</td>
<td>0.392</td>
</tr>
<tr>
<td>Therapy-naive, n</td>
<td>6</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Concomitant medication (Fisher’s exact test)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abacavir, n</td>
<td>–</td>
<td>2</td>
<td>0.278</td>
</tr>
<tr>
<td>Zidovudine, n</td>
<td>12</td>
<td>6</td>
<td>0.005</td>
</tr>
<tr>
<td>Stavudine, n</td>
<td>–</td>
<td>1</td>
<td>0.536</td>
</tr>
<tr>
<td>Didanosine, n</td>
<td>1</td>
<td>2</td>
<td>0.556</td>
</tr>
<tr>
<td>Emtricitabine, n</td>
<td>–</td>
<td>1</td>
<td>0.536</td>
</tr>
<tr>
<td>Tenofovir disoproxil fumarate, n</td>
<td>3</td>
<td>7</td>
<td>0.184</td>
</tr>
<tr>
<td>Lamivudine, n</td>
<td>11</td>
<td>13</td>
<td>0.644</td>
</tr>
<tr>
<td>Saquinavir hard-gel capsule, n</td>
<td>13</td>
<td>9</td>
<td>0.013</td>
</tr>
<tr>
<td>Saquinavir soft-gel capsule, n</td>
<td>–</td>
<td>6</td>
<td>0.013</td>
</tr>
<tr>
<td>Cotrimoxazole, n</td>
<td>–</td>
<td>2</td>
<td>0.278</td>
</tr>
<tr>
<td>Hepatitis serology (Fisher’s exact test)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBcAb-positive, n</td>
<td>7</td>
<td>5</td>
<td>0.239</td>
</tr>
<tr>
<td>HBsAg/HBeAg-positive, n</td>
<td>2</td>
<td>–</td>
<td>0.206</td>
</tr>
<tr>
<td>HCV Ab-positive, n</td>
<td>5</td>
<td>2</td>
<td>0.426</td>
</tr>
<tr>
<td>HCV PCR-positive, n</td>
<td>1</td>
<td>2</td>
<td>0.556</td>
</tr>
</tbody>
</table>

Baseline characteristics of pregnant and non-pregnant women on therapy with saquinavir/ritonavir 1,000/100 mg twice daily plus nucleos(t)ide reverse transcriptase inhibitors. *Mean (95% confidence interval). Ab, antibody; BMI, body mass index; HBcAb, hepatitis B core antibody; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; PK, pharmacokinetic assessment.

Pharmacokinetics

Significant differences were detected in the protease inhibitor pharmacokinetics of pregnant versus non-pregnant women. The geometric means (90% CI) for saquinavir Cmin, Cmax and AUC(tau) were 572 ng/ml (437–717) versus 765 ng/ml (485–1,052, P=0.064), 2,168 ng/ml (1,594–2,807) versus 3,344 ng/ml (2,429–4,350, P=0.045) and 15,512 ng•h/ml (11,657–19,943) versus 24,027 ng•h/ml (17,454–31,548, P=0.029) in pregnant versus non-pregnant women, respectively. The geometric means (90% CI) for ritonavir Cmin, Cmax and AUC(tau) were 190 ng/ml (148–234) versus 310 ng/ml (240–381, P=0.011), 781 ng/ml (580–999) versus 1,552 ng/ml (1,127–2,007, P=0.004) and 5,576 ng•h/ml (4,303–7,006) versus 10,528 ng•h/ml (8,131–13,177, P=0.003; Figures 1A & 1B) in pregnant versus non-pregnant women, respectively. The t1/2 and CL total showed no significant differences between pregnant and non-pregnant patients. The geometric means (90% CI) for saquinavir t1/2 and CL total were 3.80 h (3.25–4.15) versus 4.10 h (3.51–4.46, P=0.386) and 1,074 ml/min (803–1,374) versus 694 ml/min (499–903, P=0.285), respectively. The geometric means (90% CI) for ritonavir t1/2 and CL total were 4.24 h (3.47–4.76) versus 4.07 h (3.18–4.59, P=0.963) and 299 ml/min (229–373) versus 158 ml/min (121–196, P=0.016; Table 2).

Significant correlations (Spearman’s ρ) were found for saquinavir Cmin with ritonavir Cmin (P=0.001), Cmax (P=0.016) and AUC(tau) (P=0.017) and saquinavir Cmax with ritonavir Cmin (P<0.001), Cmax (P=0.003), AUC(tau) (P=0.006) and body weight (P=0.017). The same correlations appeared for the saquinavir AUC(tau) with ritonavir Cmin (P<0.001), Cmax (P=0.005), AUC(tau) (P=0.004) and body weight (P=0.031). Ritonavir Cmin, Cmax and AUC(tau), respectively, also correlated with either saquinavir hard-gel or soft-gel intake (P=0.029, P=0.020 and P=0.006, respectively) or body weight/BMI (P=0.014, P=0.32 and P=0.046, respectively).

The parameters, which appeared to be significant in the univariate analyses, were selected for a linear regression analysis using the stepwise deletion of variables testing their effect on the saquinavir plasma concentrations included age, pregnancy, ritonavir Cmin, ritonavir Cmax, ritonavir AUC(tau), weight, dose per
Saquinavir/ritonavir in pregnant women

Antiviral Therapy

13.8

1043

weight, baseline CD4+ T-cell count and HIV RNA, the number of previous antiretroviral treatments and protease inhibitors and the saquinavir formulation (hard-gel or soft-gel capsules).

Overall, two variables were detected as having a significant effect on the saquinavir plasma concentrations: saquinavir $C_{\text{min}}$ correlated with ritonavir $C_{\text{min}}$ ($P=0.001$) and saquinavir dose per weight ($P=0.036$).

A Pearson regression analysis detected correlations between saquinavir $C_{\text{max}}$ and ritonavir $C_{\text{max}}$ ($P<0.001$), between saquinavir $AUC_{\tau}$ and ritonavir $C_{\text{min}}$ ($P<0.001$) and between saquinavir $AUC_{\tau}$ and saquinavir dose per weight ($P=0.009$), respectively.

Pharmacodynamics

Efficacy

Pregnant women started their PMTCT therapy at a mean (±SD) of 2.71 months (±1.75) prior to giving birth. All except one patient (92.3%) exhibited a viral load $<400$ copies/ml and six pregnant women (46.2%) had a viral load $<50$ copies/ml at time of giving birth. The respective CD4+ T-cell increase was 136 cells/µl from baseline. Although these data cannot be compared directly with the group of non-pregnant women because of the highly variable duration that pregnant women spent on therapy, the results of the group of non-pregnant women revealed only marginal

Table 2. Plasma concentrations of saquinavir and ritonavir in pregnant and non-pregnant women treated with saquinavir/ritonavir twice daily plus nucleos(t)ide reverse transcriptase inhibitors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pregnant ($n=13$)</th>
<th>Non-pregnant ($n=15$)</th>
<th>Difference (pregnant versus non-pregnant)</th>
<th>Student’s t-test ($P$-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saquinavir</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{min}}$, ng/ml</td>
<td>572 (437–717)</td>
<td>765 (485–1,052)</td>
<td>0.75 (0.68–0.90)</td>
<td>0.064</td>
</tr>
<tr>
<td>$C_{\text{max}}$, ng/ml</td>
<td>2,168 (1,594–2,807)</td>
<td>3,344 (2,429–4,350)</td>
<td>0.65 (0.37–1.16)</td>
<td>0.045</td>
</tr>
<tr>
<td>$AUC_{\tau}$, ng•h/ml</td>
<td>15,512 (11,657–19,943)</td>
<td>24,027 (17,454–31,548)</td>
<td>0.65 (0.37–1.14)</td>
<td>0.029</td>
</tr>
<tr>
<td>$t_{1/2}$, h.min*</td>
<td>3.80 (2.35–4.15)</td>
<td>4.10 (2.51–4.46)</td>
<td>0.93 (0.73–1.18)</td>
<td>0.386</td>
</tr>
<tr>
<td>$CL_{\text{tot}}$, ml/min</td>
<td>1,074 (803–1,374)</td>
<td>694 (499–903)</td>
<td>1.58 (0.89–2.76)</td>
<td>0.285</td>
</tr>
<tr>
<td>Ritonavir</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{min}}$, ng/ml</td>
<td>190 (148–234)</td>
<td>310 (240–381)</td>
<td>0.61 (0.39–0.97)</td>
<td>0.011</td>
</tr>
<tr>
<td>$C_{\text{max}}$, ng/ml</td>
<td>781 (580–999)</td>
<td>1,552 (1,127–2,007)</td>
<td>0.50 (0.29–0.89)</td>
<td>0.004</td>
</tr>
<tr>
<td>$AUC_{\tau}$, ng•h/ml</td>
<td>5,576 (4,303–7,006)</td>
<td>10,528 (8,131–13,177)</td>
<td>0.53 (0.33–0.86)</td>
<td>0.003</td>
</tr>
<tr>
<td>$t_{1/2}$, h.min*</td>
<td>4.24 (3.47–4.76)</td>
<td>4.07 (3.18–4.59)</td>
<td>1.04 (0.76–1.50)</td>
<td>0.963</td>
</tr>
<tr>
<td>$CL_{\text{tot}}$, ml/min</td>
<td>299 (229–373)</td>
<td>158 (121–196)</td>
<td>1.89 (1.17–1.90)</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Plasma concentrations of saquinavir and ritonavir in pregnant and non-pregnant women on therapy with saquinavir/ritonavir 1,000/100 mg twice daily plus nucleos(t)ide reverse transcriptase inhibitors. All listed values are geometric means (90% confidence interval). *Half-life ($t_{1/2}$) is presented in decimal form. $AUC_{\tau}$, area under the plasma time–concentration curve ($t=0–12$ h); $CL_{\text{tot}}$, total clearance; $C_{\text{max}}$, maximum concentration; $C_{\text{min}}$, minimum concentration.

Figure 1. Time versus plasma concentration curves of saquinavir (1,000 mg) and ritonavir (100 mg) twice daily in pregnant and non-pregnant women treated with saquinavir/ritonavir twice daily plus nucleos(t)ide reverse transcriptase inhibitors

A

B

Non-pregnant  Non-pregnant  Pregnant  Pregnant  Difference (pregnant versus non-pregnant)
differences when analysed after a mean of 12 weeks on therapy. The mean decrease of HIV RNA from baseline was 2.87 log₁₀ copies/ml in non-pregnant women and all (93.3%) except one had a viral load <400 copies/ml, six of these patients (40%) had a viral load <50 copies/ml at week 12. The mean CD4⁺ T-cell increase was 49 cells/µl from baseline.

The 13 pregnant women gave birth to 13 children (6 girls and 7 boys), in all cases by caesarean section after a mean (±sd) pregnancy duration of 36.1 weeks (±1.3). All children were HIV-negative at 18 months of age.

Safety and tolerability
Six grade 2 or 3 clinical adverse events of variable appearance were reported for pregnant women: two patients suffered from nephrolithiasis and cystitis and were hospitalized because of these diagnoses, one woman reported two vaginal infections during late pregnancy (one bacterial and one with Candida albicans and she was treated as an outpatient in both cases), one pregnant woman had nausea and vomiting and in one pregnant woman, zidovudine was exchanged with tenofovir because of severe anaemia. One pregnancy terminated early at week 34 and six because of preliminary labour after 7 weeks on therapy with saquinavir/ritonavir, zidovudine and lamivudine.

Eight adverse events, of moderate and severe characteristics, were reported for non-pregnant women during the first 12 weeks on therapy: two women reported severe anaemia, which required a switch in therapy from zidovudine, in both cases, to tenofovir. One case of erythema nodosum in conjunction with peripheral oedema was reported, which was unlikely to be caused by medication. One woman reported the aggravation of hyperpigmentation and heartburn, which already existed prior to therapy onset. One woman developed a cutaneous Kaposi's sarcoma after 18 days on therapy, which was subsequently treated and resolved without complications. Eczema caused by an immune reconstitution syndrome occurred in one patient. Two women developed major depression and panic attacks during the first 12 weeks of therapy.

The majority (64%) of reported adverse events were of an unspecified kind and might not be directly associated with therapy with the exception of cases of anaemia, which were likely to be correlated to the intake of zidovudine and which improved when zidovudine was exchanged with tenofovir.

Regarding laboratory adverse events [30], pregnant women and non-pregnant women showed cases of grade 1–2 leucopaenia (n=2 and n=6), haemoglobinemia (n=2 and n=3), thrombocytopaenia (n=0 and n=3), lactate dehydrogenase increase (n=0 and n=2), creatinine kinase increase (n=0 and n=1), amylase (n=4 and n=4) and lipase (n=1 and n=5) increase and increases of blood glucose (n=0 and n=2) and serum creatinin (n=0 and n=2). In pregnant women, the majority of adverse laboratory events were of mild to moderate kind, except two grade 3 increases of amylase that resolved following cessation of therapy after giving birth. In non-pregnant women, one of each case, grade 3–4 leucopaenia, haemoglobinemia, AST, ALT, γ-GT and amylase increase occurred during the first 12 weeks on therapy.

In general, significant differences between pregnant and non-pregnant women were not observed and the therapy regimen was well tolerated in both groups. However, one has to take into account the small number of patients compared in this study.

Discussion
Although saquinavir exhibited significantly lower plasma concentrations in pregnant women, it appeared to be effective in the PMTCT and HIV treatment during late pregnancy. The viral load decrease was significant over a mean time of 11 weeks of treatment before giving birth. The immunological and virological response to therapy was comparable to that of non-pregnant women taking a similar therapy regimen during the first 12 weeks of treatment. This is in accordance with a number of previously published data [13–16,31], although these were given for different doses and at dosing intervals. As previous publications show the efficacy and safety of saquinavir dosing regimens that are no longer used because of a lack of availability of the 200 mg capsules [13–16,31], we aimed to evaluate a standard regimen of twice-daily saquinavir 1,000/100 mg, taken during late stage of pregnancy. This regimen was also recently presented by a number of posters [20–22]. It is well known that pregnancy decreases the plasma concentrations of various antiretrovirals, which include NNRTIs, nevirapine [6,12] and the protease inhibitors nelfinavir, indinavir, saquinavir and lopinavir [32–35]. Such decreases might be caused by changes in metabolism that affect the clearance of antiretrovirals as well as causing a significant weight gain during pregnancy and the opening of a new compartment, at least for reverse transcriptase inhibitors [12]. Because HIV protease inhibitors are highly lipophilic substances and are likely to be at least partially trapped in cellular membranes, they do not pass the placenta barrier up to a reasonable amount [33,36–38]. Thus, changes in protease inhibitor plasma levels are mostly because
of an increased hepatic clearance of the drug and an increased volume of distribution caused by physiological changes in the mother.

Pregnant and non-pregnant women showed significant differences in the mean baseline viral load, CD4⁺ T-cell count and age. These differences were most likely attributed to the fact that pregnant women were treated at an earlier stage of HIV infection, as HIV therapy is indicated by the PMTCT and not the stage of disease.

It was shown, however, that of all covariates only weight and ritonavir plasma concentrations were significantly correlated to saquinavir Cₘₘₐₓ and AUCₘₘₐₓ. In general, pregnant women exhibited significantly lower saquinavir Cₘₐₓ and AUCₘₐₓ and a trend towards a decreased Cₘₘₐₓ. However, the small CI for Cₘₘₐₓ in pregnant women suggests that saquinavir is a pharmacokinetically safe regimen during pregnancy. Saquinavir plasma concentrations measured in pregnant women did not fall below the recommended Cₘₘₐₓ of 150 ng/ml and AUCₘₐₓ of 10,000 ng*h/ml [2] and the overall safety and efficacy of this regimen proved satisfactory in all 13 mothers. None of the newborn children were HIV-infected at the age of 18 months.

Taking into account the limitations of this study, for example, the observational study design, the small number of participants and the different saquinavir gel capsule formulations taken, the results of this study have to be valued carefully. This study included only HIV protease inhibitor-naive women. An indication for saquinavir therapy during the last trimester of pregnancy should at least consider the possibility of low and intrindividually variable saquinavir plasma concentrations [39], which might be inefficient against HIV protease inhibitor-resistant virus strains. If plasma drug concentrations are which might be inefficient against HIV protease inhibitor-natived patients [40], then these results point towards the potential benefit of TDM for the individual patient and intraindividually variable saquinavir plasma concentrations [39], which might be inefficient against HIV protease inhibitor-resistant virus strains. If plasma drug concentrations are which might be inefficient against HIV protease inhibitor-natived patients [40], then these results point towards the potential benefit of TDM for the individual patient and

**Disclosure statement**

The authors declare no competing interests.

**References**


