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A Combined-Formulation Tablet of Lamivudine/Nevirapine/Stavudine: Bioequivalence Compared With Concurrent Administration of Lamivudine, Nevirapine, and Stavudine in Healthy Indian Subjects

Vishal S. Narang, PhD, Amar Lulla, MS, Geena Malhotra, BS, and Shrinivas Purandare, PhD

Generic fixed-dose combinations of antiretrovirals are frequently prescribed for the treatment of human immunodeficiency virus infection. A randomized, 2-way study was conducted in 24 fasting, healthy, Indian male subjects to assess bioequivalence between a single combination tablet containing lamivudine, stavudine, and nevirapine (treatment A) with respect to separate marketed tablets administered simultaneously (treatment B). Each subject received treatments A and B separated by 19 days of a drug-free washout period. Plasma concentrations of antiretrovirals, determined by a validated liquid chromatography/tandem mass spectrometry assay, were used to assess pharmacokinetic parameters such as maximum observed plasma concentration and area under the plasma concentration curve. Pharmacokinetic param-

eters were comparable for either treatment. As geometric mean ratios (% treatment A/treatment B) of log-transformed parameters of area under the plasma concentration curve and plasma concentration, as well as their resultant 90% confidence intervals, were within 80% to 125% and 75% to 133%, respectively, 2 treatments were considered bioequivalent in the extent and rate of absorption. Both treatments exhibited similar tolerability under fasting conditions.

Keywords: Pharmacokinetics; bioequivalence; lamivudine; stavudine; nevirapine

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It is estimated that approximately 40 million people are infected with human immunodeficiency virus (HIV).¹ Treatment guidelines recommend highly active antiretroviral therapy (HAART) that comprises various classes of antiretroviral drugs such as nucleoside analog reverse transcriptase inhibitors (NRTIs), nonnucleoside analog reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs).² Initial HAART recommended a combination of a single PI and 2 NRTIs. However, complicated dosing regimens, coupled with long-term side effects of PIs, including dyslipidemia and abnormal body fat distribution, have in turn favored an initial antiretroviral regimen that substitutes

PIs with NNRTI.³ Several randomized clinical trials in antiretroviral therapy-naïve patients have demonstrated that NNRTIs can provide an equally effective and more convenient alternative.^{4,5} A regimen consisting of NRTIs (lamivudine, stavudine) and NNRTI (nevirapine) has been shown to be efficacious and safe in both treatment-experienced and treatment-naïve patients, regardless of baseline viral loads.⁶⁻⁸

Lamivudine (3TC) and stavudine (d4T) are synthetic nucleoside analogs that are being increasingly used in comprising the core of an antiretroviral regimen for the treatment of HIV infection.^{9,10} In vivo, nucleoside analogs are phosphorylated intracellularly by endogenous kinases to putatively active 5'-triphosphate (3TC-TP and d4T-TP) derivatives that prevent HIV replication by competitively inhibiting viral reverse transcriptase and terminating proviral DNA chain extension.¹¹⁻¹³ Nevirapine (NVP), a frequently used NNRTI, has been shown to induce supe-

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rior virological suppression, improve immune function (increased CD4⁺ cell counts), and prevent clinical progression of multisymptomatic disease.¹⁴ Unlike NRTIs, NNRTIs do not require sequential intracellular phosphorylation to exert their antiretroviral effect. Highly lipophilic and essentially un-ionized at physiological pH, NVP disrupts the catalytic site of the enzyme reverse transcriptase, thereby inhibiting RNA- and DNA-dependent DNA polymerase activities.¹⁵

Single- and multiple-dose pharmacokinetic (PK) studies in nonhuman primates, healthy volunteers, and patients with HIV infections have demonstrated that mean absolute bioavailability of 3TC, d4T, and NVP after oral administration ranges between 84% and 99%. Following oral administration, the antiretrovirals are rapidly absorbed and extensively distributed. Secondary PK parameter plasma elimination half-lives ($t_{1/2\beta}$) are reported to be within 6 to 9 hours for 3TC, 1.2 to 2 hours for d4T, and 24 to 76 hours for NVP.¹⁶⁻¹⁹ The apparent oral plasma clearance (CL/F) for 3TC, d4T, and NVP ranges from 25 to 28 L/h, 22 to 26 L/h, and 1.5 to 2.5 L/h, respectively. In addition, apparent oral volume of distribution (Vd/F) is 150 to 233 L, 52 to 67 L, and 83 to 96 L for 3TC, d4T, and NVP, respectively.¹⁹⁻²¹

With the growing epidemic of acquired immunodeficiency syndrome (AIDS) in subcontinent and sub-Saharan African countries, particularly India and South Africa, it is essential that low-cost, disease-combative drug formulations are made available. HIV-infected patients in resource-poor countries are increasingly using relatively cheaper, generic, fixed-dose combinations that are preapproved by the World Health Organization (WHO). The generic-formulation fixed-dose combination of 3TC, d4T, and NVP is widely used in African countries.⁷ However, the PK of each component after the administration of the combination formulation has not yet been investigated. Hence, the objective of this study was to assess bioequivalence (BE) between the generic formulation with respect to the simultaneous administration of "proprietary-name formulations" of 3TC, d4T, and NVP. This single-center, open-label, randomized, crossover, 2-treatment study was conducted in 24 Indian, adult healthy volunteers under fasting conditions with at least 19 days of a drug-free washout period between the 2 treatments.

METHODS

Independent Ethics Committee Review, Subject Selection, and Safety Analysis

The study protocol and informed consent forms were reviewed and approved by the independent ethics

committee (IEC) of the study site (Lambda Therapeutic Laboratories Pvt Ltd, Ahmedabad, India). A favorable written informed consent was obtained before each subject's participation in the trial. Healthy adult males aged 18 to 45 years who weighed within 20% of appropriate weight range were allowed to participate in the study. Health of subjects was determined by medical history, physical examination, and laboratory-investigational tests performed within 15 days prior to the commencement of the study. Subjects were excluded from the study if they had tested positive for hepatitis or HIV; had a history of allergic conditions, hematological/psychiatric/bleeding disorders, peptic ulceration, or alcohol and substance abuse; exhibited hypersensitivity or an idiosyncratic reaction to 3TC, NVP, or d4T; had ingested any prescribed medication or over-the-counter drugs, including any enzyme-modifying or systemic medication within 15 days prior to participation in the study; or had renal or hepatic insufficiency. In addition, the subjects were asked to refrain from consuming any xanthine-containing products (caffeine and chocolates), alcohol, and tobacco for at least 48 hours prior to receiving study medication and throughout the study period. During the biostudy, subjects were continuously monitored and periodically questioned for adverse events. After the study, laboratory tests were performed on each volunteer to record any clinically abnormal finding.

Study Design, Sample Collection, and Handling

The study was single center, randomized, and crossover in nature. The subjects were confined to the clinical study unit at least 16 hours prior to drug administration and until 24-hour postdose blood sample collection. The rest of the blood samples were collected as ambulatory samples. Food and water intake was controlled during the first 24 hours of the study. A computer-generated randomization code was used to ensure balanced permutation of the treatments. The subjects were randomly assigned to receive each of the following 2 treatment regimens:

Treatment A: Combined-formulation tablet consisting of 3TC (150 mg), d4T (40 mg), and NVP (200 mg) (Triomune, batch no. K10235, manufactured by Cipla Ltd, Goa, India), administered following an overnight fast of at least 12 hours.

Treatment B: One tablet of 3TC (150 mg, Epivir, batch no. B028891, GSK, Basingstoke, UK) in conjunction with 1 tablet of d4T (40 mg, Zerit, batch no. 0290, Bristol Myers Squibb, Princeton, NJ) plus 1 tablet of NVP (200 mg, Viramune, batch no. 002320d, Boehringer

Ingelhiem, Cambridge, UK), swallowed simultaneously following an overnight fast of at least 12 hours.

Subjects received each treatment once in a crossover manner separated by at least 19 days of a drug-free washout period. A predose (0 hours, blank control) blood sample of 8 mL was collected from each volunteer in pre-labeled glass tubes containing EDTA. On the study day at 7 AM, each volunteer ingested 1 tablet of either treatment A or 3 tablets of treatment B with 240 mL of water. Mouth and tongue checks were performed to ensure the subject had ingested all of the medication. Blood samples were then collected at 0.25, 0.5, 0.75, 1.0, 1.25, 1.75, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, 12.0, 24.0, 48.0, 72.0, 120.0, 168.0, 216.0, 264.0, and 288.0 hours postdose. Within 30 minutes of blood collection, samples were centrifuged at 4°C at 3000 rpm for 10 minutes. Harvested plasma for each time point was aliquotted into 2 adequately labeled tubes. The samples were stored at -20°C until analysis.

Analysis of Plasma Sample

Plasma concentrations of 3TC, d4T, and NVP were simultaneously determined using a validated high-performance liquid chromatographic (HPLC) separation with tandem mass spectrometric (MS/MS) detection. The cartridges were initially preconditioned by sequential treatment with methanol and 50 mM phosphate buffer (pH 2.5) on a solid-phase extraction (SPE) vacuum manifold under low vacuum. Then, 0.5 mL of the thawed plasma sample was spiked with 50 µL of internal standard (IS) acetaminophen (15 µg/mL) and 0.5 mL of 50 mM phosphate buffer (pH 2.5). The plasma samples were then loaded on the conditioned C₁₈ cartridges, followed by washing with water and subsequent drying under full vacuum for 2 minutes. The cartridges were again washed with 0.5 mL hexane under low vacuum followed by 0.5 mL hexane under full vacuum for 2 minutes. The antiretrovirals were eluted using the elution mixture. The elution mixture consisted of methanol and dichloromethane (80:20, % v/v). The eluate was evaporated under nitrogen gas and reconstituted with 400 µL of reconstitution solution. The reconstitution solution consisted of methanol and water (90:10, % v/v). Then, 20 µL of the sample was injected onto the HPLC column. Chromatography was performed on a C₁₈, 5-µm, 40 × 4.6-mm Kromasil column (Flexit, Pune, India), with the mobile phase consisting of methanol/2 mM ammonium acetate buffer (pH 4.5) (85:15, % v/v) at a flow rate of 0.5 mL/min. Unless otherwise noted, all reagents were of HPLC grade (Sigma, St. Louis, Mo).

Using the mass spectrometer (PE SCIEX API 2000, Foster City, Calif), antiretroviral drugs and IS were monitored in the positive ion mode using the multiple-reaction monitoring (MRM) transitions. The mass transitions of 230.2 amu to the product ion 111.9 amu, 225.2 amu to 126.9 amu, 267.3 amu to 226.0 amu, and 152.2 amu to 109.9 amu were monitored with dwell times of 50, 500, 100, and 400 msec for 3TC, d4T, NVP, and IS, respectively. The retention time was around 1 minute. The MacQuan software (Version 1.6, PE SCIEX) was used for the evaluation of chromatograms. Calibration and quality control standards were prepared in normal human plasma. Calibration standards demonstrated acceptable linearity ($r^2 > 0.996$) over a concentration range of 50 to 3000 ng/mL, 51 to 3000 ng/mL, and 47 to 4000 ng/mL for 3TC, d4T, and NVP, respectively. Quality control standards, ranging from 150 to 3590 ng/mL, determined that mean interassay accuracy and precision (percent coefficient of variation [%CV]) ranged from 93% to 106% and 2% to 5.4%, 96.1% to 103.2% and 2.3% to 8.4%, and 94.4% to 105.3% and 2.6% to 9.7% for 3TC, d4T, and NVP, respectively. The lower limit of quantification (LOQ) for the validated assay was 50, 51, and 47 ng/mL for 3TC, d4T, and NVP, respectively. Absolute percent recoveries of the antiretroviral from the plasma were 65%, 47%, 54%, and 49% for 3TC, d4T, NVP, and IS, respectively. The matrix effect, which may influence ionization of the analyte, was investigated by extracting "blank" plasma samples from at least 10 different sources. The final extract was reconstituted by injecting a solvent containing a known amount of analyte. The reconstituted extracts were analyzed and then compared to peak areas of the analytes. No matrix effect for the analytes was observed for 10 different plasma pools tested.

Pharmacokinetic Analysis

Noncompartmental PK analysis was employed to determine PK profiles of 3TC, d4T, and NVP for both kinds of treatment regimens and to analyze plasma drug concentration-time data. Concentrations below LOQ were assigned a zero value during estimation of PK parameters. The parameters C_{max} and t_{max} were calculated directly from experimental observations of plasma concentrations. The $AUC_{0 \rightarrow t}$, the area under the plasma concentration-time curve from 0 hours to the last measurable concentration (C_{last}), was calculated by a combination of linear and logarithmic trapezoidal methods. The AUC extrapolated to infinity (AUC_{∞}) was calculated by using the following equation: $AUC_{\infty} =$

$AUC_{0 \rightarrow t} + C_{last}/\lambda_z$, where λ_z is the terminal elimination rate constant. The λ_z was estimated by performing log-linear regression on the concentration versus time data points that were determined to describe the terminal, linear elimination phase. Elimination half-life ($t_{1/2\beta}$) of the terminal log-linear phase was calculated by using the equation $0.693/\lambda_z$; CL/F was calculated as dose/ AUC_{∞} ; and V_d/F was calculated by using the equation CL/λ_z .

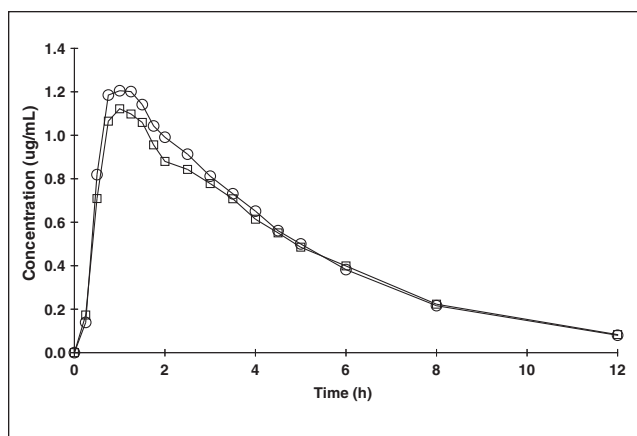
Statistical Analysis

Presuming 25% CV,^{20,22} a sample size of 24 subjects was considered to adequately power (>80%) the study to determine a 20% difference on a log scale in C_{max} and AUC_{∞} between treatments A and B. Primary PK parameters, C_{max} and AUC_{∞} , required in determining BE and statistical evaluation were performed by PK software WinNonlin Standard (Version 3.2, Pharsight, Mountain View, Calif) and by means of PROC GLM of SAS Release 8.2 (SAS Institute, Cary, NC). As per Committee for Proprietary Medicinal Products (CPMP) guidelines, the BE of 2 treatments would be concluded BE if 90% parametric confidence intervals (CIs) constructed for ratios (treatment A/treatment B) of the means of log-transformed PK parameters, AUC_{∞} and $AUC_{0 \rightarrow t}$, were within the range of 80% to 125% and that for C_{max} were within the range of 75% to 134%. The wider interval for C_{max} was due to the inherent property of large intrasubject variability (>40%-50%) of 3TC.²⁰ Pharmacokinetic parameters such as AUC_{∞} , $AUC_{0 \rightarrow t}$, and C_{max} (relative to treatment B) were evaluated using the 2 one-sided tests procedure for logarithmic transformed data. Wilcoxon's signed rank test was used to compare t_{max} . Comparisons of secondary PK parameters between Indians and other study populations were performed using the Student *t* test (Graphpad Prism v3.0, San Diego, Calif). Analysis of variance (ANOVA) was performed to assess period, treatment, and cross-over effects.

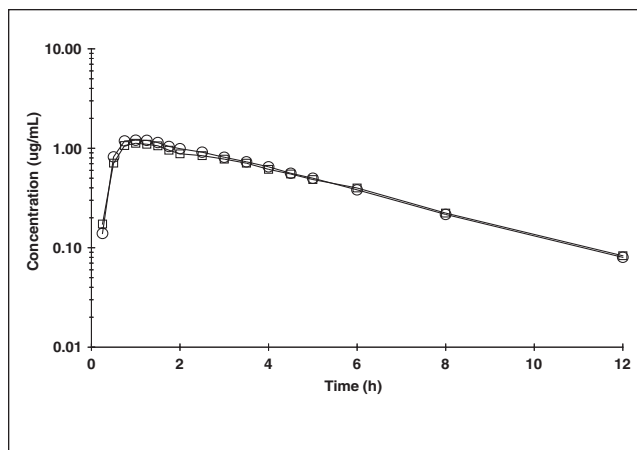
RESULTS

Estimation of Pharmacokinetic Parameters

The BE study presented here was conducted in 24 healthy, Indian adult male subjects who ranged from age 19 to 32 years (mean \pm SD; 23.84 ± 3.30), weighed 62.96 ± 5.22 kg, and averaged 168.84 ± 6.70 cm in height. All 24 subjects who participated in the study completed it and were therefore included in PK, safety, and statistical analyses. Under fasting conditions, plasma concentrations of 3TC, d4T, and NVP attained



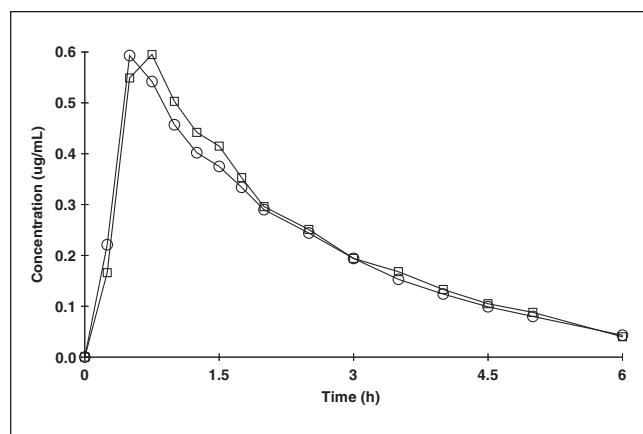
(A)



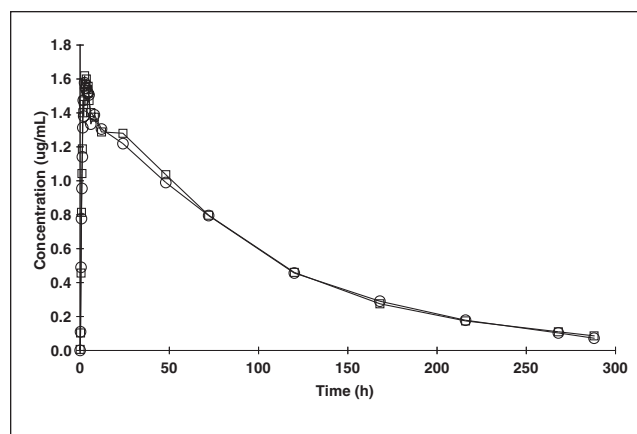
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Figure 1. Mean plasma concentration-time curve of lamivudine in 24 adult, fasting, healthy Indian subjects when administered as a combination tablet (treatment A, open squares) and as an individual tablet (treatment B, open circles) (A = linear plot; B = log plot).

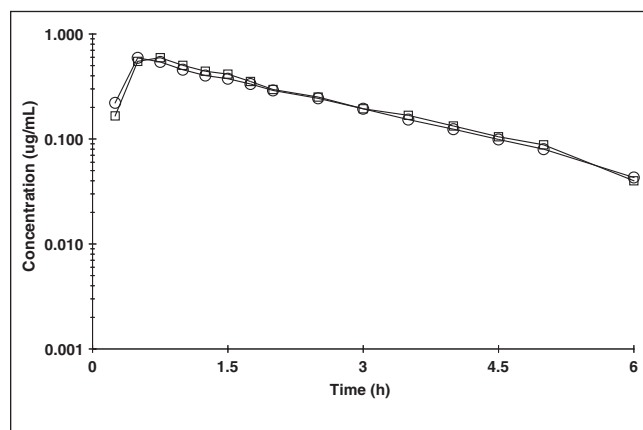
after administration of generic and proprietary-name formulations were comparable (Figures 1-3). The primary PK characteristics C_{max} and AUC_{∞} were comparable when administration was either as treatment A or B. The antiretrovirals were rapidly absorbed from either of the treatments, with median t_{max} for 3TC, d4T, and NVP being 1 hour, 0.5 hours, and 2.5 hours, respectively (Tables I, II, and III for 3TC, d4T, and NVP, respectively). Furthermore, the percentage of AUC extrapolated to infinity was similar for treatment A (5.8%, 11.28%, 5.85%) and treatment B (6.14%, 11.2%, 6.42%) for 3TC, d4T, and NVP, respectively. Moreover, the generic-formulation and single-simultaneous administered tablets exhibited no significant difference with respect to their secondary PK



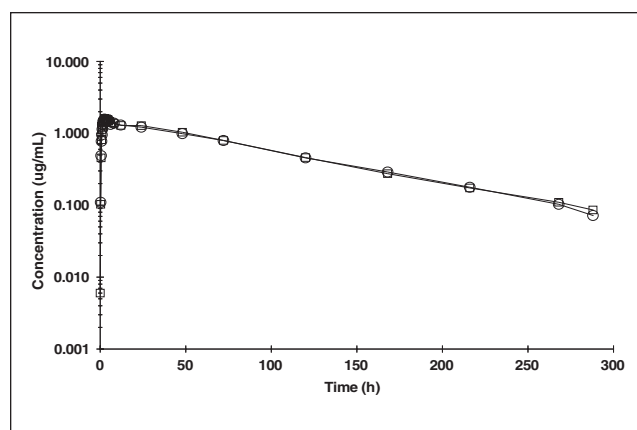
(A)



(A)



(B)



(B)

Figure 2. Mean plasma concentration-time curve of stavudine in 24 adult, fasting, healthy Indian subjects when administered as a combination tablet (treatment A, open squares) and as an individual tablet (treatment B, open circles) (A = linear plot; B = log plot).

Figure 3. Mean plasma concentration-time curve of nevirapine in 24 adult, fasting, healthy Indian subjects when administered as a combination tablet (treatment A, open squares) and as an individual tablet (treatment B, open circles) (A = linear plot; B = log plot).

parameters (Table IV). Taken together, these results suggest that the PK profile of antiretrovirals was comparable in nature for either treatment. The secondary PK characteristics of d4T were consistent with literature reports.^{21,23} However, for 3TC, $t_{1/2\beta}$ and V_d/F among Indian subjects were significantly (2- to 3-fold; $P < .001$) altered from the reported values for other study populations (Caucasians and Hispanics^{24,25}; Table IV). For NVP, $t_{1/2\beta}$ among Indian subjects was significantly higher than historical controls¹⁹ (Table IV).

Assessment of Bioequivalence

To establish if the 2 treatments were BE, the PK parameter estimates were analyzed using natural log-

transformed data. The geometric mean ratio (GMR; treatment A/treatment B) of C_{max} , $AUC_{0 \rightarrow t}$, and AUC_{∞} and their resultant 90% CI for 3TC, d4T, and NVP were within the range of 75% to 134% (Tables I-III). Untransformed results for the same parameters were consistent with the natural log-transformed data (data not shown). As 90% parametric CIs constructed for ratios were within the specified limits, the 2 treatments were considered BE in nature. ANOVA demonstrated significant formulation (treatment) ($P < .05$) and subject (sequence) effects ($P < .005$) for 3TC. Despite incorporating a sufficiently long washout period (>5 half-lives), a significant ($P < .005$) period effect for d4T was encountered. The intersubject %CV observed for all PK parameters, with the exception of t_{max} , was in the range of

Table I Pharmacokinetic (PK) Parameters of Lamivudine and Relative Bioavailability After Treatment A and Treatment B

PK Parameters	Treatment A	Treatment B	Ratio (A/B)
C_{max} , $\mu\text{g/mL}$			
Arithmetic mean \pm SD	1.414 \pm 0.37	1.220 \pm 0.33	
Geometric mean	1.367	1.19	
GMR			114.9
90% CI			102.96-128.25
$AUC_{0 \rightarrow t}$, $\mu\text{g}\cdot\text{h/mL}$			
Arithmetic mean \pm SD	5.682 \pm 0.96	5.44 \pm 1.07	
Geometric mean	5.59	5.33	
GMR			104.8
90% CI			96.63-113.58
AUC_{∞} , $\mu\text{g}\cdot\text{h/mL}$			
Arithmetic mean \pm SD	6.023 \pm 0.97	5.80 \pm 1.13	
Geometric mean	5.93	5.69	
GMR			104.3
90% CI			96.4-112.82
t_{max} , h			
Arithmetic mean \pm SD	1.38 \pm 1.03	1.32 \pm 0.934	
Median	1	1	
Range	0.5-5.00	0.5-5.00	
Median difference			0.04
90% CI			-0.15-2.49

CI, confidence interval; GMR, geometric mean ratio.

Table II Pharmacokinetic (PK) Parameters of Stavudine and Relative Bioavailability After Treatment A and Treatment B

PK Parameters	Treatment A	Treatment B	Ratio (A/B)
C_{max} , $\mu\text{g/mL}$			
Arithmetic mean \pm SD	0.64 \pm 0.11	0.65 \pm 0.10	
Geometric mean	0.634	0.64	
GMR			98.9
90% CI			90.72-107.88
$AUC_{0 \rightarrow t}$, $\mu\text{g}\cdot\text{h/mL}$			
Arithmetic mean \pm SD	1.325 \pm 0.22	1.364 \pm 0.19	
Geometric mean	1.30	1.35	
GMR			96.7
90% CI			92.33-101.31
AUC_{∞} , $\mu\text{g}\cdot\text{h/mL}$			
Arithmetic mean \pm SD	1.486 \pm 0.23	1.536 \pm 0.22	
Geometric mean	1.468	1.52	
GMR			96.6
90% CI			92.73-100.59
t_{max} , h			
Arithmetic mean \pm SD	0.61 \pm 0.23	0.67 \pm 0.11	
Median	0.5	0.75	
Range	0.5-1.5	0.5-0.75	
Median difference			-0.10
90% CI			-0.22-0.04

CI, confidence interval; GMR, geometric mean ratio.

Table III Pharmacokinetic (PK) Parameters of Nevirapine and Relative Bioavailability After Treatment A and Treatment B

PK Parameters	Treatment A	Treatment B	Ratio (A/B)
C_{max} , µg/mL			
Arithmetic mean ± SD	1.82 ± 0.38	1.80 ± 0.36	
Geometric mean	1.78	1.77	
GMR			100.8
90% CI			95.05-106.98
$AUC_{0 \rightarrow t}$, µg•h/mL			
Arithmetic mean ± SD	146.75 ± 34.28	148.85 ± 30.33	
Geometric mean	143.19	146.00	
GMR			98.1
90% CI			94.78-101.5
AUC_{∞} , µg•h/mL			
Arithmetic mean ± SD	156.95 ± 42.32	160.28 ± 37.95	
Geometric mean	152.24	156.37	
GMR			97.4
90% CI			94.13-100.7
t_{max} , h			
Arithmetic mean ± SD	3.38 ± 2.38	4.62 ± 6.12	
Median	2.5	2.5	
Range	1.25-8.00	1.25-24.00	
Median difference			-0.26
90% CI			-0.58-0.05

CI, confidence interval; GMR, geometric mean ratio.

Table IV Arithmetic Means (± SD) of Secondary Pharmacokinetic (PK) Parameters of 3TC, d4T, and NVP in Indian Subjects

Secondary PK Parameter	Treatment A	Treatment B	Reported Values for Individual Drugs
Lamivudine (3TC)			
λ_z , h ⁻¹	0.259 ± 0.029	0.257 ± 0.028	0.069-0.106 (NR)
$t_{1/2\beta}$, h	2.7 ± 0.30*	2.73 ± 0.29*	6.5-9.5 (±2.78) ^{24,25}
CL/F, L/h	25.87 ± 5.06	27 ± 5.3	24.69-25 (NR)
V_d , L	101.71 ± 25.98*	107.05 ± 28.5*	233-362 (±28) ^{24,25}
Stavudine (d4T)			
λ_z , h ⁻¹	0.41 ± 0.05	0.42 ± 0.05	0.40 ± 0.04 ²³
$t_{1/2\beta}$, h	1.69 ± 0.23	1.68 ± 0.27	1.7 ± 0.2 ²³
CL/F, L/h	27.55 ± 4.64	26.58 ± 3.73	21.2 ± 6.8 ²³
V_d , L	67.48 ± 14.88	63.27 ± 11.01	52 ± 15 ²³
Nevirapine (NVP)			
λ_z , h ⁻¹	0.011 ± 0.002	0.011 ± 0.002	0.017 (NR)
$t_{1/2\beta}$, h	67 ± 16.13*	70.04 ± 18.97*	47.27 ± 19.57 ¹⁹
CL/F, L/h	1.35 ± 0.31	1.31 ± 0.28	1.53 ± 0.62 ¹⁹
V_d , L	126.63 ± 30.3	128.1 ± 28.5	89.65 ± 36.75 ¹⁹

NR, not reported.

* $P < .001$ (Indians vs reported values).

19% to 35% (data not shown). Nonparametric statistical tests performed to calculate the 95% CI for t_{max} of the antiretroviral drug and 90% CI for the difference in t_{max}

between treatments A and B revealed no statistically significant difference (Tables I-III).

Safety

Both the treatments were well tolerated, with little or no differences in the nature and frequency of adverse events. No serious clinical adverse events causing death, disability, hospitalization, or dropouts of the subjects were encountered. Reported mild adverse events, possibly related to treatments, were dizziness (8% for treatment A and 4% for treatment B), headache (4% for treatment A and 8% for treatment B), and skin rashes (4% for treatment A and 8% for treatment B). Poststudy clinical lab tests revealed normal results.

DISCUSSION

Previous studies have shown that all 3 antiretrovirals used in this study exhibit similar PK profiles in healthy male subjects and in asymptomatic HIV-infected individuals.^{17,18,24} Therefore, for the BE study, healthy male subjects were considered as a suitable study population. This study is the first to evaluate the PK of 3TC, d4T, and NVP following administration as a combination tablet under fasting conditions. As suggested by Azoulay et al,²⁶ BE can be assessed by the simultaneous measurement of NVP in the plasma and in peripheral blood mononuclear cells (PBMCs), the sites of HIV replication and drug action. However, due to intrinsic pharmacological activity of NVP, direct correlations between the plasma drug concentrations and virological response are anticipated. Similarly, BE could also be assessed by simultaneous measurement of d4T and 3TC in the plasma and metabolites d4T-TP and 3TC-TP in PBMCs.^{12,13,27} However, the present study specifically used plasma concentrations to estimate PK characteristics and assess BE.

The PK profiles obtained for 3TC, d4T, and NVP in healthy subjects used in this study were consistent with the previously reported PK data in HIV-1-infected individuals.^{21,28,29} In both types of treatments, the C_{max} of antiretrovirals was achieved within a few hours, suggesting rapid absorption and distribution. Moreover, the estimated value of $AUC_{0 \rightarrow t}$ being more than 80% of the estimated value of AUC_{∞} implied that the sampling scheme was sufficiently long to ensure an adequate description of the absorption phase. The results of the present study suggest that under fasting conditions, the generic formulation was BE to simultaneously co-administered proprietary-name formulation tablets. The GMR of C_{max} , $AUC_{0 \rightarrow t}$, and AUC_{∞} and the 90% CI were within 80% to 120% for untransformed data and within 80% and 125% for log-transformed data. In addition, a sample size of 24 subjects provided >90%

power to detect a difference of at least 20% in AUC_{∞} and C_{max} between treatments A and B. A significant subject nested-within-sequence effect for 3TC suggests that between-subject variability (CV ~30%) significantly contributed toward total variability observed. This behavior is consistent with the characteristic of 3TC as a highly variable drug.^{20,24} A significant treatment effect for 3TC suggested that the study might have employed a sufficiently high number of volunteers. Although the exact cause of the period effect is not known, an insufficient washout period is unlikely to explain the period effect for d4T. However, as the effect is not coupled with sequence effect, the period effect appears to be insignificant in nature. With no serious clinical adverse events, it was concluded that both kinds of treatments were well tolerated.

It has been shown that a PK drug interaction did not exist between 3TC and NVP in HIV-1-infected individuals,^{29,30} and no statistically significant changes in d4T-PK were observed following the addition of NVP.³¹ Therefore, a PK drug interaction was unlikely to occur. Corroborating these observations, the present study suggests that primary PK characteristics of the antiretrovirals remain uninfluenced in the presence of each other. The lack of significant PK interactions between the antiretrovirals was predictable as the agents were excreted via different elimination pathways: 3TC is primarily eliminated via renal excretion, d4T undergoes metabolism via pyrimidine degradation and salvage mechanisms, and NVP undergoes oxidative metabolism followed by glucuronidation.^{22,28,32} This is an important finding in view of the fact that the activity profiles of the antiretrovirals are believed to be dependent on systemic exposure instead of the absorption rate. Nonsignificant differences among the AUC_{∞} values observed between the 2 treatment regimens suggest that no changes in the dosing of antiretrovirals in the combination tablet are warranted with respect to their antiviral activity.

The present study demonstrated that the estimate of $t_{1/2\beta}$ of NVP in this study was significantly ($P < .001$) higher in comparison to the reported elimination half-life of 47 hours.¹⁹ However, apart from significant differences in study design, extent of sampling time points (32-96 hours¹⁹ vs 288 hours), and sampling times (4 datum points¹⁹ vs 25 datum points), there were significant differences in the sensitivities (25 ng/mL) of assay methodologies between the literature report and the present study (47 ng/mL). For 3TC, in comparison to historical data,²⁵ Indian subjects used in this study exhibited a significantly lower elimination half-life. However, analogous to NVP, there were significant differences in study design and sampling times. Impor-

tantly, there were significant differences in the sensitivities of assay methodologies between the historical studies (5 ng/mL) and the present study (50 ng/mL). These differences may, in turn, significantly affect the estimation of $t_{1/2\beta}$ and V_d/F . A shorter $t_{1/2\beta}$ for 3TC has been previously reported in adult and pediatric patients.^{29,33} However, a major limitation of these studies was that the sampling period was until 12 hours, during which elimination was still taking place. The current study, despite being profiled until 288 hours, may suffer from a similar limitation as samples collected after 12 hours were below LOQ. These differences in the estimation of secondary PK characteristics of 3TC and NVP appear to be a reflection of differences in assay methodologies rather than study populations. Endorsing the observation, significant differences in secondary PK characteristics were not reflected in significant alterations in the extent of absorption.

It is noted that race, ethnicity, and disease state may have profound effects on the antiretroviral drug disposition of the generic formulation. Although there are insufficient data on the effects of gender, race, and concurrent underlying conditions on the PK of d4T, a recent study suggested that the PK profile of d4T in healthy South African volunteers³⁴ was similar in nature to that presented in the present study. As antiretrovirals exhibit similar PK profiles in healthy subjects and in asymptomatic and symptomatic HIV-infected individuals, disposition of d4T is likely to be similar in nature in asymptomatic and symptomatic South African patients. In other studies, it was concluded that race and gender were not associated with the PK of 3TC³⁵ and NVP,³⁶ thus requiring no dose adaptations. Although the results presented here refer only to kinetics in healthy Indian subjects, it is likely that the drug disposition of the "generic formulation" in the symptomatic South African and Indian patient population would remain unaltered by race and disease state. A recent PK and pharmacodynamic study conducted in HIV-infected African patients in Cameroon demonstrated that antiretroviral drug concentrations achieved by the generic formulation were comparable with brand-name components of the regimen.³⁷

Generic fixed-dose therapy offers various clinical advantages. As the extent of absorption (AUC_{∞}) of single formulations of 3TC, d4T, and NVP appears to be similar under fasting and fed states,^{22,24,38} it is highly unlikely that PK characteristics of 3TC, d4T, and NVP generic-formulation tablets may be altered under the fed state. In addition, dose titration of 3TC/d4T/NVP need not be performed in patients with severe hepatic and renal impairment.^{20,23,39} HAART-containing NVP can be effectively used as a long-term PI-sparing regi-

men, thereby circumventing the metabolic side effects associated with PIs. In addition, the NVP-comprising generic formulation may be used to prevent maternal-fetal transmission during labor. Furthermore, a recent study demonstrated that a combination tablet of 3TC, d4T, and NVP significantly aided in the reduction of pill burden, which in turn is likely to enhance adherence to therapy, a critical component to ensure virological suppression.⁴⁰ Availability of relatively cheaper yet effective generic antiretroviral formulations may aid in enhancing the quality of life of treatment-naive and treatment-experienced HIV patients.

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