Nevirapine and efavirenz pharmacokinetics and covariate analysis in the 2NN study

Objective: The aim of this 2NN pharmacokinetic substudy was to investigate the population pharmacokinetics of nevirapine and efavirenz.

Methods: Treatment-naive, HIV-1-infected patients received nevirapine (once or twice daily), efavirenz or a combination with lamivudine and stavudine. Blood samples were collected on day 3 and weeks 1, 2, 4, 24 and 48. Using non-linear mixed effects modelling, pharmacokinetics of nevirapine and efavirenz and factors involved in the inter-individual variability were investigated.

Results: Clearance of nevirapine in the induction phase (<14 days) and at steady state (>28 days) were 2.02 l/h and 2.81 l/h, respectively. Volume of distribution and absorption rate constant were 77.0 l and 1.66 h⁻¹, respectively. Clearance of nevirapine was lower in females (13.8%) and in patients with hepatitis B (19.5%). Patients from South America and Western countries had higher clearance of nevirapine compared with Thai and South African patients.

The clearances of efavirenz in the induction phase and at steady state were 7.95 l/h and 8.82 l/h, respectively. The volume of distribution and absorption rate constant were 418 l and 0.287 h⁻¹, respectively. Concomitant use of nevirapine increased clearance of efavirenz (43%). Patients from Thailand had lower clearance than the rest of the population.

Conclusions: The population pharmacokinetics of nevirapine and efavirenz were assessed in the 2NN trial. For both drugs, an induction phase was distinguished from the steady-state phase. Gender, hepatitis B and geographical region were involved in the variability of the pharmacokinetics of nevirapine. Region and concomitantly used nevirapine were determinants of the pharmacokinetics of efavirenz.

Introduction

Nevirapine and efavirenz are non-nucleoside reverse transcriptase inhibitors (NNRTIs) and have demonstrated potency, safety and convenience in several clinical trials [1–4]. As a result, both drugs are frequently used as the basis of highly active antiretroviral therapy (HAART) for the management of both treatment-naive and treatment-experienced patients. However, there have been no large-scale, randomized clinical trials comparing nevirapine with efavirenz. Apart from two small studies [5,6], the value of combining these NNRTIs has also not been studied in humans. The double non-nucleoside study (2NN) is the first large-scale, international, multicentre, open-label, randomized study to compare the efficacy and safety of nevirapine, efavirenz and the combination of these drugs, each in combination with background therapy including stavudine and lamivudine. More details of the trial have been reported separately [7].

Except for the DONUT study [8], no pharmacokinetic studies are available about the concomitant use of efavirenz and nevirapine.

The aim of the present 2NN pharmacokinetic (2NN-PK) substudy was to investigate the pharmacokinetic parameters of nevirapine and efavirenz, and their determinants in a large and diverse global population.

Both drugs are metabolized in the liver, predominantly by the cytochrome P450 (CYP) 3A4 and 2B6 isoenzymes. Since the autoinduction of CYP3A4 is completed in 14 days, the increase in clearance caused by induction of CYP2B6 isoenzymes occurs between 14 and 28 days [9,10]; the clearance of nevirapine and efavirenz will change during this induction phase. Therefore, in addition to the pharmacokinetics of nevirapine and efavirenz at steady state, we present pharmacokinetics during the induction phase of both antiretroviral agents.
Methods

Patients
HIV-1-infected patients were enrolled between February 2000 and June 2001 from several study sites in Europe, South Africa, Canada, United States, Argentina, Brazil, Australia and Thailand. The main eligibility criterion was HIV RNA >5000 copies/ml at screening, without prior antiretroviral therapy. Full details have been reported recently by van Leth et al. [7]. Upon inclusion, patients were randomly assigned to either nevirapine 400 mg once daily, nevirapine 200 mg twice daily, efavirenz 600 mg once daily or a combination of nevirapine 400 mg plus efavirenz 800 mg once daily. Nevirapine was given as a 200 mg once-daily dose for the first 2 weeks. All patients also received stavudine 40 mg twice daily (30 mg twice daily if weight was less than 60 kg) and lamivudine 150 mg twice daily. From the 1216 patients included in the 2NN study, patients with apparent poor adherence to the study treatment regimen, defined as less than 95% compliance with study drugs, were excluded from the 2NN-PK substudy. These subjects were identified based on the reported treatment interruption.

Sampling and bioanalysis
Plasma samples for evaluation of study drug concentrations were collected at day 3 and weeks 1, 2, 4, 24 and 48. The time of ingestion of the last dose and the sampling time were recorded. Time after ingestion was extracted from this information.

The concentrations of nevirapine and efavirenz were quantitatively assessed by a validated high-performance liquid chromatography (HPLC) with an ultraviolet detection (UV) method in a Good Laboratory Practice (GLP) licensed laboratory. Briefly, sample pre-treatment consisted of protein precipitation with acetonitrile. Subsequently, nevirapine and efavirenz were separated from endogenous compounds by isocratic, reversed-phase HPLC. Absorbance was measured at 275 nm. The method was validated over the range of 0.25–15.0 mg/l and 0.05–15.0 mg/l for nevirapine and efavirenz, respectively. Samples with concentrations above the upper limit of quantification were reanalysed after dilution. The assay proved to be accurate and precise. The average accuracy at three different concentrations ranged from −1.27–8.5%. Within- and between-day precisions were less than 5.9% for all quality control samples [11].

Covariates
To identify possible relationships between the pharmacokinetics of nevirapine and efavirenz and patient characteristics, the following covariates were collected at baseline: gender, age, weight, body mass index, geographical region and treatment arm. Patients were considered to have a chronic hepatitis B infection when the hepatitis surface antigen (HbsAg) could be detected at baseline. When anti-hepatitis C antibodies were present at baseline, patients were considered to have a chronic hepatitis C infection. The effect of a combined use of nevirapine and efavirenz on the pharmacokinetics of both drugs was also investigated. Age, weight, and body mass index were examined as continuous variables. Gender, region, treatment arm, hepatitis B co-infection and hepatitis C co-infection were examined as dichotomous variables.

Population pharmacokinetic analysis
The non-linear mixed effect modelling software program NONMEM (level 1.1; GloboMax LLC, Hanover, MD, USA) was used to perform the analyses. The first-order conditional estimate method (FOCE) with interaction between the inter-individual and residual error was used throughout the study. The adequacy of the developed structural models was evaluated using both statistical and graphical methods. The minimal value of the objective function (OFV) provided by NONMEM was used for the comparisons of nested models. Discrimination between these models was based on the OFV using the likelihood ratio test [12]. A $P$ value of 0.05, representing a decrease in OFV of 3.84, was considered statistically significant (chi-square distribution, df=1). Standard errors for all parameters were approximated using the COVARIANCE option of NONMEM. Individual Bayesian pharmacokinetic estimates of the pharmacokinetic parameters were obtained using the POSTHOC option [12].

Basic pharmacokinetic models. For nevirapine, a previously developed population pharmacokinetic model that was built with data from a cohort of 173 HIV-1-infected patients was used as starting point for model development [13]. This pharmacokinetic model consisted of a single-compartment with first-order absorption and elimination. The pharmacokinetic model of efavirenz was initially built with data from 172 HIV-1-infected patients and comprised a central and a peripheral compartment with first-order elimination. The fast absorption with slow onset was best described with a chain of three transition compartments between the absorption and the central compartment [14]. Population pharmacokinetic parameters including clearance, volume of distribution and absorption rate constant were estimated. Since clearance of nevirapine and efavirenz increased in time due to autoinduction [10], special attention was paid to the pharmacokinetics of nevirapine and efavirenz during the first few weeks of antiretroviral therapy. The induction phase of
both drugs was modelled using the same equation as
used for the covariate analysis:

\[
TVCL = \theta_1 + \theta_2^{IND}
\]

in which TVCL is the typical value of clearance in the
population; \(\theta_1\) is the typical value of CL of an
individual during steady state (with IND=0); and \(\theta_2\) is
the relative difference in clearance for individuals in the
induction phase (with IND=1).

Inter-individual variability in the pharmacokinetic
parameters were estimated with an exponential error
model. For instance, variability in clearance was esti-
mated using:

\[
CL/F_i = \theta_1 \times \exp(\eta_i)
\]

in which CL/F\(i\) represents the apparent clearance of the
\(i^{th}\) individual; \(\theta_1\) is the typical value of clearance; and \(\eta_i\)
the inter-individual random effect with a mean of 0
and a variance of \(\sigma^2\). Residual variability was modelling
with a combined additive and proportional error
model.

Covariate pharmacokinetic models. To identify factors
influencing the pharmacokinetics of nevirapine and
efavirenz, covariates were introduced in the basic
models. For instance, the influence of a dichotomous
covariate \(X\) on clearance was modelled as:

\[
TVCL = \theta_1 + \theta_2^X
\]

in which TVCL is the typical value of clearance in the
population; \(\theta_1\) is the typical value of an individual with
\(X=0\); and \(\theta_2\) is the relative difference in clearance for
individuals with \(X=1\).

Efficient screenings of covariates in the population
models of nevirapine and efavirenz were carried out
using the Wald approximation to the likelihood ratio
test statistic in conjunction with Schwartz’s bayesian
criterion [15]. This Wald approximation method
(WAM) is constructed to compare submodels (that is,
one or more covariates removed or set to zero) with the
full model (that is, all covariate parameters in the
model).

The inclusion of a covariate relationship in a phar-
macokinetic model is ideally based on a combination of
the scientific plausibility, the clinical importance and the
statistical significance of the relationship. A covariate
was considered statistically significant when the inclu-
sion was associated with a decrease in the minimal value
of the objective function associated with a \(P\) value of
\(\leq 0.05\) (log-likelihood ratio test). Clinical relevance was
considered when the typical value of the pharmacoki-
netic parameter of interest changed at least 10% in the
range of the covariate, as observed in the population to
prevent the detection of an irrelevant, albeit significant,
relationship. A covariate was retained in the model
when the influence of this parameter was statistically
significant and clinically relevant.

Statistical refinement. The validity of the inter-
individual variability model was checked by evaluating
the correlations between individual random effects (\(\eta\))
for all of the pharmacokinetic parameters [16]. When
a substantial correlation was present, covariance
between these parameters was included in the model.

Model validation. The bootstrap resampling technique
was applied as an internal validation for the final
models [17]. Bootstrap replicates were generated by
randomly sampling approximately 65% of the original
datasets with replacement. The final models were fitted
to the replicate datasets using the bootstrap option in
the software package Wings for NONMEM (by
N Holford, version 406, May 2004, Auckland, New
Zealand). Parameter estimates for each of the replicate
datasets were obtained in this way [17]. The precision
of the models was evaluated by visual inspection of
distribution of model parameters. Furthermore, the
median parameter values and 95% prediction intervals
of the bootstrap replicates were compared with the
estimates of the original datasets.

Wings for NONMEM was also used to perform a
randomization test by randomly permuting a covariate
in the original dataset to validate the significant and
relevant covariates. The purpose of the procedure is to
confirm the significance of a covariate in the original
dataset and to permute a covariate value randomly to
each subject in order to create a new randomized
dataset. The model is fitted to the new permuted
dataset and the actual significance level can be esti-
mated by repeating this procedure (\(n \times 1000\)) [18].

Since Bayesian estimates of \(C_{min}\), \(C_{max}\) and AUC will
be calculated using the developed pharmacokinetic
models for the investigation of relationships between
pharmacokinetic parameters and viral efficacy and
adverse events, the posterior predictive check tech-
nique was also applied [19]. This method was used to
assess whether simulated data have the same charac-
teristics as the original data. Index datasets were
created by selecting trough concentrations from the
concentration data of the original datasets. A nevi-
rapine concentration was considered a trough
concentration when the sample was taken 10 or 20 h
after ingestion of a twice-daily dose or a once-daily
dose, respectively. Due to the long half-life of efavirenz,
a concentration was considered a trough concentration
when the sample was taken 16 h after ingestion. The
median trough concentrations were determined.
Subsequently, 1000 datasets were simulated based on the index datasets and the final pharmacokinetic parameters of the nevirapine and efavirenz models. The median in the index dataset was compared with the 90% prediction intervals of the median values, calculated in all 1000 simulated datasets [19]. Also, the median value and interquartile ranges of Bayesian estimated trough concentrations were calculated for all patients and compared with the actual troughs and the results of the posterior predictive check. Bayesian estimates of the trough concentrations were made using the POSTHOC option of NONMEM, taking both the data from the individual patient and the population into account. Trough concentrations were defined as the concentrations at t=0 h, which is the time of drug intake.

Results

Patients
In total, 3127 nevirapine and 1728 efavirenz plasma concentrations from 1091 naive HIV-1-infected patients were included in this 2NN-PK substudy. However, some data were excluded due to unknown time of sampling or unknown regimen. Finally, a total of 3024 nevirapine and 1694 efavirenz plasma samples from 1077 patients were included, analysed and used to investigate the pharmacokinetics of nevirapine and efavirenz in the 2NN-PK substudy. An overview of the analysed plasma samples is shown in Table 1. In Figures 1 and 2 the concentration time data of nevirapine and efavirenz are illustrated.

The study included a diverse global population, many of whom are usually under-represented in clinical trials. A total of 17.8% of the patients were from Thailand, 35.7% from South Africa, 21.3% from South America and 25.6% from Western countries (Australia, Canada, Europe and United States). Furthermore, a substantial proportion of the patients was female (36.3%). From current literature, it is known that co-medication can have a significant influence on the pharmacokinetics of nevirapine and efavirenz [20]. Unfortunately, no data on co-medication were available for covariate analysis. Characteristics at baseline from included patients are shown in Table 2.

Population pharmacokinetics

Nevirapine. In agreement with the earlier developed pharmacokinetic model for nevirapine [13], the pharmacokinetics of nevirapine in the 2NN study were best described with a one-compartment model with first-order absorption and elimination. Since blood samples were collected at day 3 and weeks 1, 2, 4, 24, 48 after the start of the regimen, the data contained information about both the induction phase and steady state.

In a first attempt, the possibility of estimating a separate value for clearance for each time point was investigated. It appeared that the clearance of nevirapine was not statistically different between day 3 and week 2 as it was from week 4 and onwards. Therefore, in the final model, clearance was estimated for the first period (until week 2) and for the second period (week 4 onwards) as 2.02 and 2.81 l/h, respectively. The introduction of an induction phase in the model significantly improved the model ($\Delta$OFV=399 points, $P<0.001$).

The population pharmacokinetic estimate of volume of distribution was 77.0 l. Due to the irregular absorption phase of nevirapine [13,21] and only single concentration–time points instead of complete pharmacokinetic curves, it proved to be impossible to characterize the absorption phase. In previous studies, it has been demonstrated that both fast and slow absorbers exist. Furthermore, both high inter-patient and intra-patient variability in the absorption process was found. According to Wade et al. [22], it is a good alternative to fix the estimate of the absorption rate constant at 1.66 h$^{-1}$ according to the information available from the earlier developed pharmacokinetic model of nevirapine [13]. In addition, a sensitivity analysis has been performed. With this analysis it was

| Table 1. Analysed plasma samples of included patients |
|---------------------------------|---------|---------|---------|---------|---------|
| NVP once daily | NVP twice daily | EFV | NVP+EFV | Total |
| Number of patients in study | 205 | 373 | 378 | 135 | 1091 |
| Number of patients in analysis | 205 | 373 | 376 | 123 | 1077 |
| Day 3 | 184 | 329 | 325 | 0 | 838 |
| Week 1 | 181 | 348 | 332 | 0 | 861 |
| Week 2 | 175 | 342 | 329 | 0 | 846 |
| Week 4 | 167 | 322 | 1 | 0 | 490 |
| Week 24 | 147 | 290 | 304 | 123/123 | 987 |
| Week 48 | 141 | 273 | 279 | 0 | 603 |

NVP once daily, nevirapine 400 mg once daily; NVP twice daily, nevirapine 200 mg twice daily; EFV, efavirenz 600 mg once daily; NVP+EFV, nevirapine 400 mg plus efavirenz 800 mg once daily.
confirmed that 1.66 h⁻¹ was a reasonable value for \( k_a \), because fixing \( k_a \) at other values resulted in the same outcomes. The residual error in nevirapine pharmacokinetics incorporated an additive and a proportional component of 0.388 mg/l and 27.3%, respectively.

In view of statistical refinement, a correlation between the individual random effects of clearance and volume of distribution (\( \eta_{CL} \) and \( \eta_{V} \)) of nevirapine was observed and covariance between these parameters was added to the model. The correlation coefficient was 0.240.

After the screening of covariates using WAM [15], gender (\( \Delta \text{OFV}=49.7 \), change in CL (\( \Delta \text{CL}=13.8\% \)), hepatitis B co-infection (\( \Delta \text{OFV}=16.3 \), \( \Delta \text{CL}=19.5\% \)) and geographical region (\( \Delta \text{OFV}=88.9 \), \( \Delta \text{CL}=11\% \) and 28%) had a statistically significant increase in goodness-of-fit and a clinically relevant effect on clearance of nevirapine. Neither the concomitant use of efavirenz nor the dose or frequency of administration of nevirapine showed a relationship with clearance of nevirapine. The results of the final model are summarized in Table 3. The model population and individual predicted concentrations versus observed concentrations of nevirapine using the final model are presented in Figure 3.

**Efavirenz.** The pharmacokinetics of efavirenz were best described with a one-compartment model showing first-order absorption and elimination. Also for efavirenz, the induction phase was investigated in more
Table 2. Baseline characteristics of included patients

<table>
<thead>
<tr>
<th></th>
<th>NVP once daily</th>
<th>NVP twice daily</th>
<th>EFV</th>
<th>NVP+EFV</th>
<th>Total, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>205</td>
<td>373</td>
<td>376</td>
<td>123</td>
<td>1077</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>129/76</td>
<td>228/145</td>
<td>240/136</td>
<td>89/34</td>
<td>686/391 (63.7/36.3)</td>
</tr>
<tr>
<td>Age, years, mean ± SD</td>
<td>35.0 ± 8.5</td>
<td>36.0 ± 9.2</td>
<td>35.9 ± 8.9</td>
<td>35.5 ± 8.8</td>
<td>35.7 ± 8.9</td>
</tr>
<tr>
<td>Weight, kg, mean ± SD</td>
<td>65.1 ± 12.7</td>
<td>68.1 ± 14.5</td>
<td>66.7 ± 13.3</td>
<td>67.1 ± 12.0</td>
<td>66.9 ± 13.5</td>
</tr>
<tr>
<td>BMI, kg/m², mean ± SD</td>
<td>19.3 ± 3.3</td>
<td>20.2 ± 3.9</td>
<td>19.7 ± 3.5</td>
<td>19.7 ± 3.1</td>
<td>19.8 ± 3.6</td>
</tr>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td>49</td>
<td>43</td>
<td>69</td>
<td>26</td>
<td>187 (17.4)</td>
</tr>
<tr>
<td>South Africa</td>
<td>66</td>
<td>139</td>
<td>134</td>
<td>46</td>
<td>385 (35.7)</td>
</tr>
<tr>
<td>South America</td>
<td>38</td>
<td>87</td>
<td>80</td>
<td>24</td>
<td>229 (21.3)</td>
</tr>
<tr>
<td>Western countries</td>
<td>52</td>
<td>104</td>
<td>93</td>
<td>27</td>
<td>276 (25.6)</td>
</tr>
<tr>
<td>HBV</td>
<td>15</td>
<td>16</td>
<td>14</td>
<td>9</td>
<td>54 (5.0)</td>
</tr>
<tr>
<td>HCV</td>
<td>21</td>
<td>34</td>
<td>32</td>
<td>9</td>
<td>96 (8.9)</td>
</tr>
</tbody>
</table>

NVP once daily, nevirapine 400 mg once daily; NVP twice daily, nevirapine 200 mg twice daily; EFV, efavirenz 600 mg once daily; NVP+EFV, nevirapine 400 mg plus efavirenz 800 mg once daily; M, male; F, female; BMI, body mass index; HBV, hepatitis B co-infection; HCV, hepatitis C co-infection; SD, standard deviation.

Table 3. Parameter estimates of the final pharmacokinetic model of nevirapine and the results of the randomization test and bootstrap analyses

<table>
<thead>
<tr>
<th>NVP model</th>
<th>Estimate</th>
<th>RSE, %</th>
<th>P value*</th>
<th>Median</th>
<th>95% PI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F ≤ week 2, l/h</td>
<td>2.02</td>
<td>4.99</td>
<td>&gt;0.001</td>
<td>2.01</td>
<td>1.95–2.08</td>
</tr>
<tr>
<td>CL/F ≥ week 4, l/h</td>
<td>2.81</td>
<td>2.60</td>
<td>&lt;0.001</td>
<td>2.80</td>
<td>2.67–2.96</td>
</tr>
<tr>
<td>( \theta_{\text{Female gender}} )</td>
<td>0.862</td>
<td>2.99</td>
<td>&lt;0.001</td>
<td>0.863</td>
<td>0.813–0.910</td>
</tr>
<tr>
<td>( \theta_{\text{Hepatitis B infection}} )</td>
<td>0.805</td>
<td>7.11</td>
<td>&lt;0.001</td>
<td>0.808</td>
<td>0.705–0.931</td>
</tr>
<tr>
<td>( \theta_{\text{Thailand}} )</td>
<td>1</td>
<td>–</td>
<td>&gt;0.001</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>( \theta_{\text{South Africa}} )</td>
<td>1</td>
<td>–</td>
<td>&gt;0.001</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>( \theta_{\text{South America}} )</td>
<td>1.11</td>
<td>3.29</td>
<td>&lt;0.001</td>
<td>1.11</td>
<td>1.04–1.19</td>
</tr>
<tr>
<td>( \theta_{\text{Western countries}} )</td>
<td>1.28</td>
<td>3.64</td>
<td>&lt;0.001</td>
<td>1.28</td>
<td>1.18–1.38</td>
</tr>
<tr>
<td>V/F, l</td>
<td>77.0</td>
<td>2.79</td>
<td>&gt;0.001</td>
<td>77.1</td>
<td>73.1–81.3</td>
</tr>
<tr>
<td>kₐ, h⁻¹</td>
<td>1.66</td>
<td>–</td>
<td>&lt;0.001</td>
<td>1.66</td>
<td>–</td>
</tr>
<tr>
<td>Additive error, mg/l</td>
<td>0.388</td>
<td>29.1</td>
<td>&gt;0.001</td>
<td>0.393</td>
<td>0.150–0.604</td>
</tr>
<tr>
<td>Proportional error, %</td>
<td>27.3</td>
<td>3.99</td>
<td>&gt;0.001</td>
<td>27.2</td>
<td>25.0–29.2</td>
</tr>
<tr>
<td>IV CL/F, %</td>
<td>33.8</td>
<td>7.44</td>
<td>&gt;0.001</td>
<td>33.6</td>
<td>31.2–36.2</td>
</tr>
<tr>
<td>IV V/F, %</td>
<td>41.5</td>
<td>17.9</td>
<td>&gt;0.001</td>
<td>41.4</td>
<td>33.5–48.3</td>
</tr>
<tr>
<td>Correlation IVs</td>
<td>0.183</td>
<td>35.1</td>
<td>&gt;0.001</td>
<td>0.240</td>
<td>0.0810–0.400</td>
</tr>
</tbody>
</table>

*Non-parametric permutation randomization test. † Relative change in CL/F. ‡ Fixed from previous model. NVP, nevirapine; CL/F, clearance; V/F, volume of distribution; kₐ, absorption rate constant, IV, inter-individual variability; RSE, relative standard error; PI, prediction interval.

detail and resulted in a significant improvement in the model (ΔOFV=31.1, P<0.001). It appeared that clearance of efavirenz was unchanged up to week 2 (7.95 l/h) and after week 24 (8.82 l/h). Volume of distribution and absorption rate constant were estimated at 418 l and 0.287 h⁻¹, respectively. The residual error in efavirenz pharmacokinetics incorporated an additive and a proportional component, 0.216 mg/l and 24.2%, respectively.

Considering statistical refinement, a correlation between individual random effects of \( \eta_{\text{CL}} \) and \( \eta_{V} \) was observed in the efavirenz model. Covariance between these parameters was added to the model.

Using WAM, geographical region (ΔOFV=42.5, ΔCL=53% and 76%) and concomitant use of nevirapine (ΔOFV=25.6, ΔCL=43%) seemed to have a significant and relevant relationship with clearance of efavirenz. The results of the final efavirenz model are summarized in Table 4. The model population and individual predicted concentrations versus observed concentrations of efavirenz using the final model are presented in Figure 4.
Model validation

From the original datasets, 1000 replicate bootstrap datasets were generated and used for the evaluation of the precision of the parameter estimates. In addition to the parameter estimates of the final models, Tables 3 and 4 list the results of the bootstrap procedures, presented as medians and 95% prediction intervals. Median values of the bootstrap procedures were very similar to the parameter estimates of the original datasets.

Furthermore, 1000 replicate randomization datasets from the original datasets were generated for each covariate of interest to evaluate the level of significance. In Tables 3 and 4, the $P$ values of the randomization tests are also shown. As can be seen, all included covariates were statistically significant.

The median values and interquartile ranges of observed trough concentrations of nevirapine and efavirenz, as well as the results of the posterior predictive check are shown in Table 5. The results of the posterior predictive check showed that the observed median of the trough concentration was well included in the 90% prediction interval from the simulated datasets. The results of the estimated trough concentration from all patients, also listed in Table 5, also showed similar ranges, indicating that both models adequately simulate data having the same characteristics as the original data.
Discussion

The goal of the study was to investigate the population pharmacokinetic parameters of nevirapine and efavirenz and factors involved in a large and diverse global population.

The structural model of nevirapine adequately described the data in correspondence with the results of Zhou et al. [21] and De Maat et al. [13], from which the latter was used as starting point for model development. Furthermore, the estimates of the pharmacokinetic parameters were comparable with the results of these studies.

The structural model of efavirenz, which consisted of one-compartment with first-order absorption, was similar to the model presented by Pfister et al. [23]; however, it differed from the model that was used as basic consideration. The dataset contained too few concentration time points between 0 and 5 h to model the fast absorption phase with slow onset as we described earlier in an intensively sampled population [14]. The statistical and graphical methods did not support the implementation of a peripheral compartment; a one-compartment model appeared to describe the data adequately. Estimated pharmacokinetic parameters were in correspondence with results from earlier published studies [23,24].

Because both datasets contained concentration–time data from patients before reaching steady state, an induction phase was introduced in the pharmacokinetic models. Autoinduction is a known and important phenomenon for both nevirapine and efavirenz. Therefore, it was regarded as crucial to characterize this process for both drugs properly. Since sampling was only performed at some discrete and previously defined time points, extensive data on the time course...
of autoinduction was not available. In a first attempt, it was investigated whether it was possible to estimate separate values for clearance at each time point. Finally, it appeared that the clearance of nevirapine was similar between day 3 and week 2 (2.02 l/h) and from week 4 onwards (2.81 l/h). This increase in clearance of approximately 40% means that, when a dose adjustment to 200 mg once daily during the first 2 weeks is not applied, plasma levels of nevirapine with substantially more risk for adverse events would be achieved. From our data, it can be observed that no induction was noticeable between day 3 and week 2 and between week 4 and week 48 and that the induction of the enzyme system metabolizing nevirapine reached completeness between week 2 and week 4. The question remains as to whether the first phase of induction has taken place before day 3. From previous literature it is known that nevirapine is primarily metabolized by the CYP3A4 and CYP2B6 isoenzymes [25]. It is known that a small but significant part of the induction phase of CYP3A4 occurs during the first 14 days, and that between day 14 and 28 the clearance of nevirapine increases with a substantial increase in the formation of the CYP2B6 metabolite, 3-hydroxynevirapine [9]. Our results are remarkably consistent with these published results.

Between day 3 and week 2, the clearance of efavirenz was 7.95 l/h, and 8.82 l/h thereafter. The difference in clearance of efavirenz is only 10% and, as contrasted with nevirapine, dose adjustment is unnecessary. As found for nevirapine, there was no change in the induction of efavirenz between day 3 and week 2,
and thereafter the completion of the autoinduction of the CYP isoenzymes was observed.

Extensive data are available on the pharmacokinetics of both nevirapine and efavirenz [13,14,21,23,24]. These studies, however, have been executed with a limited number of mainly male Caucasian individuals. In a small cohort with little variability in patient characteristics, significant and relevant covariates will rarely be found. Key features of the 2NN-PK substudy were its size and wide variety of patients: it included a large diverse global population with a substantial proportion of female patients (36.3%) and only 25.6% of patients were from Western countries (Australia, Canada, Europe and the United States). The ethnicity of patients included was not documented, since permission was not granted for this by the ethical review committee in several countries, so only the country of residence was recorded. Unfortunately, no data on co-medication were available, which might explain a part of the residual error in both pharmacokinetic models.

Clearance of nevirapine was related to gender, geographical region and hepatitis B co-infection. The pharmacokinetics of efavirenz were influenced by geographical region and concomitant use of nevirapine. Hepatitis B co-infection led to a decrease in clearance of nevirapine of 19.5%. In addition, more than 80% of the patients with a hepatitis B co-infection had elevated liver enzymes, indicating a hepatic dysfunction, which might result in a smaller capacity of the liver to metabolize nevirapine to its inactive metabolites. Female patients showed a 13.8% lower clearance of nevirapine than did men. Since neither body weight nor BMI had an influence on the clearance of nevirapine, in contrast with the study of De Maat et al. [13], the effect of gender cannot solely be explained by body size. Compared with men, women may have a smaller capacity in the liver, resulting in a lower clearance [26]. Gender and hepatitis B co-infection also influenced the clearance of efavirenz, however this was only significant and relevant in the univariate analysis.

Patients from Western countries and South America showed a 28% and 11% increase in clearance of nevirapine, respectively, in comparison with patients from Thailand and South Africa. With efavirenz, the clearance in patients from Thailand was higher than in patients from South Africa and South America (53%), and Western countries (76%). Differences in pharmacokinetics due to ethnicity have been reported for several drugs that are metabolized by CYP3A [27–30] and CYP2B6 [28]. The presence of the variant CYP3A4*1B allele and the CYP2B6*9 genotype, the frequency of which appears to differ between races, have been suggested to be associated with altered activity of CYP3A4 and CYP2B6, respectively [28,30,31]. Besides genetic factors, environmental factors (including shared cultural and dietary habits) could be responsible for differences in pharmacokinetics.

The metabolism of nevirapine and efavirenz is an autoinducible enzymatic process. Since both drugs also induce and inhibit several CYP enzymes, it is hard to predict what will be the absolute effect on the metabolism of each drug. However, this study shows that the concomitant use of nevirapine plus efavirenz affects only the clearance of efavirenz, most likely due to induction of CYP enzyme activity. Because of increased clearance of efavirenz, which was already observed in the DONUT study [8], the dose of efavirenz was increased to 800 mg in the treatment arm in which nevirapine and efavirenz were combined.

In conclusion, pharmacokinetic models of nevirapine and efavirenz during both induction phase and steady state were developed. Patient characteristics, gender, hepatitis B co-infection and geographical region were involved in the inter-individual variability of nevirapine. The concomitant use of nevirapine and geographical region influenced the pharmacokinetics of efavirenz. These pharmacokinetic models will greatly aid further investigation relationships between pharmacokinetic parameters, viral efficacy and adverse events.

References


PK of NVP and EFV in the 2NN study

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