

Bioequivalence of Two Lamivudine Tablet Formulations

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Summary

The present study describes the determination of the bioavailability of a new commercial tablet formulation of lamivudine (CAS 134678-17-4) compared with a reference formulation. The comparative bioequivalence of the test and a reference formulation (each 3 × 150 mg) was assessed in 24 healthy volunteers by means of a randomized two-way crossover design. Prior to the study both the test and reference formulations were examined for conformation to chromatographic purity and drug content. Each volunteer received the test (T) and the reference formulation (R) with a one-week drug-free interval between administrations. The plasma concentrations of T were monitored over a period of 12 h after drug administration using a sensitive HPLC method. Pharmacokinetic

parameters for T were determined from plasma concentration-time data. Statistical tests were carried out at 90 % confidence intervals using a parametric method (three-way ANOVA) for AUC and C_{max} and non-parametric method for T_{max} . The present study showed that both formulations were bioequivalent for the geometric mean of $AUC_{(0-12)}$, $AUC_{(0-\infty)}$, C_{max} and T_{max} at the 90 % confidence interval. The bioavailability of the test (%) was 96.7, 93.3, 99.7, 100.3, respectively. The T : R ratio was, in each case, well within the acceptable range of 100 ± 20 %.

Key words

- CAS 134678-17-4
- Lamivudine, bioequivalence studies

Arzneim.-Forsch./Drug Res.
51 (I), 310-314 (2001)

Zusammenfassung

Bioäquivalenz zweier Lamivudin-Tablettenformulierungen

Die vorliegende Studie beschreibt die Bestimmung der Bioverfügbarkeit einer im Handel erhältlichen neuen Tablettenformulierung von Lamivudin (CAS 134678-17-4) im Vergleich zu einer Referenzformulierung. Die vergleichende Bioäquivalenz der Test- und der Referenzfor-

mullierung wurde an 24 gesunden Probanden mittels eines randomisierten Zweifach-Crossover-Designs bewertet. Vor der Studie wurden sowohl die Test- als auch die Referenzformulierung in Bezug auf Konformität zu chromatographischer Reinheit und Arzneistoffgehalt untersucht. Jedem Proband wurde die Test- (T) und die Referenzformulierung (R) mit einem einwöchigen medikamenten-

freien Intervall verabreicht. Die Plasmakonzentrationen von T wurden über eine Zeitdauer von 12 h nach Verabreichung mittels einer empfindlichen HPLC-Methode überwacht. Pharmakokinetische Parameter für T wurden aus den Daten der Plasmakonzentrations-Zeit-Daten bestimmt. Statistische Tests wurden mit

90prozentiger Vertrauensgrenze unter Verwendung der parametrischen Methode (dreiweg ANOVA) für AUC und C_{max} sowie der nichtparametrischen Methode für T_{max} durchgeführt. Die vorliegende Studie zeigte, daß beide Formulierungen als bioäquivalent für das geometrische Mittel von $AUC_{(0-12)}$, $AUC_{(0-\infty)}$

C_{max} im 90prozentigen Vertrauensintervall zu betrachten sind. Die Bioverfügbarkeit der Tests (%) betrug 96,7, 93,3, 99,7 bzw. 100,3. Das T : R-Verhältnis war in jedem Fall deutlich innerhalb des akzeptablen Bereiches von $100 \pm 20\%$.

1. Introduction

Lamivudine (CAS 134678-17-4), a negative enantiomer of 2'-deoxy-3'-thiacytidine, is a second-generation heterocyclic nucleoside analog, in which the methylene group at the 3' carbon of the ribose ring is replaced by a sulfur atom. This dideoxypyrimidine is a potent, highly selective inhibitor of human immunodeficiency virus type 1 and type 2. Lamivudine was found to have activity against a wide range of retroviruses, including strains of HIV which are resistant to zidovudine [1-4]. The relatively higher anti-HIV activity of the negative enantiomer of lamivudine has been attributed to the resistance to cleavage from the 3' terminals of RNA/DNA complexes by 3' 5'-exonuclease. In vivo lamivudine is phosphorylated intracellularly to the triphosphate derivative, which inhibits HIV-1 reverse transcriptase and acts as a chain terminator [5]. However, emerged resistant virus has been observed for lamivudine in vitro [6, 7] and in vivo [8]. Monotherapy with selective human immunodeficiency virus type 1 (HIV-1) inhibitors has been rather limited by the appearance of drug-resistant virus during therapy. This fact was particularly observed in patients treated by long term zidovudine administration. Because of the rapid turnover of HIV and the consequent emergence of resistant mutants following monotherapy, combination chemotherapy has been investigated [9-12]. Clinical trials revealed a benefit of a combined therapy of lamivudine and zidovudine in reduced virus zidovudine resistance [13]. Potential advantages of combined chemotherapy may include synergistic interactions among agents, which may allow to diminish doses of individual drugs and reduce their toxicity. According to the recommendations consensus statement of antiretroviral therapy for HIV infections [12], combination of stavudine and lamivudine is well tolerated, particularly for patients with limited bone marrow reserve who are poor candidates for zidovudine-containing regimens.

This study presents a bioequivalence evaluation of a new commercial tablet formulation of lamivudine compared with a reference formulation. The bioequivalence study was carried out by means of a randomized two-way crossover design, based on the plasma concentration data obtained following oral administration of both products to a group of 24 male healthy volunteers.

2. Materials and methods

2.1. Institutional review and informed consent

The protocol and informed consent for this study were reviewed and approved by the Federal University of Pernambuco President's Committee on Ethics in Human Experimentation. The volunteers received and approved a written informed consent before accepting to participate in the study. They were informed with details on the possible side effects of lamivudine.

2.2. Subjects selection

The subjects were selected from a cohort of healthy male drug-free volunteers aged 19-37 years, with standard weight-to-height ratio. Twenty-four subjects were initially entered, and completed the study (Table 1). Chosen candidates were free of cardiovascular, pulmonary, hepatic, renal, gastrointestinal, or neurological diseases, as assessed by clinical examination. The results of clinical laboratory tests (blood biochemistry, hematology and blood pressure) were found to be within the normal

Table 1: Demographic and anthropometric data of healthy male volunteers and treatment sequences for Lamivudine bioequivalence test.

Subject (No., Initials)	Age (years)	Weight (kg)	Height (cm)	Treatment sequences
1, A.M.C.	22	72.0	167	T, R
2, A.J.L.	26	68.0	178	T, R
3, A.J.S.P.	25	70.0	174	T, R
4, B.F.A.	23	67.1	171	T, R
5, C.A.S.M.	23	68.0	180	T, R
6, C.R.N.	20	62.0	173	R, T
7, C.A.C.R.	23	85.0	174	R, T
8, C.L.S.L.	20	74.0	174	R, T
9, E.L.C.	25	78.0	164	T, R
10, E.A.N.S.	20	66.0	164	R, T
11, F.B.M.	22	96.0	182	R, T
12, G.S.S.A.	21	79.0	178	R, T
13, G.W.S.A.	23	67.0	179	T, R
14, H.S.A.	26	80.0	178	R, T
15, J.C.F.	24	70.0	167	T, R
16, J.E.S.	22	98.0	192	R, T
17, J.A.B.S.	21	71.3	170	T, R
18, J.W.T.S.	37	76.0	181	R, T
19, M.G.S.	26	78.0	173	T, R
20, M.R.M.M.	27	74.0	189	R, T
21, R.J.M.	24	80.0	175	T, R
22, R.O.R.R.	19	60.0	171	R, T
23, R.R.A.	21	87.0	180	R, T
24, V.I.M.J.	25	67.2	167	T, R
Mean (n = 24)	24	74.7	175	
Min	19	60.0	164	
Max	37	98.0	192	

R = reference; T = test.

range. All the volunteers were required to abstain from alcoholic and caffeine-containing beverages 48 h prior to and throughout the entire course of the study. The volunteers were institutionalized 12 h before drug administrations until the time when the last blood samples was collected. Food and beverages were controlled during the study.

2.3. Study design, sample collection and handling

A list of random treatment assignments was computer generated, so as to ensure exactly equal treatment numbers. The administration of the test (150 mg lamivudine tablets, pilot #2; manufacturer: LAFEPE – Instituto Vital Brazil-IVB, Rio de Janeiro, Brasil) and reference (150 mg tablets lot #W0628AB) formulations to the volunteers was carried out by means of a randomized two-way crossover design with a one-week drug-free interval between administration (period 1 and period 2) [14]. After an overnight fast, a blood sample of 5 ml was collected for each volunteer in sodium heparin evacuated glass tubes (Vacutainer[®], 5 ml), as a blank control. The volunteers were separated in two groups and each volunteer ingested three 150 mg tablets of lamivudine with 240 ml of water, between 7:00 a.m. (group I) and 8:00 a.m. (group II). 1 h after the drug administration, the volunteers received a standard breakfast and two meals were offered at 12:00 p.m. and 18:00 p.m. for group I and at 13:00 p.m. and 19:00 p.m. for group II, respectively. Blood samples of 5 ml were collected at 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 6.0, 8.0 and 12 h after administration of the drug. The plasma was immediately separated by centrifugation at 3,000 rpm for 15 min and stored at –20 °C until analysis.

2.4. Analysis of plasma samples

Plasma samples were assayed for lamivudine content by a validated sensitive modified HPLC method [15]. The mobile phase was consisted by a mixture of phosphate buffer solution (pH 5.6), methanol and acetonitrile (94 : 3 : 3). Elution was performed at a flow rate of 1.0 ml/min, using a reversed-phase μ BondaPak C₁₈, 10 μ m, 4.9 × 300 mm column (Waters, USA). Lamivudine peak was detected at 270 nm (0.005 AUFS). Initially, a set of calibration standard curves of lamivudine were prepared in a concentration range of 300 to 7,000 ng/ml, and in a sufficient quantity for the 24 volunteer sample analysis. Lamivudine standard solutions were aliquoted separately for each concentration and stored at –20 °C. Daily, each concentration of the test formulation standard curve was charged with 20 μ l of zalcitabine solution (2,000 ng/ml) as an internal standard. During the analysis for each volunteer a standard curve was placed between the plasma samples obtained from period 1 and period 2, in a randomized way. The stability of lamivudine in the frozen plasma samples was evaluated by comparative analysis of the area under the curve peak obtained with fresh plasma standard curves. A plasma sample of 1.0 ml was transferred into a 1.5 ml Eppendorf centrifuge tube and treated with 200 μ l of 20 % acid trichloroacetic solution. The centrifuge tube was vortex-mixed and charged with 20 μ l of zalcitabine standard solution corresponding to 2,000 ng/ml. The tube was vortexed again for 10 s and then centrifuged at 23,000 g for 5 min. After centrifugation, the supernatant was collected and 20 μ l were injected.

2.5. Pharmacokinetic lamivudine analysis

The comparison between the absorption rates was essentially carried out regarding both the maximum plasma concentration

C_{max} and its corresponding occurrence time T_{max} . These parameters were obtained directly from the experimental volunteer curves.

The relative extent of absorption, i.e. the amount of drug reaching the systemic circulation, was expressed by the area under the plasma concentration versus time curve, ($AUC_{(0-t)}$ and $AUC_{(0-\infty)}$). $AUC_{(0-12)}$ was determined by numerical integration using the linear trapezoidal rule with a 0.10 step, after a smoothing of the curve descendent part. The $AUC_{(0-\infty)}$ parameter was estimated by assuming an exponential profile kinetic after oral lamivudine dose, i.e.

$$AUC_{(0-\infty)} = AUC_{(0-last)} + C_{last} / k_{ep}$$

where (C_{last}) refers to the last measurable point at the plasma concentration-time curve [16]. In order to calculate AUC a few missed experimental curve points were obtained by the linear interpolation method.

2.6. Statistical data analysis

The formal comparison between the two treatments was performed on traditional bioequivalence assessments using the parametric method for AUC and C_{max} and non-parametric method for T_{max} . The parameter variances for the test and reference formulations were evaluated by the variance ratio test (F). For bioequivalence purposes, effects of treatment, period and intersubjects on the total variation for pharmacokinetic relevant parameters were investigated by three-way analyses of variance (ANOVA) [17]. The bioequivalence between the test and reference formulations was also examined by two one-side t-tests [18]. Statistical tests were carried out using a software for bioequivalence study at a 90 % confidence level ($\alpha = 0.1$).

3. Results

3.1. Lamivudine bioavailability and bioequivalence

Lamivudine tablets at 6.0 mg/kg average dose were rapidly absorbed after oral administration, and well tolerated by the subjects. No clinical side effect was observed in any volunteer during the entire study.

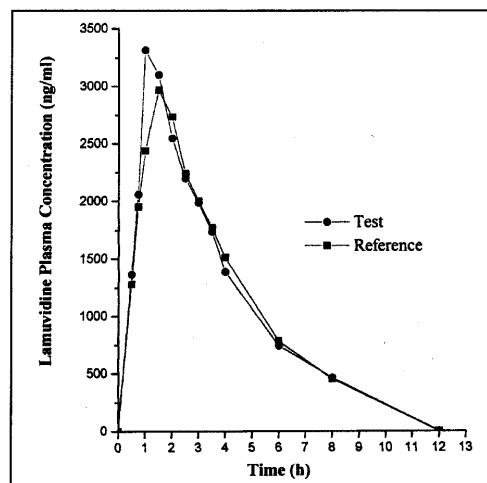


Fig. 1: Mean plasma concentration profile after a single oral dose (3 × 150 mg tablet) of the test and reference formulations.

The standard curves for lamivudine in plasma, based on the peak area response, were linear over a wide concentration range of 300 to 7,000 ng/ml. Coefficients of determination for adjusted experimental points were $r \geq 0.9938$ for all volunteers, except one that presented $r = 0.9846$. All the standard curve data indicated that the proposed HPLC method had good precision and accuracy, and lamivudine maintained stability in plasma under storage and assay conditions. Fig. 1 depicts the corresponding average plasma concentration-time curve for the lamivudine test and reference formulations. A remarkable curve balance is observed.

4. Discussion

A good agreement between test and reference mean pharmacokinetic parameters is observed (Table 2). The geometric means of C_{max} were 3,591 $\mu\text{g/ml}$ (26.3 %) and 3,605 $\mu\text{g/ml}$ (25.8 %) for the test and reference lamivudine formulations, respectively. Geometric mean values of T_{max} were 1.15 (38.3 %) and 1.13 (41.4 %) for the test and reference products, respectively. These results confirm those of a previous lamivudine phase I study [19].

Coefficients of variation (CVs) below 30 % have been obtained for all parameters, except for T_{max} . The maximum lamivudine concentration was 3,6 $\mu\text{g/ml}$ ($T : R = 0.99$), achieved about 1 h after drug administration. The mean relative bioavailability of the test calculated

as percentage $T : R$ ratios for the arithmetic mean of $AUC_{(0-12)}$, $AUC_{(0-\infty)}$, C_{max} and T_{max} values was found to be within the range of 93.3–100.3 %.

Analysis of variance was carried out supposing normal-distributed parameters (Table 3). ANOVA results of pharmacokinetic parameters indicated that there were no significant effects of formulation on any parameter. A wide intersubject variation (large CV) was observed for all the pharmacokinetic parameters. As expected, significant subject effects were found on all the parameters ($p < 0.002$). Then the variation between periods and treatments was analyzed. The null hypothesis $H_0: \mu_T = \mu_R$ versus the alternative hypothesis $H_1: \mu_T \neq \mu_R$ was tested. Regarding the period, the null hypothesis cannot be rejected at a significance level of 90 % for almost all parameters, i.e., there was no significant effect on any period or parameter except in the case of T_{max} ($p < 0.05$), which was a borderline case by 95 %.

Concerning the difference of the test and reference treatments, the H_0 hypothesis cannot be rejected at 90 % level for all parameters. $AUC_{(0-12)}$, $AUC_{(0-\infty)}$, C_{max} and T_{max} did not show any significant effect of the formulation. No significant difference (probe value $p > 0.1$) between T and R treatments was observed in these parameters.

Besides parametric statistical tests, a non-parametric bioequivalence test at 90 % confidence intervals was examined for T_{max} . The asymmetric 90 % confidence limits for the $T : R$ ratio of lamivudine formulations with log-transformed data were 90.96–114.90 % for T_{max} . The confidence limits were narrow and confined in the 80–120 % criterion of bioequivalence, surrounding the ideal relative bioavailability (100 %).

The Hauck-Anderson distribution-free approach for bioequivalence assessments was also examined (Table 4). The two formulations were found to be bioequivalent at $p < 0.002$. All values unequivocally indicated the bioequivalence of both lamivudine formulations.

The present study clearly demonstrated that pharmacokinetic parameters for lamivudine after oral administration of the test and reference formulations are comparable. ANOVA statistics indicated no significant effect of formulation on any parameters. The non-

Table 2: Mean pharmacokinetic parameters for lamivudine and relative bioavailability of the test and reference formulation.

Formulation Parameters	$AUC_{(0-12)}$ (ng · h · ml ⁻¹)	$AUC_{(0-\infty)}$ (ng · h · ml ⁻¹)	C_{max} (ng/ml)	T_{max} (h)
Test ^{a)} (CV%)	12208 (22.1)	12425 (21.5)	3591 (26.3)	1.15 (38.3)
Reference ^{a)} (CV%)	12679 (19.8)	13291 (22.7)	3605 (25.8)	1.13 (41.4)
Bioavailability of the Test % ^{b)}	96.7	93.3	99.7	100.3
T : R ratio ^{a)}	0.962	0.936	0.993	1.028
n	23	23	23	23

^{a)} Geometric mean. ^{b)} Relative bioavailability based on arithmetic mean.

Table 3: Three-way analyses of variance (ANOVA): effects for subjects, periods and treatments on the total variation of pharmacokinetic parameters.

Variance Source	df	F-value	Prob (F)	T_{max}
	$AUC_{(0-12)}$	$AUC_{(0-\infty)}$	C_{max}	
Subjects	22 10.0900 <0.0001	22 4.0568 0.0011	22 3.6995 0.0020	22 5.8422 0.0001
Periods ^{a)}	1 0.3687 >0.30	1 0.7324 >0.30	1 1.8004 0.1935	1 4.3343 0.0498
Treatments	1 1.5903 0.2271	1 2.8487 0.1062	1 0.0045 >0.30	1 0.0023 >0.30
Total	45	45	45	45
Residual variance coefficient ^{b)}	8.75	13.48	16.89	21.33

^{a)} n = 11 + 12 (R, T). ^{b)} % of reference mean.

Table 4: Bioequivalence hypothesis tests for lamivudine pharmacokinetic parameters.

Parameter	Two one-sided t-test ^{a)}		p = p (r ≤ 0.8) - p (r ≥ 1.25) (equivalent to hauck and Anderson)
	H ₀ : μ _T /μ _R < 0.8	H ₀ : μ _T /μ _R > 1.25	
AUC ₍₀₋₁₂₎	< 0.0002	< 0.0001	< 0.0002
AUC _(0-∞)	< 0.002	< 0.0001	< 0.002
C _{max}	< 0.0005	< 0.0001	< 0.0005
T _{max}	< 0.005	< 0.0005	< 0.002

^{a)} Untransformed values.

parametric bioequivalence ± 20 % criterion was respected for the test formulation. According to plasma concentration levels, the bioequivalence of both products was established based on all parameters (AUC₍₀₋₁₂₎, AUC_(0-∞), C_{max} and T_{max}) for a confidence interval of 90 %.

5. References

- [1] Soudeyns, H., Yao, X. J., Gao, Q., Anti-human immunodeficiency virus type 1 activity and in vitro toxicity of 2'-deoxy-3'-thiacytidine (BCH-189), a novel heterocyclic nucleoside analog. *Antimicrob. Agents Chemother.* **35**, 1386 (1991)
- [2] van Leeuwen, R., Katlama, C., Kitchen, V. et al., Evaluation of safety and efficacy of 3TC (lamivudine) in patients with asymptomatic or mildly symptomatic human immunodeficiency virus infection: A phase I/II study. *J. Inf. Dis.* **171**, 1166 (1995)
- [3] Coates, J. A. V., Cammack, N., Jenkinson, H. J., (-)-2'-deoxy-3'-thiacytidine is a potent, highly selective inhibitor of human immunodeficiency virus type 1 and type 2 replication in vitro. *Antimicrob. Agents Chemother.* **36**, 733 (1992)
- [4] Pluda, J. M., Cooley, T. P., Montaner, J. S. G. et al., A phase I/II Study of 2'-deoxy-3'-thiacytidine (lamivudine) in patients with advanced human immunodeficiency virus infection. *J. Inf. Dis.* **171**, 1438 (1995)
- [5] Skalski, V., Chang, C. N., Dutschman, G. et al., The biochemical basis for the differential anti-human immunodeficiency virus activity of two enantiomers of 2',3'-dideoxy-3'-thiacytidine. *J. Biol. Chem.* **268**, 23234 (1993)
- [6] Tisdale, M., Kemp, S. D., Parry, N. R. et al., Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in 4MDD region of reverse transcriptase. *Proc. Natl. Acad. Sci. USA* **90**, 5653 (1993)
- [7] Merrill, D. B., Moanis, M., Chou, T. C. et al., Lamivudine or stavudine in two and three-drug combinations against human immunodeficiency virus type 1 replication in vitro. *J. Inf. Dis.* **173**, 355 (1996)
- [8] Gao, Q., Gu, Z., Parniak, M., the same mutation that encodes low-level human immunodeficiency virus type-1 resistance to 2',3'-dideoxyinosine and 2',3'-dideoxycytidine confers high-level resistance to the (-) enantiomer of 2',3'-dideoxy-3'-thiacytidine. *Antimicrob. Agents Chemother.* **37**, 1390 (1993)
- [9] Katlama, C., Ingrid, D., Loveday, C. et al., Safety and efficacy of lamivudine-zidovudine combination therapy in antiretroviral-naïve patients. A randomized controlled comparison with zidovudine monotherapy. *JAMA* **276**, 118 (1996)
- [10] Caliendo, A. M., Hirsch, M. S., Combination therapy for infection due to human immunodeficiency virus type 1. *Clin. Infect. Dis.* **18**, 516 (1994)
- [11] Wilson, C., Hirsch, M. S., Combination antiretroviral therapy for the treatment of human immunodeficiency virus type-1 infection. *Proc. Assoc. Am. Phys.* **107**, 19 (1995)
- [12] Carpenter, C. C. J., Fischl, M. A., Hammer, S. M. et al., Antiretroviral therapy for HIV infection in 1996. Recommendations of an international panel. *JAMA* **276**, 146 (1996)
- [13] Larder, B. A., Kemp, S. D., Harrigan, P. R., Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. *Science* **269**, 696 (1995)
- [14] Food and Drug Administration. In vivo bioequivalence guidances. *Pharmacop. Forum* **19**, 6501 (1993)
- [15] Zhou, X. J., Sommadossi, J. P., Rapid quantitation of (-)-2'-deoxy-3'-thiacytidine in human serum by high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. B* **691**, 417 (1997)
- [16] Midha, K. K., McKay, G., Charkraborty, B. S. et al., Bioequivalence of two thiazine tablet formulations using radioimmunoassay and gas chromatographic-mass spectrometric methods. *J. Pharm. Sci.* **79**, 196 (1990)
- [17] Schuurmann, D. J., A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J. Pharmacokinet. Biopharm.* **15**, 657 (1987)
- [18] Hauck, W. W., Anderson, S., A new statistical procedure for testing equivalence in two-group comparative bioavailability trials. *J. Pharmacokinet. Biopharm.* **12**, 83 (1984)
- [19] van Leeuwen, R., Lange, J. M. A., Hussey, E. K. et al., A safety and pharmacokinetics of a reverse transcriptase inhibitor, 3TC, in patients with HIV infection: a phase I study. *AIDS* **6**, 1471 (1992)

Acknowledgements

The authors gratefully acknowledge LAFEPE and IVB laboratories for the partial financial support for this study. The first author is thankful to the Brazilian Council for Scientific Research-CNPq, for the partial financial assistance (Grant No. 304946/85).

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