Pharmacokinetics of nelfinavir in HIV-1-infected pregnant and nonpregnant women

P. Villani, M. Floridia,¹ M. F. Pirillo,¹ M. Cusato, E. Tamburrini,² A. F. Cavaliere,³ G. Guaraldi,⁴ C. Vanzini,⁴ A. Molinari,⁵ A. degli Antoni⁵ & M. Regazzi

Department of Pharmacology, IRCCS Policlinico S. Matteo, Pavia, ¹Department of Drug Evaluation and Research, Istituto Superiore di Sanità, ²Department of Infectious Diseases and ³Department of Obstetrics and Gynaecology, Università Cattolica, Rome, ⁴Department of Medical Specialties, Infectious Diseases Clinic, University of Modena and Reggio Emilia, Modena and ⁵Department of Infectious Diseases and Hepatology, Azienda Ospedaliera di Parma, Parma, Italy

Correspondence

Mario Regazzi, Department of Pharmacology, IRCCS Policlinico San Matteo, Piazzale Golgi 2, 27100 Pavia, Italy.

Tel: + 39 3 8250 3471

Fax: + 39 3 8242 2701

E-mail: regazzim@smatteo.pv.it

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Aims

To compare steady-state nelfinavir (NFV) pharmacokinetics in pregnant and nonpregnant HIV-infected women.

Methods

Twenty-five pregnant HIV-infected women were selected from an ongoing observational study evaluating the pharmacokinetics of antiretroviral agents during pregnancy. Twenty of them were in the third and five in the second trimester. Data for the control group of 21 HIV-infected nonpregnant women were taken from a previous multicentre pharmacokinetic trial. All the participating women achieved steady-state plasma concentrations while on a highly active antiretroviral therapy (HAART) regimen including NFV (1250 mg bid) and two nucleoside reverse transcriptase inhibitors (NRTIs). Blood samples for NFV measurement were collected predose (C_{trough}) and at 0.5, 1, 2, 3, 4, 5, 6, 8 and 12 h post dose.

Results

During the third trimester of pregnancy NFV $AUC_{0-12\text{ h}}$ median (range) values were 25.76 (12.61–42.74) $\mu\text{g h}^{-1}\text{ ml}^{-1}$, and were 32.49 (19.16–63.81) $\mu\text{g h}^{-1}\text{ ml}^{-1}$ in the control group [mean difference $-9.30\text{ }\mu\text{g h}^{-1}\text{ ml}^{-1}$; 95% confidence interval (CI) $-15.76, -2.83$; $P < 0.05$). Median oral clearance (CL/F) was significantly higher in pregnant women than in the control group (48.5 l h^{-1} , range 29.3–99.1 l h^{-1} vs. 38.5 l h^{-1} , range 19.6–65.2 l h^{-1} ; mean difference 12.6 l h^{-1} ; 95% CI 3.3, 21.9) but the difference disappeared when CL/F was adjusted for body weight. C_{trough} was significantly ($P < 0.01$) lower in pregnant compared with nonpregnant women (median 0.8 $\mu\text{g ml}^{-1}$, range 0–2.6 $\mu\text{g ml}^{-1}$ vs. 1.5 $\mu\text{g ml}^{-1}$, range 0.5–4.9 $\mu\text{g ml}^{-1}$; mean difference $-1.0\text{ }\mu\text{g ml}^{-1}$; 95% CI $-1.7, -0.31$). The median elimination half-life of NFV observed during pregnancy was 3.7 h (range 1.4–6.6 h), compared with 5.2 (range 3.1–10.1 h) in the control group (mean difference -1.7 ; 95% CI $-2.8, -0.51$).

Conclusions

Our results indicate that women in the later stages of pregnancy may be exposed to subtherapeutic concentrations of NFV. Thus, adjustments in drug dosage or frequency of administration may be required.

Introduction

The use of antiretroviral combination regimens during pregnancy has improved maternal health and significantly decreased mother-to-child HIV-1 transmission from 20.5% in untreated women to less than 2% [1, 2]. However, several pregnancy-related factors can affect the disposition of antiretroviral drugs. In particular, the gastrointestinal disturbances (nausea, vomiting and diarrhoea) that are common over the first 3 months of pregnancy may decrease drug intake or absorption. The complex physiological changes occurring during pregnancy may also affect drug absorption (by increased gastric and intestinal emptying time induced by progesterone), distribution (by increased blood flow and cardiac output and increased total body water and fat) and elimination (by increased kidney filtration rate and changes in hepatic metabolizing capacity), thus having a significant effect on antiretroviral pharmacokinetics [3, 4]. As a result, HIV-infected women may be exposed to subtherapeutic concentrations of some antiretroviral agents during pregnancy, especially in the later stages, and this can lead to incomplete virological suppression, the possible development of resistance and ultimately to an increased risk of vertical transmission of HIV infection.

Current treatment guidelines for HIV-infected pregnant women stress the importance of combination regimens that can maximize viral load suppression. Therefore, in order to protect the health of both HIV-infected women and their newborn, standard antiretroviral therapy in pregnancy is now increasingly based on the use of combination regimens, frequently including protease inhibitors (PIs) [1, 5].

The protease inhibitor nelfinavir (NFV) is widely recommended by current guidelines for use in pregnancy, because of its safety, tolerability and potency. Preliminary data in animal studies have not demonstrated any fetal toxicity of this agent [6].

NFV is mainly metabolized by cytochromes P450 (CYP) 3A4 and 2C19 [7], whose metabolic activity has been shown to be altered during pregnancy, which, together with the physiological changes in gastrointestinal motility, volume of distribution and hepatic blood flow, may significantly affect the pharmacokinetics of the drug.

Previous studies, in relatively small numbers of pregnant women, have shown decreased exposure to PIs. Similar trials with NFV have yielded conflicting results [4, 8].

The objective of this work was to compare the pharmacokinetics of NFV after multiple oral doses in pregnant and nonpregnant women infected with HIV. In

order to assess the transplacental passage of the drug, we also increased NFV concentrations in mothers and their newborn at delivery in a subgroup of subjects.

Materials and methods

Subjects

HIV-infected pregnant women receiving an antiretroviral combination regimen including NFV were selected from an ongoing observational study evaluating the pharmacokinetics of antiretroviral agents during pregnancy and at delivery in HIV-infected women. This pharmacokinetic study is part of an ongoing national surveillance programme on the use of antiretroviral drugs in pregnancy conducted in Italy (Progetto Nazionale per la Sorveglianza sul Trattamento Antiretrovirale in Gravidanza). The studies were approved by the Ethics Committees of the participating centres and each patient provided written informed consent to both. All the women selected achieved steady-state plasma concentrations while on treatment with NFV (at the standard dose of 1250 mg bid) plus two nucleoside reverse transcriptase inhibitors (NRTIs). As part of the standard counselling for patients receiving NFV, the recommendation that the drug should be taken with food was made before the start of treatment and at each visit.

Pharmacokinetic data for the control group of HIV-infected nonpregnant women were taken from a previously conducted multicentre trial [9], using the same method for the determination of NFV as that used in the present study. All subjects achieved steady-state plasma concentrations while on treatment with a HAART regimen including NFV (1250 mg bid) and two NRTIs. Patients with opportunistic infections or liver disease, or who were taking drugs known to interact with NFV, were not included in the control group. For both groups, information from medical histories, haematological, biochemical and virological tests was documented. The pregnant women and nonpregnant controls were matched for age, ethnicity, viral load, CD4, biochemical measurements and clinical assessments. As expected, body weight was the only variable that differed significantly between the two groups.

Sample collection

In pregnant HIV+ women, the steady-state plasma pharmacokinetics of NFV were assessed during the second or the third trimester of pregnancy. Blood samples (5 ml) for NFV trough concentration (C_{trough}) measurement were collected before the morning dose (1250 mg orally). All pregnant women were sampled at the same time of the day (between 08.00 h and 08.30 h). Blood

samples were collected, whenever possible, at 0.5, 1, 2, 3, 4, 6, 8 and 12 h following NFV administration.

Placental transfer was determined by comparison of drug concentrations in blood samples simultaneously collected from a peripheral maternal vein and the umbilical cord at delivery.

For nonpregnant HIV-infected women, blood samples were collected during a dosing interval at time 0 (C_{trough}), 0.5, 1, 2, 3, 4, 5, 6, 8 and 12 h post dose.

Plasma samples were separated and submitted to a heat treatment (56 °C for 45 min) to inactivate HIV and then frozen at –20 °C until analysis. Heat treatment has been reported not to affect NFV concentrations [10], but calibration standards and quality controls were treated in the same way as the samples.

Bioanalytical method

NFV concentrations in plasma were measured by a sensitive, selective and validated method using high-performance liquid chromatography with ultraviolet detection (HPLC-UV). NFV was purchased from Agouron Pharmaceuticals, Inc. (San Diego, CA, USA) and the internal standard, 6,7 dimethyl - 2 - 3 - (2 - pyridil)-quinoxaline, from Sigma Aldrich (St Louis, MO, USA). Briefly, samples were thawed and NFV was extracted from 250 µl of plasma by liquid-liquid extraction with 2.5 ml of a hexane–ethylacetate mixture (50 : 50, v/v), after addition of the internal standard. The organic extract was evaporated to dryness under a gentle nitrogen stream and the residue was dissolved in 300 µl of mobile phase. Chromatographic separation was performed on a Hyper-sil BDS C18 analytical column (250 × 4.6 mm, 5 µm; CPS Analytica, Milano, Italy). The mobile phase consisted of acetonitrile/phosphoric acid (0.1%) and was degassed under vacuum ultrasonically. The flow rate was 1.3 ml min^{–1} and the UV detector was set at 220 nm.

The limit of quantification (LOQ) for NFV was 50 ng ml^{–1} and the limit of detection (LOD) was about 20 ng ml^{–1}.

The intra-assay and the interassay variability of the NFV quality control samples were <15% for the three concentrations (160, 1600 and 6000 ng ml^{–1}) tested. Our laboratory successfully participated in an external quality control programme for NFV and other antiretroviral agents in 2004 (International Interlaboratory Quality Control Program for Therapeutic Drug Monitoring in HIV Infection).

Pharmacokinetic and statistical analyses

The pharmacokinetic analysis was performed using P-Pharm computer software (P-Pharm, SIMED, Creteil, France). The individual pharmacokinetic parameters

were assumed to arise from a log-normal distribution characterized by a population mean and an interindividual variance. Preliminary analysis revealed that the data were best fitted by a one-compartment model (producing the smallest log likelihood, the Akaike criterion, and the best fit to the data points) and that the residuals distribution showed that the error variance was better described by a heteroscedastic (proportional to the squared value of the predictions) model.

The model was parameterized in terms of CL/ F (apparent oral clearance divided by the fraction, F , of the dose appearing in the systemic circulation), V_d/F (apparent volume of distribution divided by F), k_a (absorption rate constant) and lag time (time interval between administration of NFV dosage form and first appearance of the drug in the systemic circulation). From the individual primary pharmacokinetic parameters the area under plasma concentration vs. time curve ($AUC = D \times F/CL$) and the elimination half-life ($t_{1/2} = 0.693 \times V_d/CL$) were calculated.

From the concentration–time data obtained in the control group, we generated a ‘reference curve’ to compare the plasma concentrations obtained in the pregnant women at different time points. For each sample the drug concentration ratio was calculated by dividing the drug concentration measured in that sample by the time-adjusted value in the standardized pharmacokinetic curve [11].

The Mann–Whitney U -test was used to evaluate any difference in pharmacokinetic parameters. A P -value of <0.05 was considered to be statistically significant.

All analyses were conducted using Statistics for Windows software (StatSoft, Inc. 2004; Tulsa, OK, USA).

Results

A total of 25 pregnant HIV+ women from three clinical centres participating in the pharmacokinetics substudy in the National Surveillance Program on Antiretroviral Treatment in Pregnancy were evaluated. All patients were on a stable antiretroviral regimen containing NFV.

Five were in the second trimester of pregnancy (gestational age 21–26 weeks) and 20 in the third trimester (gestational age 31–36 weeks). In six out of the 20 patients evaluated during the third trimester, two blood samples were also collected at delivery, one from the mother and one from the umbilical cord of the newborn.

All pregnant women were receiving 1250 mg of NFV twice a day, plus two NRTIs, zidovudine (300 mg bid) and lamivudine (150 mg bid).

The reference group was composed of 21 HIV+ non-pregnant women who had achieved steady-state concentrations of NFV (1250 mg bid) and who were receiving

two NRTIs. In this group, about seven plasma samples per subject were collected at different time points during the dosing interval.

Patients in both groups had normal liver function and were taking no additional drugs known to interfere with the activity of CYP enzymes. The clinical and demographic characteristics of the HIV+ women (summarized as mean $\pm$ SD) are reported in Table 1.

Sixty-nine NFV concentrations from pregnant women in their third trimester and 145 NFV concentrations from nonpregnant women were available. The regression line for the empirical Bayes-predicted values and individual observed NFV concentrations did not differ significantly from a reference line with a slope of 1 and an intercept of 0. The bias was not statistically different from 0 and the 95% confidence interval included zero.

Pharmacokinetic parameters for NFV were not determined in the pregnant women in the second trimester ($n = 5$), because they only had a C_{trough} sample taken.

The results of the pharmacokinetic analysis in the control group and in pregnant HIV+ women in their third trimester are summarized in Table 2. The mean plasma concentration–time profiles are shown in Figure 1. The median (range) value of the $AUC_{0-12\text{ h}}$ was 32.49 (19.16–63.81) $\mu\text{g h}^{-1} \text{ mL}^{-1}$ in the control group and 25.76 (12.61–42.74) $\mu\text{g h}^{-1} \text{ mL}^{-1}$ in pregnant women in the third trimester.

The absorption rate constant (k_a) of NFV was 0.86 (0.82–0.91) h^{-1} and 0.88 (0.81–0.99) h^{-1} in pregnant and nonpregnant women, respectively; the difference was not statistically significant (NS). The time interval between administration of NFV and the first appearance of drug in systemic circulation (lag time) was 0.8 (0.78–0.89) h and 0.8 (0.64–1.0) h in each group of patients, respectively (NS).

Median oral clearance (CL/F) was significantly lower ($P < 0.05$) in the control group (38.5 l h^{-1} , range 19.6–65.2 l h^{-1}) than in pregnant women (48.5 l h^{-1} , range 29.3–99.1 l h^{-1}). However, when oral clearance was

Table 1

Demographic and clinical characteristics of the patients studied (mean $\pm$ SD)

Patients	Weight (kg)	Alanine aminotransferase (U L^{-1})	Aspartate aminotransferase (U L^{-1})	CD4+ (cells μL^{-1})	Viral load (copies mL^{-1})
Pregnancy, 2nd trimester ($n = 5$)	61 $\pm$ 7	17 $\pm$ 13	24 $\pm$ 12	441 $\pm$ 225	2373 $\pm$ 2748
Pregnancy, 3rd trimester ($n = 20$)	71 $\pm$ 12	16 $\pm$ 9	22 $\pm$ 11	545 $\pm$ 336	3951 $\pm$ 10322
Controls ($n = 21$)	58 $\pm$ 8	16 $\pm$ 12	18 $\pm$ 5	389 $\pm$ 194	4176 $\pm$ 12185

Table 2

Pharmacokinetic parameters for nelfinavir

Parameters	Nonpregnancy ($n = 21$), median (range)	Pregnancy (3rd trimester) ($n = 20$) median (range)	Mean difference (95% CI)
CL/F ($\text{l h}^{-1} \text{ kg}^{-1}$)	0.62 (0.31–0.99)	0.62 (0.37–1.52)	0.02 (–0.16, 0.20)
CL/F (l h^{-1})	38.5 (19.6–65.2)	48.5 (29.3–99.1)	12.6 (3.3, 21.9)*
AUC ($\mu\text{g h}^{-1} \text{ mL}^{-1}$)	32.49 (19.16–63.81)	25.76 (12.61–42.74)	–9.30 (–15.76, –2.83)*
V_d/F (l kg^{-1})	4.9 (3.4–7.5)	3.5 (2.7–6.0)	–1.5 (–2.36, –0.73)**
V_d/F (l)	278.2 (199.0–360.0)	259.1 (182.3–309.8)	–18.9 (–43.9, 6.1)
$t_{1/2}$ (h)	5.2 (3.1–10.1)	3.7 (1.4–6.6)	–1.65 (–2.79, –0.51)**
k_a (h^{-1})	0.88 (0.81–0.99)	0.86 (0.82–0.91)	–0.01 (–0.04, 0.01)
Measured C_{trough} ($\mu\text{g mL}^{-1}$)	1.5 (0.5–4.9)	0.8 (0–2.6)	–1.0 (–1.7, –0.3)**

The 95% confidence interval (CI) is given on the differences. * $P < 0.05$; ** $P < 0.01$.

Figure 1

Plasma concentration–time profiles (mean $\pm$ SE) for nelfinavir (NFV) in the control group and in pregnant women during the third trimester. $\diamond$, Control group; $\bullet$, pregnant women.

adjusted for body weight, no significant difference was observed (Table 2).

The apparent volume of distribution divided by the fraction of the dose absorbed (V_d/F), was higher in the control group than in pregnant women, and the differences became significant when this parameter was normalized for body weight. The elimination half-life ($t_{1/2}$) was significantly shorter ($P < 0.01$) in pregnant (median 3.7 h, range 1.4–6.6 h) than in nonpregnant women (median 5.2 h, range 3.1–10.1 h).

Plasma NFV concentration immediately before the next dose (C_{trough}) exhibited considerable interindividual variability, as indicated by the high coefficients of variation (CV %) in both nonpregnant (67%) and pregnant women (83%). Compared with nonpregnant women (median C_{trough} 1.5 $\mu\text{g ml}^{-1}$, range 0.5–4.9 $\mu\text{g ml}^{-1}$), women in the second trimester showed a trend towards lower C_{trough} values (median 1.0 $\mu\text{g ml}^{-1}$, range 0–3.0 $\mu\text{g ml}^{-1}$), and two of the latter had undetectable drug concentrations. The limited number of patients with a second trimester sample ($n = 5$) precluded a statistical comparison with nonpregnant women.

NFV C_{trough} values in pregnant women were decreased further during the third trimester of pregnancy and were significantly ($P < 0.01$) lower than those from nonpregnant women (median 0.8 $\mu\text{g ml}^{-1}$, range 0–2.6 $\mu\text{g ml}^{-1}$ vs. median 1.5, range 0.5–4.9 $\mu\text{g ml}^{-1}$, respectively). Four out of these 20 patients had undetectable NFV concentrations.

The concentration ratio varied during the dosing interval, ranging from 0.12 at 12 h to 0.9 at 3 h. There was no significant difference between groups in plasma NFV concentrations measured at times expected to be close to the peak plasma concentrations. Median (range)

$C_{3\text{h}}$ value was 3.8 (1.1–5.5) $\mu\text{g ml}^{-1}$ in pregnant women and 4.1 (1.8–6.5) $\mu\text{g ml}^{-1}$ in the controls.

In the six women evaluated at delivery, we found a median plasma NFV concentration of 0.15 $\mu\text{g ml}^{-1}$ (range 0–1.82 $\mu\text{g ml}^{-1}$) and three had undetectable concentrations. NFV was not detectable in any of the six cord blood samples obtained at delivery.

Discussion

Important physiological changes occurring during pregnancy may alter the pharmacokinetics of many drugs. Increases in body weight, distribution volume, hepatic blood flow, and changes in metabolic activity all contribute to decreasing plasma concentrations of and whole body exposure to a range of drugs. Decreased plasma concentrations of PIs during pregnancy have been described previously [8]. Elevated concentrations of endogenous glucocorticoids, peaking during the third trimester of pregnancy, may induce CYP3A4, the main enzyme involved in the metabolism of PIs, thereby decreasing their plasma concentrations. Moreover, changes in absorption and protein binding may also contribute to this change, although the effect of pregnancy on both these variables needs to be evaluated further.

The addition of ritonavir to saquinavir therapy significantly improved bioavailability of the latter in pregnant women ($n = 13$) on an 800-mg SQV/100-mg RTV twice a day regimen. The combinations produce C_{trough} and AUC values high enough to suppress the replication of SQV-sensitive virus strains. However, the C_{max} , C_{trough} and AUC values for ritonavir were significantly different between gestational and post-partum periods, with a 57% increase during the latter [12].

A study in two pregnant women during their second and third trimesters and post partum found a marked difference in the AUC of indinavir between the third trimester and post partum. Despite an unchanged indinavir dose, the two patients had 63% and 86% lower AUCs during the third trimester [13].

A study evaluating the pharmacokinetics of indinavir during pregnancy and post partum (four women) and nelfinavir (nine women) demonstrated a decrease in indinavir concentrations during pregnancy. However, the results were highly variable for NFV and the influence of pregnancy was not clearly defined. In this study, increased CYP activity was also demonstrated based on the urine 6 beta-hydroxycortisol to cortisol ratio [14].

NFV plasma concentrations were found to be decreased in pregnant women (nine subjects) when 750 mg was administered three times daily [15]. Conversely, when NFV was given at a dose of 1250 mg

twice daily to a group of 21 pregnant women, exposure was equivalent to that in nonpregnant adults [16].

In a recent study during pregnancy and post partum in 11 women receiving the standard dose of 1250 mg bid, NFV exposure (AUC) was decreased by 24% during late pregnancy, with the decrease being statistically significant for C_{trough} (57% lower) when compared with the postpartum period [17]. Exposure to the active metabolite M8 was decreased by about 70% during pregnancy and the authors suggested that pregnancy may affect the balance of CYP2C19-mediated formation and the CYP3A4-mediated clearance of M8 [17].

In our study, we observed the same trend towards lower trough plasma concentrations of NFV in pregnant women during the second trimester of pregnancy. This trend became statistically significant at the third trimester, with more than 50% lower trough concentration observed in pregnant women compared with HIV-infected nonpregnant women. At delivery, concentrations were even lower.

C_{trough} may be a clinically important parameter in the assessment of virological response to treatment. A clear relationship has been demonstrated between antiretroviral drug concentrations and virological response, particularly for PIs [18]. For each of these agents, a 'target' trough value has been suggested as the minimum effective concentration [19].

The suggested therapeutic threshold for NFV has been set at $0.8 \mu\text{g ml}^{-1}$ for antiretroviral treatment-naïve patients [19], which is presumably higher for chronically treated subjects due to the presence of resistant viral strains. The majority of our pregnant patients (55%) had subtherapeutic drug concentrations at the third trimester, compared with a minority of nonpregnant women (four out of 21, 19%). At delivery, which represents a crucial time for HIV transmission, 4/6 patients (67%) had NFV concentrations below the threshold of $0.8 \mu\text{g ml}^{-1}$.

The decrease in NFV plasma concentrations has been attributed to several factors, such as: changes in CYP3A4 or CYP2C19 activity, which occur during pregnancy [20, 21]; an increase in liver blood flow and volume of distribution (blood and plasma volumes increase 40–50% during pregnancy); an increase in total body water (because of new tissue growth and fluid pooling in the extracellular extravascular space) [22, 23]. In addition, the increased production of progesterone affects gastrointestinal motility. Protein binding does not seem to be involved, as NFV binds primarily to α_1 -acid glycoprotein, the concentration of which is unchanged in pregnancy [24].

Consistent with the findings of van Heeswijk *et al.*

[17], we observed that the difference in AUC and CL/F values, which are mainly due to body weight differences, was not so marked in pregnant compared with nonpregnant patients. Furthermore, we found no significant difference between groups in the 3-h post-dose concentrations.

In pregnancy, despite a decrease in intestinal motility, the higher (30–50%) cardiac output increases intestinal blood flow, which might enhance absorption for drugs where membrane transport is the rate-limiting step. The lower plasma NFV concentrations observed during the drug elimination phase than those during the absorption phase could indicate that increased drug elimination seen in pregnant women is partially compensated for by a greater amount of drug absorbed. Alternatively, the overlapping CL/F values (when normalized for body weight) observed in the two patient groups and the marked decrease in V_d/F in pregnant women (in contrast to the decrease of $t_{1/2}$ and C_{trough}) could be explained by an increased bioavailability of NFV during pregnancy. Such an increase might mask the expected rise in systemic clearance during pregnancy and could account for the variable results previously reported on the influence of pregnancy on NFV pharmacokinetics.

NFV concentrations in all cord blood samples obtained from six patients at delivery were below the lower limit of detection of the assay. This confirms the limited passage of the drug across the placenta observed for NFV and other PIs [25, 26]. This limited transfer may result from the high degree of plasma protein binding of NFV and from active P-glycoprotein from the placenta to maternal blood. Low concentrations of NFV in the fetus decrease the risk of possible toxic effects, but could fail to exert antiviral activity and therefore decrease protection against mother-to-child transmission of HIV-1.

In conclusion, our results indicate that pregnant women during the later stages of pregnancy may be exposed to subtherapeutic concentrations of NFV, which may require adjustments in dosage or frequency of administration. Moreover, given the high interindividual variability in plasma drug concentrations, we believe that therapeutic drug monitoring of NFV could be helpful in the selection of dosing regimens in this population, to ensure optimal efficacy to prevent the possible development of resistance and perinatal transmission. Further trials are warranted to confirm our observations.

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