Pregnancy-Related effects on Tenofovir Pharmacokinetics:
A Population Study with 186 women.

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According to The European AIDS Clinical Society (EACS), Tenofovir Disoproxil Fumarate (TDF) can be used in HIV-infected pregnant women if started prior to pregnancy, although no data is available on the pharmacokinetics (PK) of Tenofovir (TFV) during pregnancy. The aim of this study was to describe TFV pharmacokinetics in HIV-infected women and to evaluate the effect of pregnancy on TFV disposition. Samples were collected according to a therapeutic drug monitoring in 186 women including 46 pregnant women treated with tenofovir and retrospectively analyzed by a population approach. Tenofovir pharmacokinetics was ascribed to an open 2-compartment with linear absorption and elimination. Mean population parameter estimates (between-subject variability) were: absorption rate constant 0.56 h\(^{-1}\), elimination clearance 59.9 L.h\(^{-1}\) (0.436) central volume of distribution 552 L (1.96), intercompartmental clearance 172 L/h and peripheral volume of distribution 1390 L. Pregnant women had a 39% higher apparent clearance compared to non pregnant women. Apparent clearance significantly decreased with age. In order to obtain an exposure similar to the known exposure in adults and guarantee similar trough concentrations (C\(_{\text{min}}\)) as observed in adult, an increase of tenofovir dose should be considered for women from the second trimester to delivery.
46 Introduction


48 Because of the lack of data on the use of TDF in pregnancy and concerns over possible bone toxicity, US guidelines recommend that TDF-based HAART should be used only after careful considerations of alternatives (8). However, for women who were already treated by TDF prior to pregnancy, EACS guidelines recommend to continue the TDF treatment during pregnancy. Thus in these therapeutic drug monitoring data (TDM) data, some women were under TDF during their pregnancy in the first, second and third trimester.

49 TDF is already taken during pregnancy at the same dose as in adults although physiological changes associated with pregnancy can lead to significant variations in pharmacokinetics (modified absorption, distribution and elimination). No pharmacokinetic data on the use of TDF before the 38th week of pregnancy are available. Two studies restricted to late pregnancy and labour suggest that TFV exposure is lower than nonpregnant adult exposure (9,16).

50 In this work, a population pharmacokinetic study was performed during pregnancy, at delivery, and out pregnancy; in order to investigate TFV pharmacokinetics all pregnancy long.
Methods

Patients-treatments

The population included nonpregnant women, pregnant women and women on the day of delivery receiving oral tenofovir for treatment of HIV infection and whose antiretroviral drug plasma concentrations were monitored on a routine basis. Tenofovir was administered chronically as a 300-mg once-daily. For each woman, the time elapsed between administration and sampling times, time of dosing, body weight (BW), age and weeks of gestation were carefully recorded. Ethics committee approval and patient consent are not compulsory in France in order to use therapeutic drug monitoring data, and thus they were not obtained.

Analytical method

The tenofovir assay was performed according to a previously published method (10) with an LOQ, interassay precision, and bias of 0.01 mg/l, 9.6, and 11.4%, respectively.

Population pharmacokinetic analysis.

Data were analysed using the nonlinear mixed effect modelling software program Monolix version 3.1s (11, 12) (http://wfn.software.monolix.org). Parameters were estimated by computing the maximum likelihood estimator of the parameters without any approximation of the model (no linearization) using the stochastic approximation expectation maximization (SAEM) algorithm combined to a MCMC (Markov Chain Monte Carlo) procedure. The number of MCMC chains was fixed to 10 for all estimations. The Likelihood Ratio Test (LRT) including the log-likelihood, the Akaike information criterion (AIC) and the bayesian information criterion (BIC) were used to test different hypotheses regarding the final model, covariate effect(s) on
pharmacokinetic parameter(s), residual variability model (proportional versus proportional plus additive error model), and structure of the variance-covariance matrix for the between subject variability (BSV) parameters. Diagnostic graphics and other statistics were obtained using the R program (http://wfn.sourceforge.net/).

Simulated tenofovir profiles and observed data were compared thanks to visual predictive check in order to validate the model. The vector of pharmacokinetic parameters from 1000 patients was simulated using the final model. The 5th, 50th and 95th percentiles of the simulated concentrations at each time were then overlaid on the observed concentration data using the R program and a visual inspection was performed.

Population pharmacokinetic modelling

One and two compartments models were tested. Tenofovir concentrations below the LOQ were set to half of the LOQ (2). Several error models were investigated (i.e. multiplicative and additive error models) to describe residual variability. Exponential model was used for between-subject variability (BSV). Only significant BSVs on pharmacokinetic parameters were retained. The covariates tested were women age and bodyweight on the day of sampling, labour status and pregnancy status.

The effect of each patient covariate was systematically tested via generalized additive modeling on the basic model.

- Continuous covariates (CO): age and bodyweight, were tested according to the following equation, using CL for example, $CL = \theta_{CL} \times \left( \frac{CO}{\text{median}(CO)} \right)^{\beta_{CO}^{CL}}$,

where $\theta_{CL}$ is the typical value of clearance for a patient with the median covariate value and $\beta_{CO}^{CL}$ is the estimated influential factor for the continuous
covariate. When a covariate was missing, it was set to the median value from all the other women.

• Delivery was considered as a binary covariate and its influence was tested as follows: 
  \[ \text{CL} = \theta_{\text{CL}} \times (\beta_{\text{DEL}}^{\text{CL}})^{\text{DEL}} \]
  where \( \text{DEL} = 1 \) if delivery and 0 otherwise, and \( \beta_{\text{DEL}}^{\text{CL}} \) is the estimated influential factor for delivery.

• The influence of pregnancy on TFV PK parameters was investigated using two different approaches:

(i) with a continuous relation between clearance and gestational age as follows:

\[ \text{CL} = \theta_{\text{CL}} \times \left( \frac{\text{GA}}{\text{median(GA)}} \right)^{\beta_{\text{GA}}^{\text{CL}}} \]

Where \( \text{PREG} = 1 \) if pregnant or parturient and 0 otherwise, and \( \beta_{\text{GA}}^{\text{CL}} \) is the estimated influential factor for pregnancy.

\[ \text{CL} = \theta_{\text{CL}} \times [1 + \left( \frac{\text{GA}^\gamma}{\text{GA}_{50}^\gamma + \text{GA}} \right)^{\text{PREG}}] \]

Where \( \text{PREG} = 1 \) if pregnant and 0 otherwise, \( \gamma \) and \( \text{GA}_{50} \) are the estimated parameters of the Hill equation.

(ii) By splitting the clearances according to gestational age in different classes: first trimester of Pregnancy (TR1), second trimester (TR2), and third trimester (TR3), the classes were grouped together at each step when non significant.

A covariate was kept if its effect was biologically plausible; it produced a reduction in AIC/BIC criterions (verified by the LRT) and a reduction in the variability of the pharmacokinetic parameter, assessed by the associated between-subject variability.

An intermediate model with all significant covariates was obtained. A backward
elimination phase was finally performed by deleting each covariate from the intermediate model, to obtain the final model.

Results

Demographic data

Data from 186 women (156 non pregnant and 46 pregnant women) were available for TFV pharmacokinetic evaluation. Table 1 summarizes the patients’ characteristics. At sampling times, among all non pregnant women 66 % were co-treated with a protease inhibitor (PI) (including 49.1% lopinavir/r), 76.7% were co-treated with a nucleoside reverse transcriptase inhibitor (NRTI) and 14.9% were co-treated with non-nucleoside reverse transcriptase inhibitors (NNRTI). Among pregnant women 85 % were co-treated with a PI (including 66% lopinavir/r), 89.6% were co-treated with a NRTI and 12.3% were co-treated with NNRTI.

Population pharmacokinetics

A total of 326 TFV concentrations were used for the pharmacokinetic analysis, among these concentrations 52 samples were obtained during pregnancy at different gestational ages (6 concentrations in the first trimester of pregnancy, 18 in the second trimester of pregnancy and 28 in the third trimester of pregnancy) and 25 on the day of delivery (70 women had more than one sample and the maximum number of sample per women was 7). The range of sampling times was 0.8–28.8 hours after the dose. Two TFV concentrations were lower than the LOQ, so they were set to half of the LOQ. All plasma samples were collected at steady state. A two compartment model with first order absorption and elimination best described the data. Parameters of the model were the absorption rate constant (ka), elimination clearance (CL),
central volume of distribution ($V_c$), intercompartmental clearance ($Q$) peripheral volume of distribution ($V_p$). Since TFV was orally administered, $CL/F$, $V_c/F$, $Q/F$, $V_p/F$ were apparent parameters and $F$ is the unknown bioavailability. The available data were not sufficient to estimate between-subject variability for $k_a$, $Q/F$, and $V_p/F$. and fixing the variance of these random effects to zero had no influence on the goodness-for-fit criteria. Variabilities were thus estimated: for $CL/F$ and $V_c/F$. A significant covariance of 0.92 was found between $CL/F$ and $V_c/F$. The residual variability was best described by a proportional error model. Pregnancy had a significant effect on $CL/F$: Pregnant women and women on the day of delivery had a 39% higher apparent clearance compared to non pregnant women. Adding pregnancy as a covariate resulted in a 9.3-unit decrease in the objective function (OF) value, BSV on $CL/F$ decreased from 0.50 to 0.46, and it also improved the goodness of fit. Age had a significant effect on $CL/F$, indeed apparent clearance decreased slightly but significantly with age and adding the age as a covariate resulted in a 8.2-unit decrease in the OF value and BSV on $CL/F$ decreased from 0.46 to 0.43. Figure 1 represents individual clearances of tenofovir as a function of gestational age, showing the increase of clearance in pregnant versus to non pregnant women. A more precise relationship between gestational age and clearance could not be established probably because of a lack of plasma samples performed in the first trimester of pregnancy. Figure 2 displays TFV observed and predicted plasma concentrations as a function of time for the non pregnant and pregnant women.

Evaluation and Validation

The final model performance was appreciated by comparing population predicted and individual predicted to observed plasma concentrations and population weighted residuals versus predicted concentrations (not shown) and versus time (Figure 4) for
TFV. Figure 4 also shows the normalized predicted distribution errors vs. time. As shown in Table 2, all the parameters are well estimated with small relative standard errors (RSE %). The visual predictive check (figure 3) confirmed that the average prediction matched the observed concentrations and the variability was well estimated.

Women TFV-concentrations after a 300mg TDF administration

Table 3 summarizes area under the curve (AUC) of TFV obtained after a 300 mg daily dose of TDF for non pregnant and pregnant women compared to previous studies in adult (4,3,14).

Simulations of doses in pregnant women

Pregnant women AUCs and Cmin were simulated following an administration of two tablets of TDF (600mg TDF, 272mg TFV) (Table 4).
Discussion

In the present work, tenofovir women pharmacokinetics was satisfactorily described by a 2-compartmental model. The following observations support the validity of this model: i) the population predicted concentrations were well correlated with observed concentrations and ii) the population model was validated thanks to the VPC method. Moreover, pharmacokinetic parameters obtained from our population model were close to the values reported in previous studies (Table 3).

Pharmacokinetics of tenofovir in pregnant women was described in two previous studies: Hirt et al. (9) reported TFV pharmacokinetics in women on the day of delivery and one week postpartum and Rodman et al. (16) investigated the PK of TFV after a single dose at the start of labour, the two studies concluded that a maternal 600 mg TDF administration at the start of labour produces the same concentrations as 300mg administration in other adults (9, 16). A more recent study confirmed the necessity of an increased dose of TDF during labor (7), administration of 600 mg and 900 mfg of TDF was safe and well tolerated in HIV-infected women in active labor or prior to caesarean section. In our study, women were treated by TDF during their pregnancy and during delivery by the same 300 mg TDF dose, we confirmed the increase of clearance in parturient women and so the necessity to increase TDF dose in these women.

Burchett et al. (5) concluded in a lower TDF exposition in the third trimester of pregnancy compared to postpartum. The magnitude of the AUC decrease in pregnancy was only about 15%, however. In our study, we could analyze TFV pharmacokinetics not only in late pregnancy but from week 2 to week 41 of gestation, during labour, and out of pregnancy. We observed that TFV clearance was about 39% higher in women during pregnancy compared to non pregnant women. Tenofovir is primarily excreted by the kidney by a combination of glomerular filtration...
and active tubular secretion system with ~70%-80% of the dose excreted unchanged in the urine (15). In pregnancy, glomerular filtration rate (GFR) and effective renal plasma flow increase to levels 50% to 80% above non pregnant levels (1, 6). This increase occurs shortly after the conception and persists throughout the second trimester (6). In our study, the increased clearance observed during pregnancy could be explained by these physiological changes in GFR during pregnancy. However, no information was available about serum creatinin levels or GFR in our population to confirm it. Besides the glomerular filtration tenofovir undergoes active tubular secretion dependent on hOAT1, hOAT2 and MRP4 (15). Very little is known regarding the effect of pregnancy on these transporters (1).

In our study, women age ranged from 16 to 62 and this wide range allowed us to describe the age effect on tenofovir clearance. Indeed tenofovir clearance seemed to decrease slightly but significantly with age, which is in agreement with the pattern of the GFR decline with aging as described in the KDOQI (13). The subject age range was higher for non pregnant women (62 years vs. 43 years) but the effect of pregnancy was observed with or without the older non pregnant women. Lopinavir/ritonavir co-treatment had no effect on the apparent clearance of tenofovir, contrary to previous studies.

Since no relationship between TFV plasma concentration and virologic response has been established, a reasonable goal for TFV dosing during pregnancy is to achieve plasma exposure in pregnant women equivalent to that observed in nonpregnant adults treated with the standard 300mg TDF dose. In our study, non pregnant women $C_{\text{min}}$ were similar to those reported in non pregnant adult (Table 3), their mean TFV exposure expressed as AUCs were slightly lower but not significantly different from non pregnant adult values (2.4 vs. 2.65, $p=0.3$). Since TDF is only available in 300 mg tablets, we simulated pregnant women AUCs and $C_{\text{min}}$ following
an administration of two tablets of TDF (600mg TDF, 272mg TFV). It appears that pregnant women exposure to TFV is low after the administration of one tablet of TDF but may be too high after the administration of two tablets (table 4).

In conclusion, women tenofovir pharmacokinetics was accurately described by a 2-compartment model. Tenofovir apparent clearance was increased by 39% in pregnant women. In order to obtain an exposure similar to the known exposure in adults and guarantee similar trough concentrations as expected in adult, a tenofovir dose escalation should be considered for women from the second trimester to delivery. Since limited clinical experience at doses higher than the therapeutic 300 mg TDF dose, further investigations are needed.
References:


Individual tenofovir clearances (L/h) as a function of gestational age (weeks). (*): during labour.

Figure 2
Tenofovir concentration-time courses in a semi-log scale. Observed (circles) and predicted (lines) tenofovir concentrations vs. Time: for non pregnant women (open circles and thin line) and for pregnant women (filled circles and thick line). (*): concentrations lower than the LOQ.

Figure 3
Evaluation of the final model: comparison between the 5th, 50th and 95th percentiles obtained from 1000 simulations and the observed data (o) for tenofovir concentrations in non pregnant women (left) and pregnant women (right). (*): concentrations lower than the LOQ.

Figure 4
Weighted residuals vs. time (up) Normalized predicted distribution errors vs. time (down).
Table 1. Characteristics of the HIV-infected women median (range) (N=186)

<table>
<thead>
<tr>
<th></th>
<th>Number of patients (samples)</th>
<th>AGE (year)</th>
<th>Body weight (kg)</th>
<th>Gestational age (week)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole population</td>
<td>186 (326)</td>
<td>37 (16-62)</td>
<td>64 (36-130)</td>
<td>30 (2.3-41)</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>46 (77)</td>
<td>35(17-43)</td>
<td>71(40-122)</td>
<td>30 (2.3-41)</td>
</tr>
<tr>
<td>Non pregnant women</td>
<td>156(249)</td>
<td>38 (16-62)</td>
<td>64 (36-130)</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 2. Population pharmacokinetic parameters of tenofovir from the final model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (RSE %)</th>
<th>Parameter</th>
<th>Estimate (RSE %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structural model</td>
<td></td>
<td>Statistical model</td>
<td></td>
</tr>
<tr>
<td>$k_a$ (h$^{-1}$)</td>
<td>0.56 (49)</td>
<td>$\omega_{CL/F}$</td>
<td>0.43 (5)</td>
</tr>
<tr>
<td>CL/F (L/h)</td>
<td>59.9 (5)</td>
<td>$\omega_{V/F}$</td>
<td>1.96 (10)</td>
</tr>
<tr>
<td>$V_p/F$ (L)</td>
<td>552 (43)</td>
<td>$\sigma$ (proportional)</td>
<td>0.34 (6)</td>
</tr>
<tr>
<td>Q/F (L/h)</td>
<td>172 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_p/F$ (L)</td>
<td>1390 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\theta_{PREG}$ on CL/F</td>
<td>1.39 (6)</td>
<td>$\theta_{AGE}$ on CL/F</td>
<td>0.49 (22)</td>
</tr>
</tbody>
</table>

Key: RSE%, relative standard error; $k_a$ absorption rate constant; CL/F maternal apparent elimination clearance from the central compartment; $V_p/F$ apparent central volume of distribution; Q/F intercompartmental clearance; $V_p/F$ apparent peripheral volume of distribution; $\theta_{PREG}$ effect of pregnancy on CL/F; $\theta_{AGE}$ effect of age on CL/F; $\sigma$ residual variability estimates (proportional error model); $\omega$, between subject variability estimates.
Table 3. Comparison of concentration and area under the curve for the current study vs. Blum et al. and Ramanathan et al.

<table>
<thead>
<tr>
<th></th>
<th>Current Study (n = 186) mean (IC95%)</th>
<th>Boffito et al. mean</th>
<th>Blum et al. mean</th>
<th>Ramanathan et al. mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>non pregnant (n = 156) 300 mg TDF</td>
<td>adult (n=29) 300 mg TDF</td>
<td>adult (n= 19) 300 mg TDF</td>
<td>adult (n=24) 300 mg TDF</td>
</tr>
<tr>
<td>AUC (mg/L *h)</td>
<td>2.4 (1.1-5.2)</td>
<td>1.6 (0.9-3.3)</td>
<td>2.62</td>
<td>2.65</td>
</tr>
<tr>
<td>C_{min} (mg/L)</td>
<td>0.061(0.031-0.164)</td>
<td>0.039(0.022-0.092)</td>
<td>0.052</td>
<td>0.053</td>
</tr>
</tbody>
</table>
Table 4. Pregnant women simulated AUC and \( C_{\text{min}} \) following daily administrations of 136 mg or 272mg TFV (which corresponds to 300 mg and 600 mg TDF) and comparison to non pregnant women values.

<table>
<thead>
<tr>
<th>Mean parameter</th>
<th>Non pregnant Women</th>
<th>Pregnant Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>One tablet (136 mg)</td>
<td>One tablet (136 mg)</td>
</tr>
<tr>
<td>AUC (mg/L.h)</td>
<td>2.4 (1.1-5.2)</td>
<td>1.6 (0.9-3.3)</td>
</tr>
<tr>
<td>( C_{\text{min}} ) (mg/L)</td>
<td>0.061 (0.031-0.164)</td>
<td>0.039 (0.022-0.092)</td>
</tr>
</tbody>
</table>
Figure 2

tenofovir concentrations (mg/L)

0 5 10 15 20 25 30
0.005 0.010 0.020 0.050 0.100 0.200 0.500

time (h)
Figure 3
Figure 4

![Graph showing TIME (h) vs. WRES and NPDE](image-url)