Single-Dose Bioequivalence of a New Fixed-Dose Combination Tablet Containing Tenofovir Disoproxil Fumarate and Lamivudine

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Abstract

Tenofovir Disoproxil Fumarate, CAS 147127-20-6 is a nucleotide reverse transcriptase inhibitor with potent activity against both HIV and hepatitis B infections. Lamivudine, CAS 134678-17-4 is a nucleoside analogue reverse transcriptase inhibitor developed as a treatment for HIV infection and also with activity against hepatitis B virus. The combination of tenofovir and lamivudine associated either with non-nucleoside reverse transcriptase inhibitors or with a ritonavir-boosted or unboosted protease inhibitor are recommended as preferred regimens for antiretroviral therapy-naïve patients infected with HIV, and also for the treatment of HIV-HVB coinfected patients. The objective of this study was to compare rate and extent of absorption and to assess the bioequivalence between a new pharmaceutical equivalent tablet formulation containing a fixed-dose combination of tenofovir disoproxil fumarate/lamivudine 300/300 mg and the innovator products. A randomized, single-center, open-label, single-dose, two-way crossover bioequivalence study in 40 healthy adult subjects was conducted. Dosing was separated by a wash-out period of 14 days. All subjects signed an informed consent form. In each study period, 13 blood samples were collected in Vacutainers containing EDTA over 48 h. Plasma levels of tenofovir and lamivudine were determined by a validated HPLC/fluorescence assay and by a validated HPLC/UV assay, respectively. Rate and extent of absorption were similar between products. The 90% confidence interval(CI) of the ratio of the geometric means for log-transformed C_{max}, AUC_{last} and AUC_{inf} values were used to assess bioequivalence between the two formulations using the equivalence interval of 80 and 125%. In healthy subjects, the point estimate and 90 % C_l of the ratios of C_{max}, AUC_{last} and AUC_{inf} values for tenofovir were 100.99%(92.89-109.80%), 96.11%(90.02-102.63%) and 94.73%(88.22-101.73%), respectively; and for lamivudine were 90.37%(83.76-97.50%), 97.02%(93.27-100.93%) and 97.04%(93.41-100.82%), respectively. Both treatments exhibited similar tolerability and safety. It was concluded that the new pharmaceutical formulation was bioequivalent to the innovators.

Introduction

Tenofovir disoproxil fumarate (TDF) is a nucleotide analog reverse transcriptase inhibitor orally bioavailable as an ester-derived prodrug which requires diester hydrolysis for conversion to tenofovir (TFV) and subsequent intracellular phosphorylations by cellular enzymes to the active metabolite, tenofovir diphosphate, which is a competitive inhibitor of HIV-1 reverse transcriptase, leading to the prevention of DNA chain elongation and termination of viral DNA growth [1,2]. TDF was approved by the Food and Drug Administration (FDA) in October 2001 and is indicated for use in combination with other antiretroviral agents for the management of HIV-1 infection. TFV has also activity against hepatitis B infection and has been approved in 2009 as the first-line option in the treatment of hepatitis B [3,4]. The pharmacokinetic (PK) of TFV following oral administration of 300 mg has been well characterized in HIV-infected and healthy adults subjects. After oral administration of 300 mg, TFV concentrations increase over 1 to 3 h (T_{max}) with a maximum concentration (C_{max}) of approximately 300 ng/ml and a mean area under the plasma concentration-versus-time curve (AUC) at steady state of approximately 3000 ng*h/ml is observed [5,6]. When administered with a high fat meal (700-1000 calories containing 40-50% fat), TFV AUC and Cmax are increased by 40% and 14 %, respectively. TFV is primarily cleared unchanged in the urine by a combination of glomerural filtration and active tubular secretion. The once-daily dosing schedule is supported by TFV serum elimination half-life of 12 to 17 h and the long half-life of the intracellular metabolite between 10 to 50 h [7].

Lamivudine (3TC) is a nucleoside analogue developed as a treatment for HIV infection. It has also activity against hepatitis B virus (HBV). Intracellular, lamivudine is phosphorylated to its active metabolite, lamivudine 5`-triphosphate (3TC-TP) which prevents HIV-1 and HBV replication by competitively inhibiting viral reverse transcriptase via DNA chain termination after incorporation of the nucleotide analogue The pharmacokinetics of 3TC are similar in patients with HIV-1 or HVB infection, and healthy volunteers [8]. Following oral administration, 3TC is well absorbed from the gastrointestinal tract, being the bioavailability in adults between 80 and 85 %, and the mean time (T_{max}) to maximal serum concentrations (C_{max}) about an hour. Based on data derived from a study in healthy volunteers, at a therapeutic dose of 150 mg twice daily, mean steady-state C_{max} and 12h AUC in plasma are 1200 ng/ml and 4700 ng*h/ml, respectively. Lamivudine systemic exposure (based on the AUC) is not influenced when it is administered with food and is widely distributed into total body fluid being predominately eliminated

unchanged by renal excretion. The dominant elimination half-life of 3TC is approximately 5 to 7 h, and the intracellular in vitro half-life of its active 5'-triphosphate metabolite is approximately 10.5 to 15.5h [9].

The Working Group of the Office of AIDS Research Advisory Council (OARAC) recommends the combination of 2NRTI (nucleoside reverse transcriptase inhibitors) such as TDF and 3TC associated either with a NNRTI (non-nucleoside reverse transcriptase inhibitors) such as efavirenz (EFV) or with a ritonavir-boosted or un-boosted PI (protease inhibitor) as the preferred regimens (AI) for antiretroviral therapy (ART)-naïve patients infected with HIV. Moreover, the combination of TDF and 3TC or FTC is recommended as the preferred regimen (AII) for the treatment of HIV-HVB co-infected patients [3].

Successful long-term treatment of HIV/AIDS requires high levels of patient´s adherence to therapy to prevent drug-resistance that leads to treatment failure. Incomplete adherence to HIV regimens is associated to their complexity, frequency, discordant dosing schedules; therefore pharmaceutical strategies to simplify patient´s adherence are significant determinants of treatment outcomes. A new oral tablet formulation containing a fixed-dose combination of TDF/3TC 300/300 mg has been developed to support OARAC recommendations for first-line therapy and to help improve patient compliance with once-daily dosing of this combination tablet. The objective of the present study was to evaluate and compare the rate and extent of absorption of both TDF and 3TC from the new combination dosage form related to that of the innovators single formulations in healthy volunteers under fasting conditions.

Subjects and Methods

Study design and methodology

A randomized, balanced, open-label, single-center, single-dose, two treatment, two period, two sequence, crossover bioequivalence study in 40 healthy adults subjects under fast condition was carried out. The study was conducted at FP Clinical Pharma Clinical-Pharmacokinetic Unit located at Buenos Aires, Argentina between September and December 2010. Both, the study protocol and the Informed Consent Form (ICF) were approved by an Institutional Review Board, an Independent Ethic Committee and by the local Regulatory Agency (ANMAT) before the beginning. Clinical procedures were carried out in accordance with ICH-GCP guidance, FDA guidance for conducting bioavailability and bioequivalence studies for oral administered drugs and the principles enunciated in the latest version of the Declaration of Helsinki [10-12]. All subjects who volunteered to participate signed an approved ICF.

All volunteers were randomly assigned to receive either a single 300/300 mg of the new fixed-dose combination oral tablet of TDF/3TC Mivuten® as test preparation (batch No.1344), manufactured by Richmond Laboratories, Buenos Aires, Argentina, or a single 300 mg oral tablet of the innovator product TDF: Viread® (batch No. L05847) manufactured by Gilead Sciences plus two single 150 mg oral film-coated tablets of the innovator product 3TC: 3TC® (batch No. R397808-1) manufactured by GlaxoSmithKline as reference preparations. Both reference products were purchased at a local pharmacy. The treatments were administered under fasting condition in two different dosing periods separated by a 14-day wash-out period according to a predetermined randomized schedule. Subjects were not allowed to either crush or chew the study medication. Mouth checks were performed after each dosing. Subjects remained under fasting condition until after the 4-hour post-dosing. A standard lunch and

afternoon meal were administered after the 4th and 8th hour of drug administration, respectively.

Study population

Sample size was calculated using the formula developed by Marzo and Balant, using C_{max} CVs of 38 % for TDF and of 24 % for 3TC, according to literature [1,9,13]. A total of 40 healthy male and female subjects (non-pregnant and non-lactating) between 21 and 50 years of age were enrolled. Inclusion criteria included Body Mass Index (BMI) between 19 and 27 Kg/m². Female subjects of childbearing potential (ie. not surgically sterile or at least 2 years postmenopausal) were required to have a negative pregnancy test at screening and agree to use a highly effective contraception method (not hormonal) while on study treatment and for three weeks after the last dose of study drugs. Laboratory values, 12-lead electrocardiograms and chest x-rays for all subjects had to be within normal range. Negative test for VIH, hepatitis B and C viruses were also required.

Subjects were excluded if they had a history of alcohol or drug abuse in the last two years, history or current manifestations of gastrointestinal disease or surgery, or hepatic, cardiovascular, respiratory, renal, hematopoietic, neurological, endocrine-metabolic diseases. Volunteers were not allowed to use any kind of medicine within the previous two weeks and throughout the study execution. Other standard exclusion criteria for BD/BE studies were adopted for subject enrollment [11].

Sample collection

A total of 13 serial blood samples for pharmacokinetic assessments were collected by venipuncture over a 48 h period at the following points: 0 (pre-dose), 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 and 48 h after oral administration of each treatment. For each sample, approximately 10 ml of blood was collected into vacutainers containing EDTA as an anticoagulant. Blood samples were centrifuged and separated plasma was frozen at -20ºC until analysis.

Bio analytical procedures

Tenofovir concentrations in human plasma was determined by a validated HPLC/fluorescence method using an analytical column 100x4.6nm, Phenomenex Luna C18 (2), 3μ, fluorescence detection within 236 to 420 nm. The lower limit of quantification (LOQ) corresponding to TFV was 5.0 ng/ml. The relationship between concentration and peak area ratio for TDF (TFV: Internal Standard) was found to be linear within the range of 5.0 ng/ml to 1000.0 ng/ml. The precision and accuracy in the assay validation were evaluated using 3 separate analytical runs, with each containing quality control samples in replicates of 5. Inter- and intra-assay precision had a < 6% coefficient of variation.

Lamivudine concentrations in human plasma was determined by a validated HPLC/ UV method using an analytical column 75x4.6 nm, Phenomenex Luna C18 (2), 3μ, UV detection 271 nm. The LOQ corresponding to 3TC was 20.0 ng/ml. The relationship between concentration and peak area ratio (3TC: Internal Standard) was found to be linear within the range of 20.0 ng/ml to 4000.0 ng/ml. The precision and accuracy in the assay validation were evaluated using 3 separate analytical runs, with each containing quality control samples in replicates of 5. Inter- and intra assay precision had a < 3 % coefficient of variation.

Full methodological validation was carried out according to local and FDA guidance for bioanalytical method validation [14].

In vitro dissolution

The in vitro dissolution study of both tablets were studied in USP apparatus type II employing a paddle stirrer at 50 rpm using 900 ml of 0.1N HCl at 37±0.5°C as dissolution medium. The percentage of drug release was calculated using an equation obtained from a standard curve and results are presented in Figures 1A and 1B.

Pharmacokinetic evaluation

The plasma concentration-time data after oral administration of a single dose of test and reference products were analyzed using a Non-compartmental pharmacokinetic model (WinNonlin, version 5.3; Pharsight, Certara, USA). The maximum plasma concentration and the corresponding sampling time were defined as C_{max} and T_{max}, respectively. The slope of the log-linear regression function (λ) was the first order rate constant associated with the terminal portion of the curve estimated by linear regression of time vs. log-concentration. The elimination half-life ($T_{\frac{1}{2}}$) was estimated as $\ln 2/\lambda$. The area under the plasma concentration-time curve from the time of dosing to the last measurable concentration (AUC_{last}) was calculated using the trapezoidal rule. The AUC from dosing time extrapolated to infinity based on the last observed concentration was defined as AUC_{inf}. Which was calculated by the equation $AUC_{inf} = AUC + (C_n / \lambda)$ where C_n is the last measurable concentration and λ is the slope of the log-linear regression function. A pharmacokinetic rule was generated to treat data coming from samples presenting values less than the lower level of quantification in bio analytic assays.

Safety assessment

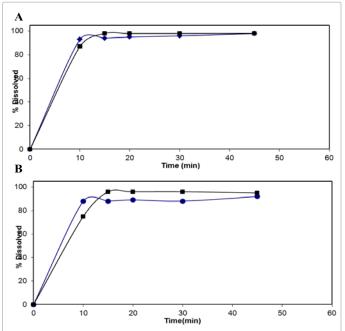Physical examination, hematology, platelets count, serum

Characteristic	Results (mean ± SD)
Age (yrs)	34.32 ± 8.84
Height (cm)	167.82 ± 8.96
Weight (kg)	69.89 ± 10.33
BMI (kg/m²)	24.71 ± 2.22

Table 1: Demographic data of investigational subjects (n=40).

chemistry (fasting glucose, liver function panel, creatinin, urea, uric acid, potassium, sodium, calcium, phosphorous, bicarbonate, lactic acid), urinalysis, were performed at screening (Day-21 to -1) and at study termination for safety purposes (Day 17). A 12-lead electrocardiogram and a chest x-ray were also carried out at screening. For female with childbearing potential, serum pregnancy test was performed at screening and on urine samples previous to each dosing period. An abbreviated physical examination was carried out on the morning before drug administration. Vital signs (systolic and diastolic blood pressure in supine position and heart rate) were recorded during screening, immediately before drug administration, and 4, 12 and 72 h after drug administration.
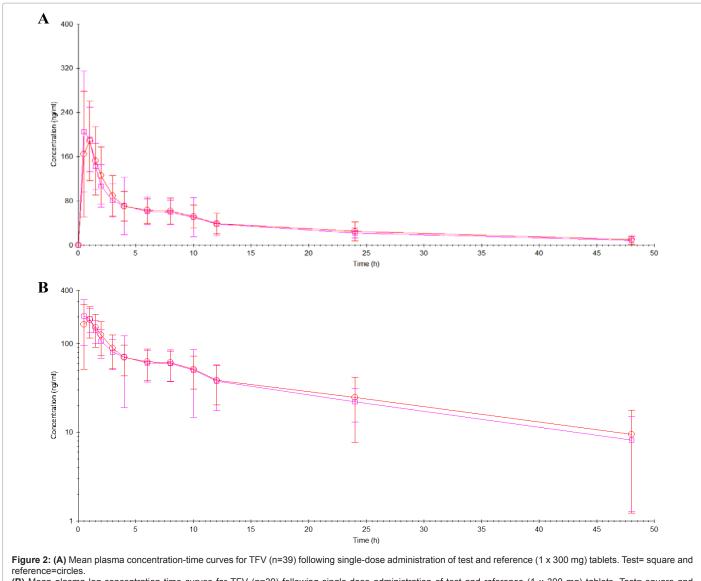
Statistical analysis

The following pharmacokinetic parameters: C_{max}, AUC_{last}, and AUC_{inf} were analyzed for TFV and 3TC using natural log-transformed data. These PK variables were compared by means of ANOVA for a 2-treatment crossover design. The model included the fixed effects of period, sequence, and treatment. In accordance with scientific standards and international guidelines for bioequivalence studies, bioequivalence was concluded if the 90% confidence interval (CI) for the ratio of the geometric least-squares means (test treatment/reference treatment) was within the bounds of 80% to 125% for the primary PK parameters. All statistical tests used a 5% level of significance [11,15].
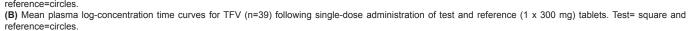
Results

A total of 40 healthy subjects were enrolled in the study. All subjects were Caucasian, being 65% (26/40) male and 35% (14/40) female. Demographic data and mean health parameters of all the participants are summarized in Table 1. One subject was excluded from the study because of personal reasons after the first period of dosing.

Pharmacokinetics

The data set for TFV and 3TC analysis included 39 subjects. Figure 2 (arithmetic scale) and Figure 3 (semi log-transformed scale) show mean plasma concentration-time curves after single dose administration of 300/300 mg (TDF/3TC) of test and reference products. The mean curves for the two treatments for TFV and 3TC followed a typical profile for a conventional immediate release formulation and were almost superimposable. Elimination phase for both TFV and 3TC showed a biphasic shape (alpha and beta elimination half-lives) on both the test and reference products. Table 2 shows a comparison of individual subject AUC_{last} values for TFV/3TC test and reference treatments. For TFV 6 out of 39 subjects showed test AUC_{last} values 30% different to reference AUC_{last} data. In the case of 3TC, individual AUC_{last} values differences between test and reference did not exceed 30% in none subjects, suggesting that extend of absorption was similar for both drugs. Plasma pharmacokinetic parameters for TFV and 3TC are summarized in Table 3. No statistical differences were observed between mean pharmacokinetic parameters for TFV and 3TC test and reference formulations.

Figure 1: (A) In vitro dissolution profiles for lamivudine for Reference (square) and test (circle) products.
(B) In vitro dissolution profiles for tenofovir for Reference (square) and test (circle) products.

Figure 2: (A) Mean plasma concentration-time curves for TFV (n=39) following single-dose administration of test and reference (1 x 300 mg) tablets. Test= square and reference=circles.
(B) Mean plasma log-concentration time curves for TFV (n=39) following single-dose administration of test and reference (1 x 300 mg) tablets. Test= square and reference=circles.

The analysis of variance did not show any statistically significant difference between test and reference formulations (p<0.05) in relation to the fixed effect of period, sequence and treatment for the pharmacokinetic parameters analyzed: ln C_{max}, AUC_{last} and AUC_{inf}.

Statistical analysis of TFV and 3TC pharmacokinetic log-transformed parameters and their geometric least squares mean ratios for the test and reference treatment are presented in Tables 4 and 5 for TFV and 3TC formulations, respectively. The limits of the 90 % confidence intervals (CI) for the ratios of C_{max}, AUC_{last} and AUC_{inf} for their log-transformed data fell well within 80 to 125%. Coefficients of intra-subject variation for C_{max}, AUC_{last} and AUC_{inf} corresponding to TFV were 0.23, 0.40 and 0.38, respectively; and corresponding to 3TC were 0.25, 0.20 and 0.21, respectively.

Test-Reference ratio for the geometric means (%) for all primary pharmacokinetic metrics (AUC_{last}, AUC_{inf}, C_{max}) and the corresponding 90% confidence intervals corresponding to TFV and 3TC were contained within the bioequivalence bounds of 80 % to 125 %. Moreover, the null hypothesis of the two one-sided t-test Schuirmann could be rejected (p<0.05) as shown in Tables 4 and 5. Power of the statistical tests were much higher than 0.80, as requested by regulatory agencies.

Safety

TFV was well tolerated by all subjects. No clinically significant changes in vital signs (blood pressure, heart rate) and safety laboratory tests were observed after single oral dose administration of TDF/3TC 300/300 mg. A total of 4 non-serious adverse events (AEs) which were considered not related to the study drug by the investigators were reported: One case of teeth ache of moderate intensity that resolved with the use of ibuprofen 400 mg, one case of pharyngitis of moderate intensity which resolved spontaneously, one case of skin injury and another case of traumatism in right knee of moderate intensity that resolved with local treatment.
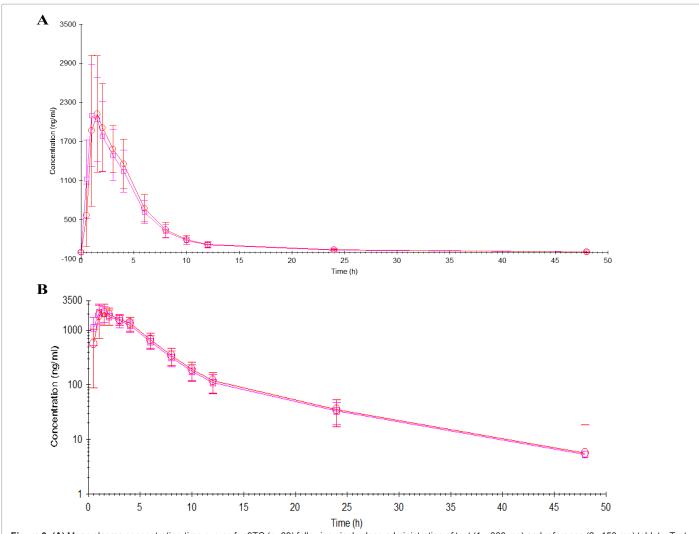
Figure 3: (A) Mean plasma concentration-time curves for 3TC (n=39) following single-dose administration of test (1 x 300 mg) and reference (2x 150 mg) tablets. Test= square and reference= circles.
(B) Mean plasma log-concentration time curves for 3TC (n=39) following single-dose administration of test (1 x 300 mg) and reference (2x 150 mg) tablets. Test= square and reference= circles.

Subject Nº	Tenofovir (N=39) AUClast (ng*h/ml)		Lamivudine (N=39) AUClast (ng*h/ml)	
	Reference	Test	Reference	Test
1	2327.3	2430.14	18327.9	16637.88
2	768	871.75	6953.47	8606.69
3	1491.65	1685.77	14047.93	14361.01
4	992.51	1369.27	10314.07	9830.74
5	1220.5	1085.97	11113.99	8054.3
6	1120.45	1452.77	11681.65	10481.75
7	1915.7	2000.04	12317.39	14065.32
8	1961.56	1217.3	9576.35	7036.73
9	1748.94	1807.27	14461.87	13880.45
10	1549.15	1261.47	5710.25	6333.19

11	852.61	1565.02	9173.39	10023.46
12	2043.18	2205.03	11780.66	9885.28
13	1637.25	1527.86	13434.67	11200.56
14	4842.91	4378.79	16641.18	14853.9
15	2275.9	2210.23	7122.95	5826.2
16	2000.89	1782.21	12072.39	11685.83
17	1410.7	1440.59	12786.89	15643.27
18	2050.43	1379.81	12828.37	11057.92
19	1342.33	966.45	12744.42	9208.99
20	1708.38	1535.18	11241.37	11558.16
21	839.62	1011.15	12170.92	11068.7
22	1053.88	927.19	10133.73	9764.9
23	2350.86	1982.18	11690.22	10876.13
24	1731.35	1545.3	10770.84	9867.83
25	2820.83	2018.06	9633.14	12070.42
26	2393.57	1585.59	10711.17	13438.01
27	969.48	1066.34	10250.17	11075.47
28	1238.2	1409.28	9335.52	10121.85
29	2995.48	2914.91	8772.56	10643.76
30	1684.65	1809.6	11422.97	11738.11
31	796.34	889.81	10766.06	8849.27
32	1960.62	1886.91	9763.98	9499.89
33	1400.22	1739.67	9282.27	9858.01
34	2121.61	2211.32	10685.38	10816.44
35	1611.14	1173.49	9859.52	9652.06
37	1819.93	1705.63	8703.72	9021.52
38	1090.51	554.46	9922.89	8733.43
39	731.41	714.04	8885.62	8193.66
40	631.69	788.5	10144.54	9750.24

Table 2: Individual subject AUClast values for TFV/3TC (n=39) of either test or reference treatment.

PK Parameter	Tenofovir (N=39)			Lamivudine (N=39)		
	Reference arithmetic mean (SD)	Test arithmetic mean (SD)	P value	Reference arithmetic mean (SD)	Test arithmetic mean (SD)	P value
C_{max} (ng/ml)	235.21 (68.66)	239.75 (81.07)	0.79	2538.80 (964.98)	2279.01 (756.76)	0.190
T_{max} (hours)	0.95 (0.55)	0.77 (0.32)	NA	1.73 (0.69)	1.41 (0.55)	NA
AUC_{last} (ng*h/ml)	1679.53 (783.72)	1592.46 (686.98)	0.603	10954.77 (2417.68)	10647.98 (2399.37)	0.575
AUC_{inf} (ng*h/ml)	2043.68 (944.63)	1894.78 (783.35)	0.451	11300.91 (2585.9)	10979.83 (2543.85)	0.582
K_e (1/h)	0.05 (0.02)	0.05 (0.02)	1.00	0.13 (0.08)	0.14 (0.07)	0.558
Terminal half-life (h)	17.07 (7.20)	16.42 (6.70)	0.681	6.99 (4.12)	6.33 (3.49)	0.447

Table 3: Pharmacokinetic parameters of TFV/3TC in healthy volunteers (n=39) following single-oral dose administration of either test or reference treatment.

Pharmacokinetic Parameter	Ref GeoLMS(*)	Test GeoLMS(*)	Ratio (% Ref)	Classical 90% CI	Two one-Sided t-test Schuirmann	Power
LnC_{max} (ng/ml)	225.21	227.22	100.99	92.89 - 109.80	P(0<80%)=0.0000 P(0>125%)=0.0001	0.99
$LnAUC_{last}$ (ng*h/ml)	1528.89	1470.23	96.11	90.02 - 102.63	P(0<80%)=0.0000 P(0>125%)=0.0000	0.99
$LnAUC_{inf}$ (ng*h/ml)	1859.83	1763.12	94.73	88.22 - 101.73	P(0<80%)=0.0001 P(0>125%)=0.0000	0.99

(*) Geo LSM= Geometric Least Squares Mean

Table 4: Bioequivalence analysis for TFV following single-oral dose administration of either test (1 x 300 mg tablet) or reference treatment (1 x 300 mg tablet).

Pharmacokinetic Parameter	Ref GeoLMS(*)	Test GeoLMS(*)	Ratio (% Ref)	Classical 90% CI	Two one-Sided t-test Schuirmann	Power
LnC_{max} (ng/ml)	2399.36	2167.62	90.37	83.76 - 97.50	P(0<80%)=0.0051 P(0>125%)=0.0000	0.99
$LnAUC_{last}$ (ng*h/ml)	10700.50	10387.45	97.02	93.27 - 100.93	P(0<80%)=0.0000 P(0>125%)=0.0000	1.00
$LnAUC_{inf}$ (ng*h/ml)	11025.27	10704.50	97.04	93.41 - 100.82	P(0<80%)=0.0000 P(0>125%)=0.0000	1.00

(*) Geo LSM= Reference Geometric Least Squares Mean

Table 5: Bioequivalence analysis for 3TC following single-oral dose administration of either test (1 x 300 mg tablet) or reference treatment (2 x 150 mg tablet).

Discussion

The objective of the present study was to evaluate and compare the rate and extent of absorption of a new pharmaceutical oral equivalent tablet formulation containing a fixed dose combination of TDF/3TC 300/300 mg to that from the same dose of the separate innovators formulations in healthy volunteers; and secondarily to assess bioequivalence between them.

Our results showed that no significance differences were found, in terms of rate and extent of absorption, between test and reference products, as indicated by C_{max} and AUC comparisons and also by the similar plasma tenofovir and lamivudine concentration-time curves. Considering that 90% CIs of the ratios of µT/µR for the PK parameters (C_{max} and AUC_s log-transformed) were found to be within the predetermined range (80% - 125%) and the Schuirmann two one-sided t test procedure (probability of exceeding limits of acceptance) found all probability values <0.05, the null hypothesis that the estimated parameters exceeded limits of acceptance was rejected, both combination regimen tested were found to be bioequivalent.

This is the first study showing single-dose bioequivalence between a new fixed-dose combination tablet formulation of TDF/3TC 300/300 mg versus the regular single tablet regimen of TDF and 3TC in different dosage forms. The fact that the new dosage form, formulated as a combination tablet, was developed as a pharmaceutical equivalent product related to each innovator reference respectively, with similar pharmaceutical behavior, as seen by similar *In vitro* dissolution profiles, lead to obtain similar pharmacokinetic profiles and similar pharmacokinetic endpoints values *In vivo*.

The pharmacokinetic profile of TFV described in our study were lower than previous pharmacokinetic studies carried out in HIV-infected adults with mean calculated AUC_{last} values of: 2093 ng*h/ml [5], 3000 ng*h/ml [6], 2290 ng*h/ml [1]. In a previous single-dose, crossover, two-treatment bioequivalence study of a triple-combination tablet containing tenofovir disoproxil fumarate (300 mg) in association with Efavirenz (600mg) and Emtricitabine (200 mg) carried out in 45 healthy volunteers under fasting conditions, mean calculated TFV AUC_{last} from test and reference products reported were 1950 ng*h/ml and 1970 ng*h/ml, respectively, being slightly higher than mean calculated AUC_{last} reported in our study (1592.46 and 1679.53 ng*h/ml, test and reference, respectively) [16]. This results could be explained by the inter individual variability of tenofovir pharmacokinetics which is quite large, being the sources of variability incompletely understood [1,5,6].

Pharmacokinetic parameters of 3TC reported in our study were slightly higher than a previous randomized, two-way, crossover study carried out in 60 healthy volunteers comparing the steady-state of pharmacokinetics of lamivudine following 7 days of treatment with lamivudine at 300 mg once daily *versus* the standard regimen of 150 mg twice daily where the mean steady-state C_{max} and AUC24h were 2040 ng/ml and 8870 ng/ml, respectively, at a therapeutic dose of 300 mg once daily [17]. In another study comparing steady-state of lamivudine once daily 300 mg *versus* twice daily 150 mg in HIV-infected patients as part of HAART therapy for at least three months, mean C_{max} and AUC24h reported values for 300mg once daily were 2230 ng/ml and 11800 ng*h/ml which did not differ much from our data [18]. The differences in the PK parameters of 3TC obtained in our study could be explained by the interindividual variability of lamivudine [7,8]. In our study, mean calculated 3TC AUC_{last} from test and reference formulations were higher than previously described mean values in HVB-infected patients (4300 ± 1400 ng/ml, AUC_{last} and 1280 ± 560 ng*h/ml). These results could be related to the lower dose (100 mg) of 3TC used in this previous study and also to the inter individual variability of lamivudine [8].

Mean TFV half-life values for TFV and 3TC test and reference formulations, did not differ from previous reported data [1,2,8,9].

In a previous pharmacokinetic study carried out in healthy volunteers, the most common adverse events reported during treatment with TFV were gastrointestinal disorders, fatigue, headache, somnolence and dizziness [1,2,16]. Common adverse events during therapy with lamivudine are malaise and fatigue, gastrointestinal disorders, headache, myalgia, and skin rashes [9]. However, in our study, no adverse events related to the study drug were observed.

The combination of TDF with 3TC as part of an EFV-based

regimen in ART-naïve patients has better virologic responses than with zidovudine/lamivudine or with abacavir/lamivudine regimens in patients with baseline HIV RNA > 100,000 copies/mL and are associated with a less frequency of adverse events. The working group of the OARAC recommends in the last guidance the use of dual NRTIs (TDF + 3TC) in combination with a NNRTI, a PI (usually boosted with RTV), or an INSTI (integrase strand transfer inhibitor) as preferred regimens (AI) for antiretroviral therapy-naïve patients infected with HIV, and the combination of TDF + 3TC or FTC as the preferred regimen (AII) for the treatment of HIV-HVB co-infected patients. The fixed-dose combination of TDF/3TC 300/300mg has the advantage to be administered as once daily tablet with or without food and leading to an improvement of treatment adherence, as it has been demonstrated with other fixed-dose combinations of TDF/FTC and TDF/FTC/EFV co-formulated recently [3].

In conclusion, the 90% confidence interval on the geometric mean test-to-reference C_{max}, AUC_{last} and AUC_{inf} ratios were within the bioequivalence interval 80-125 % for both TDF and 3TC.

No statistically significant differences were found for fixed effects when ANOVA test was applied to the ln C_{max}, AUC_{last} and AUC_{inf}. Both formulations were similar in terms of rate and extent of absorption. This study demonstrated that the new pharmaceutical equivalent fixed-dose combination of TDF/3TC 300/300 mg in a single oral tablet formulation is also bioequivalent to the reference products as single-drug products. Then, considering that test product is pharmaceutical equivalent and bioequivalent implies that both products are therapeutically equivalent.

Acknowledgments

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