Pharmacokinetic modelling of the placental transfer of nelfinavir and its M8 metabolite: a population study using 75 maternal-cord plasma samples

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What is already known about this subject

- Nelfinavir is an HIV protease inhibitor used in pregnant women, although no *ex vivo* and few *in vivo* data are available describing its transfer across the placenta.

What this study adds

- A population pharmacokinetic study was performed on maternal, cord plasma and amniotic fluid samples to characterize the concentration–time courses of nelfinavir and its metabolite M8 in the three compartments and to study the influence of covariates, such as weight and duration of pregnancy, on placental transfer.
- The pharmacokinetics of nelfinavir and M8 were satisfactorily described by a three compartment model. The median nelfinavir fetus : maternal concentration ratio was 25% for maternal concentrations between 0.1 and 2.5 mg l⁻¹, between the 31st and 41st week of gestation, irrespective of bodyweight. This ratio was significantly higher for M8 (a 55% increase).
- To our knowledge this is the first population pharmacokinetic integrated model to describe satisfactorily the maternal-foetal-amniotic fluid transfer of a drug.

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Aims

A population pharmacokinetic model was developed to characterize the transfer of nelfinavir and its active metabolite M8 from maternal to cord plasma and amniotic fluid.

Methods

Concentration data were obtained from 75 women on the day of delivery and for whom maternal, umbilical plasma and amniotic fluid samples were collected. Data from 53 pregnant, 61 nonpregnant and seven consecutively pregnant and non pregnant women were then added to the database, the contents of which were analyzed using NONMEM.

Results

Nelfinavir and M8 concentrations in maternal plasma, umbilical plasma and amniotic fluid were described by six connected compartments. Mean (% intersubject variability) population estimates were: absorption rate 00.67 h⁻¹, lag time 00.87 h, oral clearance and volume of distribution: 39.5 l h⁻¹ (53%), and 557 l for non pregnant and pregnant women, respectively, and 115 l h⁻¹ (132%) and 1626 l, respectively, on the day of delivery, M8 formation clearance 0.77 l h⁻¹ and M8 elimination rate constant 03.41 h⁻¹ (74%). For nelfinavir and M8, respectively, the mother-to-cord parameters were 0.058 l h⁻¹ (34%), and 00.35 h⁻¹ (76%), the cord-to-amniotic fluid rate constants were 0.23 and 00.59 h⁻¹, and the elimination rate constants from amniotic fluid were 0.36 and 00.49 h⁻¹. The nelfinavir fetus : maternal concentration ratio was 25% for maternal concentrations between 0.1 and 2.5 mg l⁻¹, between the 31 and 41st week of gestation.

Conclusions

The low transfer of nelfinavir from the placenta is unlikely to protect the fetus from vertical HIV-1 transmission.

Introduction

Nelfinavir is an HIV protease inhibitor used in highly active antiretroviral therapy. Because of its tolerability and potency it has been widely used in pregnancy to treat the mother, and for the prevention of mother-to-child transmission. *In vivo* studies of the placental transfer of nucleoside and non nucleoside reverse transcriptase inhibitors has been reviewed by Pacifici [1]. However few *ex vivo* [2–4] and *in vivo* data [5–8] are available describing the transfer of protease inhibitors (PIs) across the placenta, and *ex vivo* studies for nelfinavir are lacking. Two studies performed on pairs of maternal-cord plasma samples suggested that protease inhibitors cross the placenta poorly, and that nelfinavir transfer was greater than the other PIs, with a cord : mother concentration ratio of 0.24 [5, 8] (range 0–0.3 [6]).

The oral bioavailability of nelfinavir is highly variable in adults (20–80%) and increases 2–3 fold when administered with food [9]. The apparent volume of distribution of nelfinavir is 2–7 l kg⁻¹, and the drug binds extensively (98%) to both α_1 -acid glycoprotein (AAG) and albumin in plasma [9]. Nelfinavir is converted to an active metabolite, hydroxy-tert-butylamide (M8), by CYP2C19, and both compounds are metabolized by CYP3A4 [10, 11].

The physiological changes associated with pregnancy can lead to considerable variations in pharmacokinetics. In pregnant women, an increase in plasma progesterone concentration, which is believed to increase gastric and intestinal emptying time, could add to the variability in the absorption of nelfinavir [12]. Moreover, an increase in plasma volume, fat stores and body weight could increase the apparent volume of distribution of nelfinavir [13]. Binding to albumin is decreased during pregnancy, but it is not clear whether binding to AAG changes [14]. Furthermore, pregnancy may produce alterations in hepatic drug metabolism (involving the CYP enzymes), as a possible result of enzyme induction by progesterone [15]. Nelfinavir and M8 are subject to active transport mediated by P glycoprotein (P-gp) [16]. This transporter protein is present on the maternal side of the syncytiotrophoblast (in the placenta) where it carries xenobiotics back into the maternal circulation, thus limiting fetal exposure [17].

In the present work, a population pharmacokinetic study was performed on maternal, cord plasma and amniotic fluid samples in order to confirm some of the above findings in a larger number of pregnant women, to describe the concentration–time courses of nelfinavir and its metabolite M8 in the three compartments and to study the influence of covariates (such as weight, duration of pregnancy) on nelfinavir and M8 pharmacokinetics.

This was achieved by (i) developing an integrated pharmacokinetic model to describe simultaneously the transfer of nelfinavir and M8 from maternal plasma to cord plasma and amniotic fluid and (ii) using a pharmacostatistic model to identify the patient characteristics that could influence nelfinavir and M8 transport.

Methods

Patients

Initially, only women at delivery were studied, but samples from pregnant and non pregnant women were then included to improve the accuracy of the maternal pharmacokinetic estimates. All women were receiving oral nelfinavir for the treatment of HIV infection, and their antiretroviral drug plasma concentrations were monitored on a routine basis. Nelfinavir was administered as a 750 mg thrice daily or a 1250 mg twice daily regimen, using 250 mg tablets. The time between administration and blood sampling, the time of dosing, bodyweight (BW), age, weeks of gestation, and combined treatments, particularly other antiretroviral drugs, were recorded. All plasma samples were collected at steady state, considered to be reached after 6 days of treatment [25]. When poor compliance was consistently suspected (characterized by undetectable plasma concentrations of nelfinavir and M8), the data were not included in the analysis. When the time between administration and sampling was more than 15 h for twice daily and 11 h for thrice daily dosing, the data were not included in the analysis. The data from four women who were administered ritonavir with nelfinavir were also excluded from the analysis. The study was performed at Port Royal Hospital (Paris) and Louis Mourier Hospital (Colombes). Ethics committee approval and patient consent are not compulsory in France for the use of therapeutic drug monitoring data obtained retrospectively. Thus, informed consent was not obtained in non-pregnant women. All pregnant women were enrolled with informed consent in the French Perinatal Cohort, a study that was approved by an institutional review board, as described previously [20].

Analytical methods

Nelfinavir and M8 plasma concentrations were measured by HPLC. Drug, metabolite and internal standard (clazepam SL 72469) were extracted from 200 μ l of plasma with a 6 ml mixture of ethyl acetate : hexane (v : v) in an alkaline medium (0.2 M sodium carbonate 0.5 ml). After evaporation, the residue was dissolved in mobile phase consisting of acetonitrile-perchlorate tetramethyl ammonium (0.01 M) in trifluoroacetic acid (0.01%) (37 : 63, v : v). Chromatography was

performed using a reverse phase C8 analytical column (Nucleosil C8 125 × 4.6 mm, 3 µm, Macherey-Nagel) and gradient elution with an increase in the proportion of acetonitrile from 37 to 45%. UV detection at 205 nm was used. Interday accuracy for the two analytes ranged from 92.9 to 97.6%, and interday precision, expressed as a percent coefficient of variation, was less than 10%.

Initial data analysis

Concentrations that were below the limit of quantification (LOQ) were set to half this value [21] (i.e. 0.1 mg l⁻¹ for nelfinavir and 0.025 mg l⁻¹ for M8). The cord-to-mother plasma concentration ratio and the amniotic fluid-to-cord plasma concentration ratio were compared by a non parametric Mann-Whitney test. Ratio data were not analyzed when maternal (for cord : maternal ratio) or cord (for amniotic fluid : cord) concentrations were below the LOQ.

Population pharmacokinetic modelling of nelfinavir and M8. Data were analyzed using the nonlinear mixed effect modelling program NONMEM (version V, level 1.1, double precision) with the DIGITAL FORTRAN compiler [22]. The first-order conditional estimation (FOCE) method was used. Nelfinavir data were first analyzed according to a one-compartment open model. The pharmacokinetic parameters for the parent compound were used to produce the input function into the metabolite compartment. Parameters for the nelfinavir-M8 model were the absorption rate constant (k_a), absorption lag time (t_{lag}), apparent distribution volume of nelfinavir (V/F), apparent nelfinavir oral clearance ($CL_{Nm \rightarrow No}/F$ for nelfinavir mother to nelfinavir out only), M8 apparent formation clearance ($CL_{Nm \rightarrow M8m}/F$ for nelfinavir mother to M8 mother) and M8 elimination rate constant ($k_{M8m \rightarrow M8o}$ for M8 mother to M8 out), where F is the bioavailability. The M8 distribution volume could not be determined and was given the value of 1.

The pharmacokinetic parameters from the nelfinavir-M8 model were then used to produce the input function into cord compartments, which were then used as the input function to amniotic fluid compartments. The additional parameters for this model were the nelfinavir ($k_{Nm \rightarrow Nc}$) and M8 ($k_{M8m \rightarrow M8c}$) rate constants for transfer from mother to cord plasma, the nelfinavir ($k_{Nc \rightarrow Naf}$) and M8 ($k_{M8c \rightarrow M8af}$) rate constants for transfer from cord plasma to amniotic fluid, and the nelfinavir ($k_{Naf \rightarrow No}$) and M8 ($k_{M8af \rightarrow M8o}$) rate constants of elimination from amniotic fluid (Figure 1).

Exponential, proportional and additive error models were investigated to describe intersubject (ISV) and residual variabilities.

Figure 1

Pharmacokinetic model for the simultaneous prediction of nelfinavir and M8 concentrations in three compartments: mother plasma, cord plasma and amniotic fluid after oral dosing with nelfinavir. k_a denotes the absorption rate constant, V/F the nelfinavir distribution volume in the maternal compartment, $CL_{Nm \rightarrow No}/F$ the nelfinavir elimination clearance, $CL_{Nm \rightarrow M8m}/F$ the nelfinavir-to-M8 formation clearance in the maternal compartments, $k_{M8m \rightarrow M8o}$ the M8 elimination rate constant from the mother, $CL_{Nm \rightarrow Nc}/F$ (nelfinavir) and $k_{M8m \rightarrow M8c}$ (M8) are parameters of transfer from mother to cord plasma, $k_{Nc \rightarrow Naf}$ (nelfinavir) and $k_{M8c \rightarrow M8af}$ (M8) are rate constants of transfer from cord plasma to amniotic fluid, and $k_{Naf \rightarrow No}$ (nelfinavir) and $k_{M8af \rightarrow M8o}$ (M8) are rate constants of elimination from amniotic fluid

The influence of each patient covariate was systematically tested via generalized additive modelling according to the following equation, using CL as the example,

$$CL = TV(CL) \times [BW/\text{median}(BW)]^{\theta_{BW}}$$

where $TV(CL)$ is the typical value of clearance for a patient with the median covariate value and θ_{BW} is the estimated influential factor for bodyweight (BW). Such covariates included age and BW.

The categorical covariates (CC), pregnancy (PREG), delivery (DEL), diurnal variation in nelfinavir disposition and cotherapy with antiretroviral drugs were tested as follows:

$$CL = TV(CL) \times (1 + \theta_{CC} \times (CC = 0 \text{ or } 1))$$

(for an inducing agent)

or in the case of an inhibitory drug effect,

$$CL = TV(CL)/(1 + \theta_{CC} \times (CC = 0 \text{ or } 1))$$

(for an enzyme inhibitor)

On the day of delivery, the coding was zero for pregnancy and one for delivery.

Covariates were selected for the final population model if (i) their effect was biologically plausible, (ii) they produced a minimum decrease of 4 in the objective function value (OFV) and (iii) they produced a decrease in the variability of the pharmacokinetic parameter, assessed by the associated intersubject variability. An intermediate multivariate model was then obtained including all significant covariates. In order to retain only those covariates with the largest contribution in the final multivariate model, a change of 7 ($P < 0.01$, one degree of freedom) of the OFV was required during backward stepwise multiple regression analysis.

For evaluation of goodness-of-fit, the following relationships were compared: observed and predicted concentrations *vs.* time, observed concentrations *vs.* predictions (OBS-PRED), weighted residuals (WRES) *vs.* time and weighted residuals *vs.* PRED (WRES-PRED) as well as the corresponding relationships issued from the POSTHOC estimation step. Diagnostic graphics and distribution statistics were obtained using the R program [23].

Bootstrap validation. The accuracy and robustness of the final population model were assessed using a bootstrap method, as described previously [24]. Briefly, this includes the following steps,

- i) from the original data set of n individuals, B bootstrap sets ($B = 1000$) of n individuals are drawn with replacement (resampling),
- ii) for each of the B bootstrap sets, the population pharmacokinetic parameters are estimated,

- iii) with the B estimates of each population pharmacokinetic parameter, the corresponding mean and SD are estimated,
- iv) to validate the model, the parameters estimated from the bootstrap must be close to estimates obtained from the original population set.

The entire procedure was performed in an automated fashion using Wings for NONMEM (<http://wfn.sourceforge.net/wfninst.htm>). This also allowed non-parametric statistics (median, 2.5th, 97.5th percentiles) of the population parameters.

Concentration–time curve in mother, cord plasma, amniotic fluid for nelfinavir and M8. Based on the Bayesian estimates obtained with the POSTHOC option of NONMEM, individual pharmacokinetic parameters were determined, followed by the estimation of nelfinavir and M8 concentrations in the three compartments as a function of time.

Results

Data from 75 women on the day of delivery (77 samples) were available for pharmacokinetic modelling. The duration of pregnancy was between 31 and 41 weeks (median = 38). Data from 53 pregnant women (75 samples, mean gestation time = 32 weeks, range 10–39), 61 nonpregnant women (120 samples) and from a further seven non pregnant (eight samples) and pregnant women (12 samples) were subsequently added to this database to ensure a more accurate estimate of k_a and V . Two samples out of the 294 were excluded, because the time that had elapsed between administration and sampling was up to 15 h for twice daily dosing or 11 h for thrice daily dosing. Table 1 summarizes the characteristics of the patients: age, weight, regimen, percentage of

Table 1

Patient characteristics

	Number of women (number of samples)	
	75 at delivery ($n = 77$)	61 nonpregnant ($n = 120$) 53 pregnant ($n = 75$) 7 nonpregnant + pregnant ($n = 20$)
Age (years)	32.7 ± 4.4	33.4 ± 4.5
Weight (kg)	73 ± 14	65 ± 15
Twice daily regimen (%)	92	82
% of dosing after morning dose	68	64
Distribution of dosing time (h)	8.1 ± 5.2	6.4 ± 4.6
Number of women who were co-administered a NNRTI	8	13

Table 2

Percentage of nelfinavir and M8 concentrations under the LOQ

% concentrations under LOQ	Maternal plasma	Umbilical plasma	Amniotic fluid
Number of samples	77	77	27
Nelfinavir (%)	3	38	56
M8 (%)	23	47	33

Table 3

Comparisons between nelfinavir and M8 observed ratios

Ratios (both concentrations above LOQ)	Number of samples	Median (Min–Max) [Confidence interval]	Mann–Whitney test
Nelfinavir cord : maternal concentration ratio Nc : Nm	73	0.25 (0.05–5.18) [0.22, 0.51]	$P < 10^{-4}$
M8 cord : maternal concentration ratio M8c : M8m	55	0.55 (0.07–8.14) [0.46, 1.05]	
Nelfinavir amniotic fluid : cord plasma concentration ratio Naf : Nc	17	0.59 (0.27–2.54) [0.45, 1.06]	$P = 0.02$
M8 amniotic fluid : cord plasma concentration ratio M8af : M8c	19	1.00 (0.05–2.5) [0.74, 1.23]	

dosing after the morning dose, distribution of dosing time and other concomitant medications.

Table 2 summarizes the percentage of nelfinavir and M8 maternal, umbilical plasma and amniotic fluid concentrations below the limit of quantification. Table 3 shows the data for nelfinavir and M8 transfer from mother to cord and from cord to amniotic fluid. The median cord : mother plasma concentration ratio was significantly higher for M8 (55%) than for nelfinavir (25%) ($P < 10^{-4}$). A significant difference was also observed between the amniotic fluid : cord plasma concentrations ratio for nelfinavir and M8 (59 and 100%, respectively, $P = 0.02$).

A one-compartment model adequately described the data for nelfinavir, and the pharmacokinetics of M8 were modelled as a metabolite compartment connected to the central one (Figure 1). Inter-subject and residual variabilities were best described by exponential and additive error models, respectively. The available data were not sufficient to estimate intersubject variability for k_a and t_{lag} and $CL_{Nm \rightarrow M8m}$, and exclusion of these random effects had no influence on the OFV. Delivery (DEL) significantly increased $CL_{Nm \rightarrow No}$ by 92%, resulting in a 41 unit decrease in the OFV. When an effect of bodyweight was added to $CL_{Nm \rightarrow No}$ in the delivery group only, OFV decreased by 8 units and the correlation between

observed and predicted concentrations in this group was three times better. A 10 kg increase from mean bodyweight increased $CL_{Nm \rightarrow No}$ 1.44 fold. Adding the covariate pregnancy (PREG) also had a significant effect on $CL_{Nm \rightarrow No}$, which was increased by 22%, resulting in a decrease in OFV of 5 units. The effect of delivery on V resulted in an 8 unit decrease in the OFV. Finally the same delivery effect on both V and $CL_{Nm \rightarrow No}$, acting in fact on bioavailability, was chosen because the use of two different delivery effects did not improved the OFV. The following equations described the final covariate model for nelfinavir:

$$CL_{Nm \rightarrow No} = TV(CL) \times (1 + \theta_{DEL} \times (DEL = 0 \text{ or } 1) \times (BW/73)^{\theta_{BW}} \times (1 + \theta_{PREG} \times (PREG = 0 \text{ or } 1)))$$

$$V = TV(V) \times (1 + \theta_{DEL} \times (DEL = 0 \text{ or } 1))$$

In the 'pregnancy' group, $k_{M8m \rightarrow M8o}$ was significantly increased by 67%, resulting in a 12 unit decrease in OFV. The effect of weight was also significant, resulting in a 17 unit decrease. Thus, for every 10 kg increase above the mean weight of 63 kg, $k_{M8m \rightarrow M8o}$ was increased by 1.23. The enzyme inducing effect of non nucleoside transcriptase inverse inhibitors led to a 5 unit decrease in OFV, and increased M8 elimination by 148%. The

following equation described the final covariate model for M8 elimination was:

$$k_{M8m \rightarrow M8o} = TV(k_{M8m \rightarrow M8o}) \times [BW/\text{median}(BW)]^{0.9BW} \\ \times (1 + \theta_{\text{PREG}} \times (\text{PREG} = 0 \text{ or } 1)) \\ \times (1 + \theta_{\text{NNRTI}} \times (\text{NNRTI} = 0 \text{ or } 1))$$

The pharmacokinetics of nelfinavir and M8 were described in cord plasma and amniotic fluid by the sequential compartments, cord connected to mother and amniotic fluid connected to cord. (Figure 1) The available data from 75 women at delivery were not sufficient to estimate the intersubject variability in the nelfinavir $k_{Nc \rightarrow Naf}$ and $k_{Naf \rightarrow No}$ and the M8 $k_{M8c \rightarrow M8af}$ and $k_{M8af \rightarrow M8o}$ rate constants for transfer from cord plasma to amniotic fluid and for elimination from amniotic fluid, respectively, and exclusion of these random effects had no influence on the OFV. As intersubject variability in nelfinavir and M8 transfer parameters through the placenta could be estimated, we tested weight and age of gestation as possible covariates but neither had a significant effect on these parameters.

A backward elimination step was performed, and the deletion of pregnancy on $CL_{Nm \rightarrow No}$ resulted in a 5 unit increase in the OFV. As a result, this covariate was deleted from the model. Table 4 summarizes the covariate modelling, and Table 5 summarizes the final population pharmacokinetic estimates. The correlation coefficient between $\sigma_{\text{Nelfinavir}}$ and σ_{M8} was 0.50 ($\pm 31\%$).

The performance of the final model was tested by comparing population predicted vs. observed plasma concentrations for each of the six compartments (Figures 2 and 3). Because in most cases, only one sample per patient was available, Bayesian estimates, obtained using the POSTHOC option of NONMEM, led to very good correlation between individual predicted and observed concentrations (data not shown).

Table 4

OFV changes from the backward elimination step based on the final model

Covariate deleted	OFV increase
None	0
Bodyweight for women at delivery on $CL_{Nm \rightarrow No}$	+10
Delivery on $CL_{Nm \rightarrow No}$ and V	+69
Weight on $k_{M8m \rightarrow M8o}$	+15
Pregnancy on $k_{M8m \rightarrow M8o}$	+13
NNRTI on $k_{M8m \rightarrow M8o}$	+10

The final model obtained with the original dataset was subjected to a bootstrap analysis. As shown in Table 5, the median parameter estimates and their variability obtained from the bootstrap analysis of 1000 runs, were reasonably close to those previously obtained with the original dataset.

For the 75 women studied, pharmacokinetic parameters were estimated and used to calculate concentrations as a function of time. The curves for nelfinavir and M8 were almost parallel for 72 women, and only three who had a very low volume of distribution had a faster elimination phase. In the group of 72 women, we chose the highest, median and the lowest concentration curves for nelfinavir and M8. Maternal and fetal curves are shown in Figure 4 for nelfinavir and Figure 5 for M8. A plasma concentration of 0.8 mg l^{-1} (considered to be the lower limit of the therapeutic range in children) was not achieved in any of the newborns.

Discussion

Use of antiretroviral drugs is an integral part of prenatal care in women infected with HIV (<http://aidsinfo.nih.gov/Guidelines/GuidelineDetail.aspx?MenuItem=Guidelines&>). Beyond the issue of HIV, use of prescription drugs is increasingly frequent in pregnant women. In a French population-based survey [26], 99% of pregnant women received a prescription for at least one drug, with a mean of 13.6 medications per patient. Most of these drugs were in FDA categories B or C, meaning that 'there are no adequate and well-controlled studies in pregnant women'.

For obvious ethical reasons, *in vivo* drug studies in pregnant women have been restricted to single maternal plasma, with umbilical plasma and amniotic fluid samples obtained only at the time of delivery. Thus, most studies of placental transfer have used the ratio between the concentration in the fetal and maternal compartments as an index of relative fetal drug exposure. However, as only one maternal cord sample per woman is generally available, this ratio does not take account of the time that has elapsed between the last administration of drug and the sampling time, nor of intersubject and residual variabilities.

Population pharmacokinetics are particularly useful for drug studies in pregnancy, since they allow the use of sparse sampling protocols and the estimation of intersubject and residual variabilities. Moreover, samples can be taken at any time after drug administration. This method has been used to describe placental transfer of metformin [27], in which a one compartment maternal model with the fetus as a second compartment was fitted to data from 27 patients, including eight maternal-

Table 5

Population PK parameters for nelfinavir and M8 from the original dataset and following the bootstrap validation

Parameter	Final model original dataset	Bootstrap*	
	Mean (CV%)	Median	2.5th–97.5th percentiles
<i>Structural model</i>			
k_a (h^{-1})	0.67 (23)	0.81	0.11–1.73
t_{lag} (h^{-1})	0.87 (2)	0.90	0.31–2.26
V (l)	557 (25)	632	66–1650
$CL_{Nm \rightarrow No}/F$ ($l h^{-1}$)	39.5 (6)	38.1	31–45
$CL_{Nm \rightarrow No}/F$ and V , θ_{DEL}	1.92 (20)	1.95	1.23–3.05
$CL_{Nm \rightarrow No}/F$, θ_{BW} (on DEL)	2.81 (34)	3.25	1.49–6.06
$CL_{Nm \rightarrow M8m}/F$ ($l h^{-1}$)	0.77 (10)	0.86	0.34–4.35
$k_{M8m \rightarrow M8o}$ (h^{-1})	3.41 (14)	3.93	1.51–20.4
$k_{M8m \rightarrow M8o}$, θ_{PREG}	0.67 (31)	0.71	0.27–1.29
$k_{M8m \rightarrow M8o}$, θ_{BW}	1.41 (25)	1.39	0.53–2.26
$k_{M8m \rightarrow M8o}$, θ_{NNRTI}	1.48 (38)	1.32	0.53–2.98
$CL_{Nm \rightarrow Nc}/F$ ($l h^{-1}$)	0.058 (48)	0.08	0.01–0.71
$k_{Nc \rightarrow Naf}$ (h^{-1})	0.23 (48)	0.34	0.03–3.02
$k_{Naf \rightarrow No}$ (h^{-1})	0.36 (43)	0.53	0.06–5.09
$k_{M8m \rightarrow M8c}$ (h^{-1})	0.35 (24)	0.30	0.06–0.53
$k_{M8c \rightarrow M8af}$ (h^{-1})	0.59 (18)	0.55	0.10–0.83
$k_{M8af \rightarrow M8o}$ (h^{-1})	0.49 (8)	0.47	0.10–0.71
<i>Statistical model</i>			
$\sigma_{NELFI\ MOTHER}$ ($mg\ l^{-1}$)	1.07 (16)	1.06	0.85–1.26
$\sigma_{M8\ MOTHER}$ ($mg\ l^{-1}$)	0.24 (25)	0.24	0.17–0.30
$\sigma_{NELFI\ FETUS/CORD}$ ($mg\ l^{-1}$)	0.09 (38)	0.09	0.06–0.13
$\sigma_{M8\ FETUS}$ ($mg\ l^{-1}$)	0.03 (56)	0.06	0.02–0.08
$\sigma_{M8\ CORD}$ ($mg\ l^{-1}$)	0.12 (68)	0.13	0.01–0.25
$\omega(V)$ (%)	132 (36)	157	114–327
$\omega(CL_{Nm \rightarrow No}/F)$ (%)	53 (16)	56	46–73
$\omega(k_{M8m \rightarrow M8o}/F)$ (%)	74 (24)	63	42–84
$\omega(CL_{Nm \rightarrow Nc})$ (%)	34 (58)	37	14–57
$\omega(k_{M8m \rightarrow M8c})$ (%)	76 (32)	83	46–139

Figure 2

Population predicted (PRED) vs. observed (OBS) nelfinavir concentrations based (in $mg\ l^{-1}$) on the final model. The solid line is the line of identity

Figure 3

Population predicted (PRED) vs. observed (OBS) M8 concentrations (in mg l^{-1}) based on the final model. The solid line is the line of identity

Figure 4

Individual predicted nelfinavir concentrations based on the final model as a function of time in maternal plasma (left) and in the cord plasma (right). Solid line: median concentrations, dotted line: minimum and maximum concentrations. Data points: observed nelfinavir concentrations in mother and fetus on the day of delivery, after administration of 1250 mg twice daily

umbilical plasma samples pairs. The findings showed that metformin crossed the placenta, exposing the fetus to concentrations approaching those in the maternal circulation.

Nelfinavir was assigned FDA pregnancy category B status. Population pharmacokinetics have been used to predict nelfinavir and M8 concentrations in cord plasma. The minimum plasma concentrations for efficacy were shown to be 1 mg l^{-1} in adults [18] and 0.8 mg l^{-1} in children [19], but were not determined in the fetus. The prevalence of 3% birth defects with nelfinavir [28] may be related to fetal concentrations.

In the present work, the pharmacokinetics of nelfinavir and M8 were satisfactorily described by a compartmental model in the maternal and cord plasma, and in the amniotic fluid.

The maternal population model has already been used in children [29] and in adults [30, 31] and its development was reported in detail in our previous study in women [30]. Because on the day of delivery, plasma samples were infrequently taken during the absorption phase and data from the literature were variable, we decided to add data from pregnant and nonpregnant women to the analysis in order to estimate absorption rate and apparent volume of distribution more accurately.

The maternal nelfinavir and M8 compartments were used to produce the input function into the cord compartments, which were then used as the input function to amniotic fluid compartments. The conversion of nelfinavir to M8 was assumed to be negligible, because this pathway is catalyzed by CYP2C19 [10], which is not expressed by the fetus [32].

Figure 5

Individual predicted M8 concentrations based on the final model as a function of time in maternal plasma (left) and in the cord plasma (right). Solid line: median concentrations, dotted line: minimum and maximum concentrations. Data points: observed M8 concentrations in mother and fetus on the day of delivery, after administration of nelfinavir 1250 mg twice daily

The following observations support the use of this pharmacokinetic model:

- i) In each of the six compartments (for nelfinavir and M8 in maternal plasma, cord plasma and amniotic fluid), the population predicted concentrations were well correlated with observed concentrations.

For the mother:

- ii) In our study the oral clearance of nelfinavir was increased by 22% during pregnancy, a finding in agreement with those of Van Heeswijk *et al.* [33] (a 33% increase) and of Villani *et al.* [34] (a 26% increase). Nellen *et al.* [35] reported a 35% decrease in concentration ratio for pregnant woman compared with nonpregnant women. In the latter, fluid retention and haemodilution could cause a decrease in the plasma protein-bound fraction of drugs. This mechanism may explain lower total concentrations of phenytoin in pregnancy, despite limited changes in the free fraction [36].
- iii) During delivery, nelfinavir and M8 concentrations were lower than during pregnancy and in non pregnant women, probably due to a decrease in bioavailability, consistent with the data obtained in our previous study [30]. This effect on bioavailability has been demonstrated on mice. Thus, Matthias *et al.* [37] found that after oral but not after i.v. administration, the plasma clearance of nelfinavir was higher (by 134%, $P < 0.05$) and bioavailability was lower (by 32%, $P < 0.05$) in pregnant ($n = 3$) vs. nonpregnant mice ($n = 3$). Chappuy *et al.* [5] also reported that during human pregnancy, most

maternal protease inhibitor plasma concentrations were below the trough concentration that is recommended based on therapeutic drug monitoring. Marzolini *et al.* [6] also noted that maternal concentrations of proteases inhibitors measured at delivery were lower than those observed in the general HIV infected population.

For umbilical plasma and amniotic fluid:

- iv) Concentration–time relationships based on the Bayesian estimates of the final model were consistent with the results of the observational study.
- v) Values of the median cord to mother concentration ratio for nelfinavir were in agreement with those of Chappuy *et al.* [5] (24%), Marzolini *et al.* [6] (0 and 30%) and Gingelmaier *et al.* [8] (13–38%).

Because samples were obtained only at time of delivery, and maternal concentrations were low, concentration–time relationships for mother and cord plasma may not be similar to those during early pregnancy.

In our study we found that between 31 and 41 weeks of gestation, nelfinavir and M8 placental transfer from mother to cord plasma was not modified by any of the covariates. Gil *et al.* [38] showed a progressive two-fold decline in expression of P-glycoprotein between early pregnancy (13–14 weeks of gestation) and term (38–41 weeks of gestation). Moreover in maternal plasma, the protein-bound fraction of drug could decrease during pregnancy, improving diffusion from maternal plasma to blood cord and amniotic fluid. Thus, nelfinavir and M8 plasma concentrations in cord plasma are expected

to be lower during early pregnancy than during late pregnancy.

The median fetus : maternal concentration ratio was significantly higher for M8 than for nelfinavir. Nelfinavir, which is more lipophilic than M8, should be a better substrate for P-gp, which could explain why the placental transfer of M8 is higher than that of nelfinavir. Although M8 has antiretroviral activity comparable with that of nelfinavir [11], its concentration seems to be too low compared with that of nelfinavir to have significant activity in cord plasma.

Nelfinavir concentrations in the fetus represent approximately 20–30% of maternal exposure. None of the newborns studied achieved a plasma concentration of 0.8 mg l^{-1} , thought to be the minimum required for antiviral efficacy in children. As maternal concentrations and placental transfer were low, nelfinavir is probably not the best drug to prevent the HIV materno-fetal transmission on the day of delivery.

Exposure of the fetus to antiretroviral drugs taken by the mother may be beneficial in terms of prevention of mother-to-child viral transmission, but may also cause toxicity in the fetus. Population pharmacokinetics offer a useful tool to study the placental transfer of HIV and other drugs.

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