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Pharmacokinetic Study of Tenofovir Disoproxil Fumarate Combined with Rifampin in Healthy Volunteers


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Coinfection with Mycobacterium tuberculosis and human immunodeficiency virus (HIV) is frequent, particularly in Africa and Asia (3, 14, 18). Simultaneous treatment of tuberculosis and HIV infection may lead to complex combination therapy. Rifampin is a drug of choice for the treatment of tuberculosis. Rifampin is known to have major pharmacokinetic interactions with HIV protease inhibitors and nonnucleoside reverse transcriptase inhibitors (8, 10, 12, 13, 16, 17). Tenofovir disoproxil fumarate (tenofovir DF) is the first drug from a new class of anti-HIV agents (nucleotide reverse transcriptase inhibitors) that has been recently approved for use for the treatment of HIV infections in adults. However, no data are available regarding its pharmacokinetics in combination with tuberculosstatic drugs, in particular, rifampin. No influence of rifampin on the pharmacokinetics of tenofovir is expected, because rifampin has no effect on the pharmacokinetics of tenofovir DF and rifampin has no effect on the pharmacokinetics of tenofovir DF. Therefore, no cross-over design was used in this study because rifampin could lead to considerable carryover effects, due to its long-lasting cytochrome P450-inducing effect. To eliminate this effect a longer washout period would be necessary, but this would have significantly prolonged the duration of the study and would have significantly prolonged the duration of the study.

MATERIALS AND METHODS

Study design. The present study was designed to evaluate the effect of 600 mg of rifampin on the pharmacokinetics of 300 mg of tenofovir DF and also to assess whether tenofovir DF has a substantial impact on steady-state exposure to rifampin. This study was a multiple-dose, open-label, single-group, two-period study with 24 healthy volunteers. First, the subjects received tenofovir DF at 300 mg once daily (QD) for 10 days (period 1). At study day 10, a steady-state 24-h pharmacokinetic curve was obtained for tenofovir. During the second period of the study (period 2), tenofovir DF at 300 mg was combined with rifampin at 600 mg QD, again for 10 days. At study day 20, 24-h steady-state pharmacokinetic curves were obtained for tenofovir and rifampin. During the study both tenofovir DF and rifampin had to be taken with breakfast. On the days prior to study days 9 and 19, the subjects reported to the study center for direct observation of dosing with the medications and with a standardized breakfast. Subsequently, on the evening of study days 9 and 19, the subjects remained at the study center for two overnight stays and remained at the study center until the mornings of study days 11 and 21, respectively. On days 9, 10, 11, 19, and 20 the subjects received a standardized breakfast of 550 kcal (two slices of white bread, 15 g of low-fat margarine, 14 g of jelly, 150 ml of orange juice, and 150 ml of skim milk). The medication was administered immediately after breakfast with 200 ml of tap water. All other meals and snacks on the pharmacokinetic study days were also standardized. When the subjects took the medication at home, study drugs were administered with breakfast (at least two and at most three slices of wheat bread).

No crossover design was used in this study because rifampin could lead to considerable carryover effects, due to its long-lasting cytochrome P450-inducing effect. To eliminate this effect a longer washout period would be necessary, but this would have significantly prolonged the duration of the study and would have significantly prolonged the duration of the study.
led to difficulties with subject recruitment and retention. This study was reviewed and approved by the independent ethics committee Arnhem-Nijmegen. Written informed consent was obtained from each study subject prior to the conduct of any study-related activity.

**Study subjects.** Twenty-four healthy male and female subjects were eligible for inclusion in the study. The subjects could be between 18 and 65 years of age with a body weight of at least 50 kg and in good age-appropriate health condition, as established by the individual’s medical history; a physical examination; electrocardiography; and the results of biochemistry, hematology, and urine analyses within the 3 weeks prior to administration of the first dose. Other inclusion criteria were an ability to sign informed consent voluntarily and a willingness to refrain from the use of contact lenses during the treatment with rifampin. Exclusion criteria were as follows: positive tests for HIV, hepatitis B virus, or hepatitis C virus; a tuberculin skin test reaction of more than 15 mm with abnormalities consistent with tuberculosis; pregnancy; breast-feeding; the lack of adequate contraception (e.g., hysterectomy; bilateral tubal ligation; the use of an intrauterine device, total abstinence, or double-barrier methods; or a postmenopausal state for 2 years) among female subjects of childbearing potential; a creatinine clearance rate <60 ml/min; or a serum creatinine level above 133 μmol/liter.

**Sampling for pharmacokinetic studies.** For determination of the tenofovir and rifampin concentrations in blood plasma, samples of 5 ml of blood, recovered to obtain at least 2 ml of plasma, were collected in heparinized hard plastic tubes at the following times: just before drug intake (predosing); on day 10 and day 20; and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 h after drug intake. The blood samples were centrifuged at 2,500 × g for 10 min at 4°C. The plasma was divided into equal portions, transferred to polypropylene tubes, and stored at −70°C for samples containing rifampin and −20°C for samples containing tenofovir.

**Safety.** Blood samples for serum biochemistry analyses, including tests for glucose and hematologic analyses, and urine samples for urinalysis were taken on study days 1, 4, 9, 11, 15, 19, and 21. These samples were taken while the subjects were in a fasting condition. In females of childbearing potential, testing of blood for hCG was performed on study day 1, 9, and 19, with the Instacheck Multi-Drug Screen panel (Frontiforeign Diagnostics, San Diego, Calif.). Vital signs for cardiovascular safety (systolic and diastolic blood pressures and heart rate) were monitored, and an electrocardiogram was recorded at the screening visit. The medical and nursing staff of the trial center monitored the subjects for adverse events (AEs) throughout their confinement. Subjects voluntarily reported any AE or reported AEs in their medical history; a physical examination; electrocardiography; and the results of biochemistry, hematology, and urine analyses established by the individual’s medical history; a physical examination; electrocardiography; and the results of biochemistry, hematology, and urine analyses.

**Pharmacokinetics.** Tenofovir concentrations were determined by using a validated high-performance liquid chromatography assay with a fluorometric detector by a modified method (5). A Symmetry Shield RP18 analytical column (3.5 mm; 150 μm; 4.6 mm; Waters, Etten-Leur, The Netherlands) was used. The method involved extraction of the drug and the internal standard, adefovir (Gilead Sciences, Foster City, Calif.), from 100 μl of human plasma by adding 200 μl of acetonitrile. The supernatant was evaporated, and 200 μl of 0.34% chloroacetic-aldehyde in 50 mM acetic buffer (pH 4.5) was added. Fluorescent compounds were obtained by 40 min of incubation at 90°C. After the samples were cooled at −20°C for 5 min, 10 μl was injected onto the column. The flow rate was 1 ml/min. The mobile phase consisted of a mixture of phosphate buffer (50 mM; pH 6.8) and acetonitrile (96:4; vol/vol) that resolved the drug and the internal standard from endogenous matrix components and other drugs that were possibly present.

Chromatographic analysis was performed at 30°C under isocratic conditions with extinction and emission wavelengths of 232 and 420 nm, respectively. The retention times of tenofovir and the internal standard, adefovir, were 6.34 and 3.90 min, respectively. The chromatograms used were 0.03, 0.21, and 1.05 mg/liter. The intra- and interassay coefficients of variation were less than 4% for all quality controls. The lower limit of quantification was 0.0005 mg/liter. The rate of recovery of tenofovir from human plasma was 86%. Rifampin concentrations were determined by using a previously described (12) high-performance liquid chromatography method. The concentrations of the quality controls used were 2.85, 9.5, and 24 mg/liter. The intra- and interassay coefficients of variation were less than 1.1% for all quality controls. The lower limit of quantification was 0.50 mg/liter. Samples from the same subject were analyzed by use of the same standard curve.

**Demographics.** Twenty-four subjects (13 males, 11 females) were enrolled in this trial. One male subject was black; all other subjects were Caucasian. The mean age of the subjects was 41 years (range, 20 to 63 years). The mean body weight was 77 kg (range, 58 to 97 kg), and the mean height was 1.75 m (range, 1.59 to 1.88 m).

**Pharmacokinetic analysis.** Pharmacokinetic parameters for tenofovir and rifampin were calculated by noncompartmental methods by use of the WinNonlin software package (version 4.1; Pharsight Corporation, Mountain View, Calif.) and the log/linear trapezoidal rule. On the basis of the individual plasma concentration-time data, the following pharmacokinetic parameters were determined: the area under the plasma concentration-time curve (AUC) from time zero to 24 h (AUC0–24; in milligram · hour per liter), the maximum concentration of drug in plasma (Cmax; in milligrams per liter), the time to reach Cmax (Tmax; in hours), the minimum concentration drug in plasma (Cmin; in milligrams per liter), the apparent elimination half-life (t1/2; in hours), and the apparent oral clearance (CL/F; in liters per hour). AUC0–24, where C* is the last quantifiable concentration, was calculated for rifampin. Cmin and CL/F were not calculated for rifampin.

**RESULTS**

**Pharmacokinetic evaluation was based on data sets for subjects that completed the study on both study days (study days 10 and 20).** Data for 23 subjects were included in the pharmacokinetic analysis of tenofovir and rifampin. **Table 1** provides a summary of the values of the pharmacokinetic parameters for tenofovir, including the arithmetic means, geometric mean ratios, and 90% confidence interval estimates for the pharmacokinetic parameters for tenofovir alone (study day 10) and tenofovir in combination with rifampin (study day 20). The tenofovir AUC0–24, Cmax, and Cmin were lower in period 2 when tenofovir DF was coadministered with rifampin. However, the magnitudes of these differences were small, with geometric mean ratios (90% confidence intervals) of 0.88 (0.84 to 0.92), 0.84 (0.78 to 0.90), and...
0.85 (0.80 to 0.91) for AUC₀⁻2₄, Cₘₐₓ, and Cₘᵢₙ, respectively, suggesting pharmacokinetic equivalence when tenofovir DF was dosed with or without rifampin.

Figure 1 illustrates the effects of rifampin on the mean concentration-time profiles of tenofovir. Table 2 presents the values of the pharmacokinetic parameters for rifampin when it was combined with tenofovir and the values of the pharmacokinetic parameters of rifampin from the literature (4, 15). The values of the pharmacokinetic parameters for rifampin when it was combined with tenofovir are comparable to those in the literature when rifampin is administered with food, suggesting that tenofovir has no influence on rifampin exposure.

Safety. All 24 subjects reported one or more AEs at some time during the study. No subject experienced a grade 4 AE or a serious AE. In total, 160 grade 1 or grade 2 AEs were reported. A total of 102 AEs were judged to be possibly or probably related to a study drug(s). During treatment with tenofovir DF (period 1), 33 possibly or probably related AEs were reported, while during period 2 (tenofovir DF combined with rifampin), 69 possibly or probably related AEs were reported. Of the 69 AEs reported during period 2, 24 AEs were related only to rifampin. Each subject reported discoloration of the urine. Most of the study drug-related AEs were mild (85% were grade 1 in severity). All AEs resolved after the withdrawal of treatment.

All 24 subjects completed treatment period 1 (tenofovir DF alone). The most common AEs that were reported during treatment with tenofovir DF were fatigue, headache, and gastrointestinal disorders.

During period 2 one subject was withdrawn from the study due to several complaints, which were rash, headaches, abdominal disorders, fatigue, somnolence, and dizziness. The study medications were stopped on study day 15. At the follow-up visit, 5 days later, the subject developed elevated liver enzyme levels, which were judged to be a grade 3 AE. Nine days after the first follow-up visit the liver enzyme levels returned to normal. The AEs that occurred during the combination treatment with tenofovir DF and rifampin consisted mainly of flulike symptoms (e.g., fatigue, headache, and gastrointestinal disorders) and urine discoloration, which are well-known AEs of rifampin (7).

No clinically significant hematology or urinalysis values were observed in this study.

DISCUSSION

This study was designed to investigate whether rifampin influences the pharmacokinetics of tenofovir. The study showed that bioequivalence could be suggested for tenofovir DF combined with rifampin and tenofovir DF given alone and that the combination of tenofovir DF with rifampin was generally well tolerated, as only one patient prematurely discontinued from study.

The confidence intervals for AUC and Cₘᵢₙ were 0.84 to 0.92 and 0.80 to 0.91, respectively, while the confidence interval was

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value on day 20 (this study)</th>
<th>Value in the literature (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tₘᵢₙ (h)</td>
<td>4.43 (1.11)</td>
<td>2.4 (0.6)</td>
</tr>
<tr>
<td>Cₘᵢₙ (mg/liter)</td>
<td>7.27 (2.25)</td>
<td>10.9 (3.0)</td>
</tr>
<tr>
<td>AUC₀⁻₁₂ (mg · h/liter)</td>
<td>50.97 (14.27)</td>
<td>43.27 (15.28)</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>1.5 (0.3)</td>
<td>4.43 (1.11)</td>
</tr>
</tbody>
</table>

The data are from reference 15 and are for subjects who received rifampin with breakfast.

Values are means (standard deviations).
0.78 to 0.90 for $C_{\text{max}}$. By definition, bioequivalence was proven for AUC and $C_{\min}$ but was only suggested for $C_{\text{max}}$.

The tenofovir DF dose used in this study (300 mg QD) is the dose recommended for the treatment of HIV infection in adults (11). The rifampin dose used (600 mg QD) is an accepted regimen for the treatment of tuberculosis in patients weighing more than 50 kg (7). A previous study (2) has shown that steady-state conditions for rifampin are generally achieved after the sixth daily dose of rifampin at 600 mg. To ensure the achievement of steady-state pharmacokinetics, subjects were given tenofovir DF combined with rifampin for 10 days before pharmacokinetic assessment.

The reason for the lower observed tenofovir levels is unknown. Several mechanisms could contribute to this interaction. Because tenofovir is not metabolized and is eliminated unchanged by a combination of glomerular filtration and active tubular secretion (1, 6), it is unlikely that the inducing effect of rifampin on hepatic and intestinal cytochrome P450 enzymes (especially CYP3A4) (8) is the mechanism responsible for this effect. This is supported by no apparent changes in the tenofovir $t_{1/2}$ and no clinically relevant effects of rifampin on the tenofovir $C_{\min}$.

Similarly, as tenofovir minimally binds to proteins in human plasma or serum (≤0.7 and 7.2%, respectively) (11), altered distribution is also probably not the mechanism responsible for the pharmacokinetic differences observed. As the decrease in the tenofovir $C_{\text{max}}$ was 16% while the decrease in AUC$_{0-24}$ was 12%, the cause may be in the process of tenofovir DF or tenofovir absorption. Rifampin has been shown to be an inducer of the efflux transporter P glycoprotein (9). No information exists in the literature that P glycoprotein plays a role in the process of absorption of tenofovir in vivo. However, van Gelder et al. (19) have described the transport of tenofovir DF by a P-glycoprotein-related efflux mechanism in the Caco-2 system.

AEs led to one discontinuation in this study; grade 3 elevations in hepatic enzyme levels were reported after the medication was stopped during period 2, when tenofovir DF was combined with rifampin. Liver disturbance is a well-known side effect of rifampin. Gastrointestinal disorders are well-known AEs of both tenofovir and rifampin and occurred in a total of 46% of the study subjects during both study periods. During period 2 all subjects reported discoloration of their urine, which is a well-known AE of rifampin (7).

Some additional considerations are important for the extrapolation of the results of this study to patients. First, it should be noted that all the participants in this study were healthy subjects. It cannot be excluded that the pharmacokinetics of tenofovir and rifampin are different in HIV-infected patients coinfected with $M. tuberculosis$ due to one or both of the diseases. Second, 23 of the 24 subjects of this study were Caucasian. Race might have an effect on the values of the pharmacokinetic parameters for tenofovir, although the available pharmacokinetic data do not indicate substantial differences with regard to race (11). Finally, the subjects in this study were given tenofovir DF and rifampin only, while HIV-infected patients coinfected with $M. tuberculosis$ are treated with other antiretroviral and tuberculostatic drugs, which can cause interactions.

In conclusion, the data from this study demonstrate that the addition of rifampin to tenofovir DF is well tolerated, and the small decrease in plasma tenofovir levels during combination treatment suggests that these drugs can be coadministered without the need for dose adjustments. This implies that standard doses should be a starting point for the use of these medications by HIV-infected patients. Additional pharmacokinetic studies in a clinical setting are warranted to confirm the findings of this study.

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