Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **28** (3): 433-7 (2009)

Original Article

Received: February 16, 2009
Accepted: March 7, 2009

Bioequivalence Test Applied to a New Lamivudine/Zidovudine Combined Formulation Tablet

Jacqueline de SOUZA [1]*; Eunice K. KANO [1], Eunice E. M. KOONO [2], Simone G. SCHRAMM [2], Valentina PORTA [3] & Sílvia STORPIRTIS [3]

[1] *DEFAR, Escola de Farmácia – Universidade Federal de Ouro Preto - UFOP, Rua Costa Sena, 171 Centro – Ouro Preto MG – Brasil*
[2] *Laboratório BIOFAR, Faculdade de Ciências Farmacêuticas da USP (FCF – USP);*
[3] *Departamento de Farmácia, FCF/USP Av. Prof. Lineu Prestes, 560 Cidade Universitária – Butantã – São Paulo – SP – Brasil*

***SUMMARY*. A double-center, open-label, two-way crossover study was conducted in 24 healthy volunteers to assess the bioequivalence of a combined lamivudine/zidovudine tablet related to a reference and test drug products. The volunteers were randomly assigned to receive one lamivudine/zidovudine combination tablet of reference or test product with 7-days washout period between. Blood samples were collected up to 36 h post dose. Pharmacokinetic parameters were estimated. Drug products were bioequivalent if 90% confidence intervals for the ratio of least squares (CI 90%) means are under plasma concentration-time curve ($AUC_{0-\tau}$) and absorption rate (C_{max}) fell within 80 to 125% for log-transformed parameters. Test and reference products present data of $AUC_{0-\tau}$, C_{max} referents to lamivudine and data of ASC_{∞} referents to zidovudine, in agreement of these limits. The result of C_{max} (CI 90%) to zidovudine was: 116% (90-141%), it has confirm that the zidovudine has high individual variability of absorption.**

INTRODUCTION

Lamivudine (3TC) and zidovudine (ZDV) are nucleoside reverse transcriptase inhibitors [1,2]. Zidovudine was the first antiretroviral applied for treatment of human immunodeficiency virus (HIV) infection [3]. Its use, however, frequently leads to adverse reactions, including myelosuppression.

Zidovudine pharmacokinetics parameters show large interindividual variation and a clear therapeutic window has not yet been defined [3]. Lamivudine has antiviral activity against human immunodeficiency virus type-1 (HIV-1) or hepatitis B virus (HBV). In contrast to other nucleoside analogues, such as zidovudine, zalcitabine, didanosine and stavudine, lamivudine has little activity against mammalian DNA polymerase and is not incorporated into mammalian mitochondrial DNA. Thus, lamivudine is unlikely to induce clinically important hematological and hepatic adverse events, neuropathy or myopathy [4,5].

Since 1997, the highly active antiretroviral therapy (HAART) was the new standard of HIV treatment and care introduced into routine clinical care in some countries of the world. HAART consists of a combination of three or more of the following classes of antiretroviral (ARV) drug: reverse transcriptase inhibitors, protease inhibitors and a recently approved fusion inhibitor [6-8].

Use of the lamivudine/zidovudine combination tablets simplifies treatment of HIV-1 infected patients who require both nucleosides. Because only one dosage form needs to be taken twice daily, compliance may be higher than that observed when each agent is administered separately. A twice-day regimen may be a valuable asset to HIV-1 patient care, possibly enhancing quality of life and simplifying therapy for patients whose memory is impaired by AIDS-related cognitive disease. Facilitating compliance with antiviral medication is crucial to the treatment of HIV-1 infected patients, as failure to take prescribed regimens can lead to the rapid development of mutant HIV-1 strains and resistance [9,10].

KEY WORDS: Bioequivalence, High variability drugs, Lamivudine, HPLC, Zidovudine.

* Author to whom correspondence should be addressed. *E-mail:* jacsouza@ef.ufop.br

The determination of the area under the concentration-time curve (AUC) is the most commonly method used by regulatory agencies to assess extent of drug absorption after single-dose administration of oral products [11]. Bioequivalence or relative bioavailability testing involves comparison of measures of bioavailability of the generic and innovator formulations. Bioavailability is characterized by the rate evaluated by comparing the maximum blood plasma concentration, C_{max}) and extent (tested by comparing AUC) to which the active drug ingredient or therapeutic ingredient is absorbed from a drug product and becomes available at the site of drug action [12,13]. Bioequivalent drug products must be pharmaceutically equivalent (its contain the same active ingredient, are administered in the same dosage form by the same route of administration, and are of identical strength or concentration) and display comparable bioavailability when studied under similar experimental conditions [14,15].

Bioequivalence studies are necessary to have confidence that a generic drug product will be expected to have the same clinical effect and safety profile as the innovator (or reference) product [16,17].

The purpose of this study was compare to pharmacokinetic parameters from lamivudine and zidovudine obtained by relative bioavailability study between reference product and one Brazilian new formulation of lamivudine/zidovudine tablets.

MATERIAL & METHODS

Samples

Samples of coated tablet containing 150 mg of lamivudine and 300 mg of zidovudine produced by Fundação para o Remédio Popular (FURP), Brazil (test product) and by Glaxo-SmithKline, Brazil (Biovir®, reference product) were used.

Subject Selection

The volunteers were informed about the aims and risks of the study by the clinical investigator and signed a written informed consent statement before entering the study. Ethical Council from São Paulo University, Brazil approved the study protocol. Subjects could be include in this study if they were healthy males or no pregnant females ages 18 to 50 within 15% of ideal body weight. Twenty-four healthy volunteers (12 man and 12 women) participated in this comparative study in accord to Brazilian guidelines [17]. Their mean age was 33.0 ± 2.4 years; mean body weight was 62.0 ± 4.2 Kg and mean body height was 163.0 ± 12.3 cm. The volunteers were free from significant cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal or hematological diseases as determined by their medical history, physical examination and routine laboratory test (hematology, blood biochemistry and urine analysis) and were negative for hepatitis B antigen and HIV. They were instructed to abstain from taking any drug for two weeks prior to and during the study period.

Study design

This was a double-center, open-label, randomized, two-way crossover study that was conducted at an inpatient clinical research Paris & Paris, São Paulo, Brazil.

The volunteers were boarder in Clinical at 8:00 pm and had a standard dinner. After an overnight fasting they were given one single dose of each formulation (reference or test in a randomized design) of 150 mg of lamivudine and 300 mg of zidovudine with 200 mL of water. Food and drinks were not allowed until 4 h after the ingestion of tablet and than standard lunch, breakfast and dinner were given to all volunteers according to a time schedule. Beverages and food containing caffeine were not permitted over the entire course of study. 8 mL blood samples were drawn into evacuated heparinized glass tube through indwelling cannula before (0 h) and at 0.25; 0.50; 0.75; 1.00; 1.25; 1.50; 1.75; 2.00; 2.50; 3.00; 4.00; 6.00; 8.00; 10.00; 12.00; 24.00 and 36.00 h after dosing. Blood sample were centrifuged for 20 min. at 10,000 rpm to obtain plasma. Plasma sample were storage frozen at –20 °C pending drug analysis. After a washout period of 7 days the study was repeated of same manner to complete the crossover design.

Sample preparation for HPLC injection

50 µL of internal standard work solution (stavudine 10 µg/mL) was added to glass tube. Methanolic phase was dried in nitrogen atmosphere at 40 °C and was added 250 µL of whole plasma sample. The samples were adding of 25 µL of ammonium acetate solution and vortexed for 30 s. Double extraction of two aliquots of 4 mL of ethyl acetate with 1 minute of vortex was performed. The inorganic phase was freezing and organic phase was filtered through Millipore unity filter, type HV Millex in polystyrene with 13 mm of diameter and 0.45 µm of pore of

Durapore membrane. The extracts were joined and dried in nitrogen atmosphere at 40 °C. The residues were reconstituted with 250 µL of mobile phase A and vortexed for 30 s. All volume was transferred for vials and 25 µL was injected into a chromatographic system, where lamivudine, zidovudine and internal standard were separated from endogenous substances.

Chromatographic Conditions

Plasma samples were analyzed simultaneously for lamivudine and zidovudine according to a sensitive, selective and accurate HPLC method that was validated before the study. Standard reference of zidovudine and lamivudine were obtained from FURP (São Paulo, Brazil) and stavudine (internal standard - IS) was purchased from Labogem (São Paulo, Brazil). Acetonitrile; methanol and ethyl acetate from EM Science (Cincinnati, OH, USA) were HPLC grade. Potassium dihydrogenphosphate (KH_2PO_4) and ammonium acetate from Merck (Darmstadt, Germany) were analytical-reagent grade.

The HPLC system was from Merck-Hitach® and it consisted of a quaternary pump series L 7100, a degasser system series L 7612, column oven series L 7300 and auto sampler series L 7200. The programmable UV-visible detector was a series L 7400 used in a wavelength of 270 nm. Data processing was carried out on an automatic Merck-Hitachi® (Model LD- 7000) program system with a Chromatography data station software version 4.1. The integration parameters were 2 for noise and 30 for sensitivity.

Three mobile phases were combined in a linear gradient system with two phases. The analysis was performed in 21 min using a flow-rate of 1.0 mL/min. The phase A was composed of 10 mM KH_2PO_4 buffer (pH 5.6, without adjust) with 3% (v/v) acetonitrile, the phase B was methanol and the phase C was acetonitrile. The phases were filtered through a 0.22 µm membrane filter (Sartorius, Bedford, MA, USA). The gradient system began with 98 % of phase A and 2% of phase B from 0.0 to 7.0 min, changing for 72% of phase A and 28% of phase B from 7.1 to 10.0 min This proportion still unchanged until 15 min. After this time one drastic condition was used, changing mobile phase for 5% of phase A and 95% of C to withdraw residues. In 17.1 min the mobile phase proportion return to 98% of phase A and 2% of phase B until 21 min for system stabilization. Separation was performed at 40 °C using column heater on a C8 Column (150 x 4.6 mm I.D, 5 µm) (Shim-pack®) protected by a C18 pre-column (50 x 4.6 mm, 5 µm) (Phenomenex®). In these conditions lamivudine and zidovudine were simultaneously quantified.

Pharmacokinetic and statistical Analysis

Pharmacokinetics and statistical analysis was performed by means of a model independent method. The elimination rate constant (λ_z) was obtained as the slope of linear regression of the log transformed concentration values versus time data in the terminal phase. The elimination half-life ($T_{1/2}$) was calculated as $0.693/\lambda_z$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t} + C_t/\lambda_z$, where Ct is the last measurable concentration. C_{max} and t_{max} were obtained directly from plasma concentration time profile [16,17]. Two ways analysis of variance (ANOVA) for crossover design was used to assess the effect of products, periods, sequences and subjects on these parameters. 90% confidence intervals based on the ANOVA of the mean test/ reference (T/R) rations of AUC_{0-t} and C_{max} were computed [16,17].

RESULTS

Lamivudine and zidovudine associated tablets were well tolerated by all volunteers; unexpected incidents that could have influenced the outcome of the study did not occur. Both formulations were readily absorbed from the gastrointestinal tract and lamivudine and ziodvudine were measurable at the first sampling time (0.25 h).

The mean plasma concentration-time profiles for lamivudine and zidovudine of the two formulations are shown in Figs. 1 and 2, respectively. All calculated pharmacokinetics parameters values are shown in Table 1 for lamivudine and Table 2 for zidovudine and presents in good agreement with reported values for both drugs [18-20]. Bioequivalence of two formulations of the same drug substance requires equivalence with respect to the rate (C_{max}) and the extent (AUC) of drug absorption. Two formulations whose rate and extent of absorption differ by –20%/+25% or less are generally considered bioequivalent [12].

Pharmacokinetic parameters estimates (except t_{max}) were analyzed following log transformation. Logarithmic transformation for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} should be provide for mea-

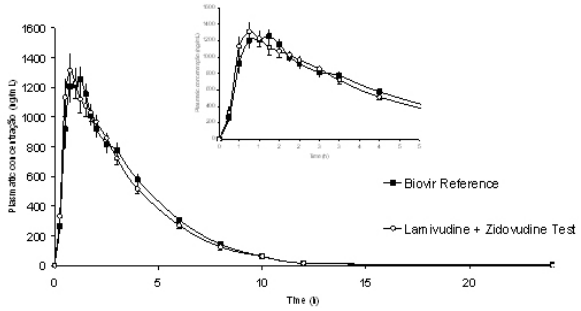

Figure 1. Mean ± MSE lamivudine plasma concentration-time profile (linear plot) after oral administration of one tablet contained 150 mg of lamivudine and 300 mg of zidovudine to 24 healthy volunteers. In detail the profiles between 0 and 5 h are presented.

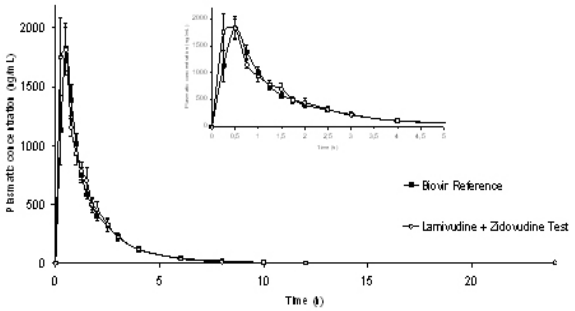

Figure 2. Mean ± MSE zidovudine plasma concentration-time profile (linear plot) after oral administration of one tablet contained 150 mg of lamivudine and 300 mg of zidovudine to 24 healthy volunteers. In detail the profiles between 0 and 5 h are presented.

Pharmacokinetics Parameters	Reference	Test	Statistical Analysis	
			ANOVA	90% CI
AUC_{0-t} (ng.h/mL)	4936.10 ± 1227.06	4760.07 ± 1409.24	0.2798 (0.5653)	88 – 103%
$AUC_{0-\infty}$ (ng.h/mL)	5305.76 ± 1313.97	5069.16 ± 1386.52	0.2400 (0.4441)	88 – 102%
C_{max} (ng/mL)	1526.33 ± 431.03	1585.61 ± 416.34	0.5860 (0.3870)	93 – 115%
t_{max} (h)	1.04 ± 0.54	0.97 ± 0.53		
$T_{(1/2)}$ (h)	1.99 ± 0.57	2.00 ± 0.53		

Table 1. Pharmacokinetics parameters and statistic analysis (ANOVA and CI 90%) calculated for product and sequence effects of lamivudine 150 mg in associated tablets (mean ± standard deviation, n= 24). Parenthesis value indicates analysis for sequence effects.

Pharmacokinetics Parameters	Reference	Test	Statistical Analysis	
			ANOVA	90% CI
AUC_{0-t} (ng.h/mL)	2341.32 ± 775.00	2538.17 ± 919.03	0.2646 (0.1842)	97 – 120%
$AUC_{0-\infty}$ (ng.h/mL)	2561.68 ± 775.66	2770.21 ± 943.22	0.2711 (0.2456)	97 – 118%
C_{max} (ng/mL)	2406.56 ± 1139.74	2652.80 ± 1153.55	0.3949 (0.8942)	90 – 141 %
t_{max} (h)	0.60 ± 0.40	0.55 ± 0.43		
$T_{(1/2)}$ (h)	1.41 ± 0.55	1.47 ± 0.61		

Table 2. Pharmacokinetics parameters and statistic analysis (ANOVA and CI 90%) calculated for product and sequence effects of zidovudine 300 mg in associated tablets (mean ± standard deviation, n= 24). Parenthesis value indicates analysis for sequence effects.

sures used in bioequivalence demonstration. Confidence interval (90% CI) values should not be rounded off, To pass a CI limit of 80 to 125%, the value should be at least 80.00 and not more than 125.00 [16,17]. Geometric LS mean ratios and 90% confidence intervals for $AUC_{0-\tau}$ and C_{max} for lamivudine were 95% (88-103%) and 104 (93-115%), respectively (Table 1). Geometric LS mean ratios and 90% confidence intervals for $AUC_{0-\tau}$ and C_{max} for zidovudine were 109% (97-120%) and 116% (90-141%), respectively (Table 2). Lamivudine and zidovudine were rapidly absorbed from test and reference formulations, with the median lamivudine t_{max} occurring 1.04 hours post dose of reference and 0.97 hours post dose of test and median zidovudine tmax occurring 0.6 hours post dose of reference and 0.55 hours post dose of test.

The standard deviation (SD %) from zidovudine was higher than 30% and has suggested that it has high variability of absorption. The standard deviation can be reduced using a great number of volunteers, but there are published studies using 24 health volunteers [21,22]. Zidovudine undergoes significant first-pass hepatic metabolism. Therefore, the serum concentrations achieved may be more dependent on the patient's metabolic capacity than on weight. Studies question the importance of individualization of zidovudine regimens on the basis of body weight for those patients who are within 20% of ideal weight [23]. There are studies that inquire about the legal requirement of use C_{max} values which bioequivalence parameter for chronicle useful drugs [24]. On the other hand additional tests and controls may be needed to ensure the quality of drug products containing narrow therapeutic range or potential toxicity which zidovudine [25].

CONCLUSION

Based on statistical results it can be concluded that test and reference products meet ANVISA and FDA requirements for bioequivalence using AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} for lamivudine AUC_{0-t} and $AUC_{0-\infty}$ for zidovudine [16,17]. Both products had presents bioequivalence between the rate and the extent of lamivudine absorption and the extent of zidovudine absorption. Beside the C_{max} value was out of 90% confidence interval, this parameter was not relevant for chronicle therapy. The results have confirmed that zidovudine has high variability of absorption (S.D. was higher than 30%).

Acknowledgements. We gratefully acknowledge financial support provided by CAPES, FURP, UFOP and BIOFAR/FCF/USP.

REFERENCES

1. Havlir, D.V. & J.M.A. Lange (1998) *Aids* **12**(A): S165-74.
2. Page, C.P., M.J. Curtis, M.C. Sutter, M.J.A. Walker & B.B Hoffamn. (1999) *Farmacologia Integrada*. Manole, São Paulo Pp. 445-460.
3. Hoetelmans, R.M.W., M. Profij, P.L. Meenhors;, J.W. Mulder & J.H. Beijnen (1998) *J. Chromatogr. B.* **713**: 387-94.
4. Johnson, M.A., G.A. Verpooten & M.J. Daniel (1999) *Br. J. Clin. Pharmacol.* **46**: 21-7.
5. Souza, J. & S. Storpirtis (2004) *Rev. Bras. Ciênc. Farm.* **40**: 9-19.
6. King, R.J., D.W. Kimberlin, G.M. Aldrovandi &.P. Acosta (2002) *Clin. Pharmacokinet.* **44**: 1115-33.
7. Beck, E.J., S. Mandalia, M. Gaudreault, C. Brewer, H. Zowal, N. Gilmore, M.B. Kein, R. Lalonde, A. Piche & C.A. Hankins (2004) *Aids* **18**: 2411-8.
8. Pereira, C.F. & J.T. Paridaen (2004) *Curr. Pharm. Des.* **10**: 4005-37.
9. Horneff, G., O. Adans & V. Wahn (1998) *Aids* **12**: 489-94.
10. Moore, K.H.P, J.E. Barrett, S. Shaw, G.E. Pakes, R. Churchus, A. Kappor, J. Lloyd, M.G Barry & D. Back (1999) *Aids* **13**: 2239-50.
11. Bois, F.Y., T.N. Tozer, W.W. Hauck, M.L. Chen, R. Patnaik & R.L. Williams (1994) *Pharm. Res.* **11**: 715-22.
12. Benet, L.Z. (1999) *Transplant. Proc.* **31**: 7s-9s.
13. Consiglieri, V.O. & S. Storpirtis (2000) *Rev. Bras. Ciênc. Farm.* **36**(1):13-21.
14. Storpirtis, S., P.G. Oliveira, D. Rodrigues & D. Marinho (1999) *Rev. Bras. Ciênc. Farm.* **35**:1-16.
15. Moraes, M.E.A. & O.M. Moraes (2000) *Fármacos & Medicamentos* **5**: 36-40.
16. Code Of Federal Regulations (2001) *Title 21. Part 320 - Bioavailability And Bioequivalence Requeriments*. Washington: Us Government Printing Office,.
17. Brasil (2003) Resolução RDC n° 894. Seção 1. *"Guia para Protocolo e Relatório Técnico de Estudos de Bioequivalência"*. Diário Oficial da União, Brasília, 2 de Junho de 2003
18. Moore, K.H.P., S. Shaw, A.L. Laurent, P. Lloyd, B. Duncan, D.M. Morris, M.J. O'mara & G.E. Pakes (1999) *J. Clin. Pharmacol.* **39**: 593-605.
19. Simon, V.A., M.D. Thiam & L.C. Lipford (2001) *J. Chromatogr. A.* **913**: 447-53.
20. King, R.J., D.W. Kimberlin, G.M. Aldrovandi, & E.P. Acosta (2002) *Clin. Pharmacokinet.* **44**(14): 1115-33.
21. Moore K.H., S, Shaw, A.L. Laurent, P. Lloyd, B. Duncan, D.M. Morris, M.J. O'Mara & G.E. Pakes (1999) *J. Clin. Pharmacol.* **39**: 593-605,
22. Estrela R. de C.; M.C. Salvadori & G. Suarez-Kurtz (2004) *Rapid Commun. Mass Spectrom.* **18**: 1147-55.
23. Sahai, J., K. Gallicano, E. Ormsby, G. Garber & D.W. Cameron (1994) *Aids* **8**: 793-6.
24. Dipiro, J.T., R.A. Blouin, J.M. Pruemer & A.J Spruill (1996) *Concepts In Clinical Pharmacokinetics,* 2 ª Ed., American Society of Health-System Pharmacists.
25. Chen, M.L., V. Shah, R. Patnaik, W. Adams, A. Hussain, D. Conner, M. Mehta, H. Malinowski, J. Lazor, S.M. Huang, D. Hare, L. Lesko, D. Sporn & R. Williams (2001) *Pharm. Res.* **18**: 1645-50.