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Research Article

Bioequivalence of Two Oral-Suspension Formulations of Oseltamivir in Healthy Mexican Adults

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ABSTRACT

Oseltamivir is an oral prodrug of oseltamivir carboxylate, which is a selective inhibitor of influenza A and B neuraminidases. It is indicated for the treatment and prophylaxis of influenza A and B infections. In this study, we investigated the bioavailability and the bioequivalence of a test oral-suspension oseltamivir formulation (6 mg/ mL) with respect to the corresponding reference oral-suspension formulation. A single-dose randomized, open-label, two treatment, two-sequence, two-period crossover design under fasting conditions with a 4-day washout interval between the two periods was used.

A single dose of 75 mg of oseltamivir (in 12.5 mL of oral suspension) was administered to healthy adult Mexican subjects.

Samples were drawn at baseline and then at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00 and 12.00 hours after administration.

The 90% CIs for oseltamivir C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 98.34%-114.47%, 95.27%-100.49% and 95.93%-101.10%, respectively, which fell within the predetermined range of 80% to 125%. Thus, these results show that the bioequivalence criteria were met.

Keywords: Oseltamivir; Suspension; Bioavailability; Bioequivalence

INTRODUCTION

Oseltamivir is an oral prodrug of oseltamivir carboxylate (an antiviral drug) which is a selective inhibitor of influenza A and B neuraminidases. It is indicated for the treatment and prophylaxis of influenza A and B infections [1,2].

In particular, the oral oseltamivir suspension is indicated for pediatric patients >1 year or adult patients who cannot swallow a capsule [3].

After oral administration of oseltamivir (as its phosphate salt), it is absorbed from the gastrointestinal tract and transformed by hepatic esterases into the oseltamivir carboxylate, with an absolute bioavailability of 80%. At least 75% of the oseltamivir is transformed into the oseltamivir carboxylate. Plasma concentrations of oseltamivir decay rapidly with an apparent elimination half-life ($t_{1/2}$) of 1–3 h, while oseltamivir carboxylate has a longer apparent elimination half-life of 6–10 h, thus allowing twice-daily dosing [4-6].

Oseltamivir has a linear-pharmacokinetic profile at doses of up to 500 mg twice daily [6,7]. In addition, concomitant food intake has little effect on its bioavailability [6].

In Mexico, oseltamivir is available as capsules at the strengths of 30, 45 and 75 mg [8]. However, at the time of this research there was no oral oseltamivir suspension marketed in Mexico. The sponsor of this research (Laboratorios Liomont, S.A. de C.V., Mexico City. Mexico) wished to obtain the marketing authorization for oseltamivir powder for oral suspension in Mexico.

Therefore, the goal of this research was to study both the bioavailability and the bioequivalence of a test powder for suspension formulation containing oseltamivir and compare it with the corresponding reference formulation.

METHODS

Formulations

Both reference and test formulations containing oseltamivir phosphate (equivalent to 360 mg of oseltamivir free base) were in powder form intended for oral suspensions, which after constitution with water yields a working volume of 60 mL. Thus, the resulting concentration of oseltamivir free base is 6 mg/ mL. The test formulation was manufactured by Laboratorios Liomont, S.A. de C.V. (Mexico City, Mexico), the lot number was 314J0027, and the expiration date was February, 2020. The reference formulation was manufactured in Switzerland and distributed by Genentech USA, Inc (South San Francisco, CA). The lot number was 3031362, and the expiration date was April 2019.

Ethical considerations

An independent ethics and research committee (Comité de Ética en Investigación y Clínica de Enfremeddades Crónicas y de Procedimientos Especiales, S.C.) reviewed and approved the study protocol (OSL-08-LIO) and the informed consent documents on January 15, 2018. This study was authorized by the Federal Commission for Protection against Sanitary Risks (Comision Federal Para La Proteccion Contra Riesgos Sanitarios [COFEPRIS]) on April 5, 2018.

The trial was performed in accordance with the Declaration of Helsinki (and its amendments) and the International Conference on Harmonization for Good Clinical Practice Guideline.

The principal investigator informed the subjects about the anticipated risks and potential discomfort associated with the study drug, the procedures, and study duration. A written informed consent was given by all of the subjects prior to study initiation. The clinical stage of the study was conducted in September of 2018.

Subjects

Healthy Mexican adults of both genders between 18 and 55 years were considered eligible for this study. The clinical evaluation included an interview and a physical examination of vital signs, blood pressure, heart rate, temperature and 12-lead electrocardiogram. Moreover, laboratory tests (hematology and blood chemistry, urinalysis, and tests for alcohol, drug-abuse and a pregnancy test for women) and serological tests (hepatitis B and C in addition to HIV antibodies) were performed.

Study design and drug administration

A single-dose randomized, open-label, two treatment, two-sequence, two-period crossover design under fasting conditions with a 4-day washout interval between the two periods was employed.

The subjects were admitted to a clinical unit (Investigación Farmacológica y Biofarmacéutca) on the day prior to the drug administration. They were randomly assigned to one of the two sequences: the reference formulation followed by the test formulation and vice versa.

The subjects received a single-dose of 12.5 ml of the oral

suspension containing 75 mg of oseltamivir (equivalent to a capsule dose at the 75 mg strength) of the reference or the test formulation with 250 ml of water after fasting for at least 10 hours. Blood samples (6 mL) were drawn from each subject using an indwelling cannula. These samples were placed into tubes containing Citrate-Phosphate-Dextrose-Adenine (CPDA) as an anticoagulant. The samples were obtained at baseline (pre-dose) and then at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50,1.75, 2.00, 2.50, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 10.00 and 12.00 hours after administration. After the washout period, the subjects received the alternative formulation and the samples were obtained using the same procedure.

The blood samples were centrifuged at 4500 rpm for 5 minutes. Finally, the plasma samples were kept at -70°C ± 10°C until being transported to the analytical unit (Biokinetics) in which they were kept at 75°C ± 5°C until analysis.

The subjects' diet consisted of standardized meals (breakfast, lunch and dinner) which were provided at 4, and 8 hours after drug administration.

Determination of oseltamivir plasma concentrations

Chemicals: Oseltamivir phosphate (lot: R00490), the reference standard was obtained from the USP (MD, USA) and oseltamivir *d*5-phospate (internal standard, lot: PA/OMV/11800 D5) was obtained from Pharmaffiliates Analytics & Synthetics (P) Ltd. (Haryana, India). The water and the solvents were LC-MS grade (Avantor Performance Materials, LLC, PA, USA and Honeywell International Inc. MI, USA) and all reagents were obtained as analytical grade (Sigma-Aldrich, Inc. Missouri, USA).

Method and sample preparation: Plasma oseltamivir plasma concentrations were determined using a HPLC method coupled to a mass spectrometer (MS/MS); this method was developed and validated by Biokinetics personnel in Mexico City, Mexico.

A sample containing 10 µL of internal standard (1.5 µg/ mL) and 250 µL of plasma and was extracted with 1000 µL of ethyl acetate. This mixture was vortexed for one minute and centrifuged at 8000 rpm for 5 minutes at 20°C. The organic phase (800 µL) was separated and placed into a test tube in which it was evaporated to dryness under a nitrogen current, at 50°C for 6 minutes. The residue was reconstituted with a mixture of 100 µL consisting of formic acid (0.1%) and acetonitrile (50:50 v/v), which was vortexed for 15 seconds, transferred to a glass vial and two µL of this reconstituted solution was injected into the chromatographic system (HPLC, Agilent Technologies, model 1200, CA, California).

Chromatographic conditions

The analytical column was a Zorbax® SB-C18, (50 × 2.1-mm internal-diameter column of 3.5-µm particle size (Agilent Technologies)). Oseltamivir and the Internal Standard (IS) were eluted with a mobile phase consisting of a mixture (77:23 v/v) of aqueous formic acid (0.1%) and acetonitrile. The column temperature was 25°C, the flow of the mobile phase was 0.3 mL/minute, and both analytes were detected by a triple-quadrupole mass spectrometer (Agilent Technologies, model G6410B). The spectrometric (MS/MS) analysis was performed by monitoring the transition 313.2 m/z → 166.2 m/z for oseltamivir and 318.1 m/z → 171.2 m/z for the internal standard. The spectrometric conditions were positive-ionization mode, fragmenter energy (87 V for oseltamivir and 75 V for IS), and collision energy (18 V for oseltamivir and 75 V for IS), and collision energy (18 V for oseltamivir and 75 V for IS). The typical retention times for oseltamivir and IS were 1.90 and 1.85 minutes, respectively. The peak areas were measured in order to calculate the peak area ratio of oseltamivir with respect to that of the IS. We then calculated the concentration.

Method validation

The analytical method was validated according to Mexican and international guidelines [9,10].

Analysis of blank human plasma samples from six different subjects, blank human (hemolyzed and lipemic) plasma samples, as well as anticoagulants (CPDA), xanthines (caffeine and theobromine), and other drug substances commonly used as analgesics (acetylsalicylic acid, diclofenac, paracetamol, ibuprofen and naproxen) were used to test the method's selectivity.

The calibration curve consisted of the following oseltamivir concentrations: 0.5, 1, 12, 35, 60, 90 and 100 ng/ mL. Thus, the range was 0.5-100 ng/ mL, and the Lower Limit of Quantification (LLOQ) was 0.5 ng/ mL. The method was linear over this range of concentrations, and the coefficient of determination was > 0.99 (average from four calibration curves). The intra-assay %CV and accuracy (relative error) of oseltamivir were 13.71% and 2.01 to 6.85%, respectively; and the inter-assay %CV and accuracy were 9.43% and 2.56% to 4.17%, respectively.

Oseltamivir was found to be stable in plasma for at least 25 hours at room temperature (25°C), after three freeze-thaw cycles and after 16 weeks at –75 ± 5°C.

Sample dilution was also tested to account for oseltamivir concentrations beyond the upper bound of the calibration curve's range.

Quality-control samples were prepared at three different concentration levels (designated as low (15 ng/ mL), medium (45 ng/ mL) and high (80 ng/ mL) of oseltamivir independent of the calibration curve and they represented 5% of the all of the tested samples.

The acceptance criteria for the approval of the analytical runs and the quality control samples, in addition to the criteria for performing sample reanalysis, were consistent with Mexican and international guidelines.

Tolerability

The subjects were interviewed by the principal investigator and/or the study coordinator in order to determine the occurrence of Adverse Events (AEs) during the study and at the end of the study's clinical stage. The subjects were asked to report any AEs to the principal investigator at any time over the entire duration of the study, including washout period.

Pharmacokinetic and statistical analyses

The sample size calculation was based on the within-subject variability of oseltamivir C_{max} with a %CV of 31.87% (calculated from its reported %90 CI of 91.10%-124.00%, n = 24) obtained from the information available from the Center for Drug Evaluation and Research (CDER) regarding the application for oseltamivir powder for suspension [11], because at the time of the present research, there was no available data from published bioequivalence studies regarding the oral-suspension. This calculation, was performed using the following values: 1 - β = 0.8, α = 0.05, expected ratio (μ_T/μ_R) = 1

and an equivalence range of 80% to 125%, yielded a sample size of 38 subjects [12]. Thus, we planned to recruit 44 subjects to consider potential dropouts.

We directly obtained C_{max} and T_{max} values from the plasma concentration–time curves.

We estimated the rate constant (k_e) from the terminal log-decay phase by using linear regression, and the $t_{½}$ was estimated with the following equation:

$t_{½} = \ln 2/k_e$, in which *ln* is the natural logarithm.

In order to assess the bioequivalence between the test and reference formulations, C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were considered to be primary variables.

Using log-transformed data for these parameters, analysis of variance for a 2 x 2 crossover design, was carried out at the significance level of 5% ($\alpha = 0.05$).

The 90% confidence intervals of the geometric mean ratios (test/reference) of the C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were estimated. The test and the reference formulations were regarded to be bioequivalent if the 90% confidence intervals of these parameters fell within a predetermined acceptance range of 80% to 125%. All of the pharmacokinetic and statistical analyses were performed using WinNonlin Version 8.1 (Certara, NJ, USA).

RESULTS

Table 1 lists the demographic characteristics of the 44 subjects who were enrolled in the study.

One subject was withdrawn from the study because she had a dental infection at the time of the second period of the study. Hence, the sample size for the bioequivalence evaluation was 43 subjects.

Pharmacokinetic parameters

The mean plasma concentration-time curves of the two formulations are shown in figure 1. The results indicate that the two formulations have similar mean plasma concentration-time curves.

The pharmacokinetic parameters (C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t} and $AUC_{0-\infty}$) for both formulations are listed in table 2.

The analysis of variance of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ did not detect significant period or sequence effects (data not provided).

The bioequivalence statistics using the log-transformed data for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$; including geometric means, geometric mean ratios (test/reference), 90% CIs, and the intra-subject %CV are listed in table 3.

The 90% CIs for oseltamivir C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ were 98.34%-114.47%, 95.27% -100.49% and 95.93% -101.10%, respectively. The 90% CIs of the geometric mean ratios of the parameters fell within the predetermined range of 80% to 125%. Hence, these results show that the bioequivalence criteria were satisfied.

Table 1: Demographic characteristics of subjects.

Characteristic	Values
Total No. of recruited subjects (female/male)	44 (23/21)
Age, mean (SD), range, years	35 (10),18-55
Weight, mean (SD), range, kg	63.6 (7.2), 49.5-87.2
Height, mean (SD), range, m	1.64 (0.08), 1.50-1.83
BMI , mean (SD), range, kg/m^2	23.78 (2.26), 18.17-26.78

BMI: Body Mass Index; SD: Standard Deviation.

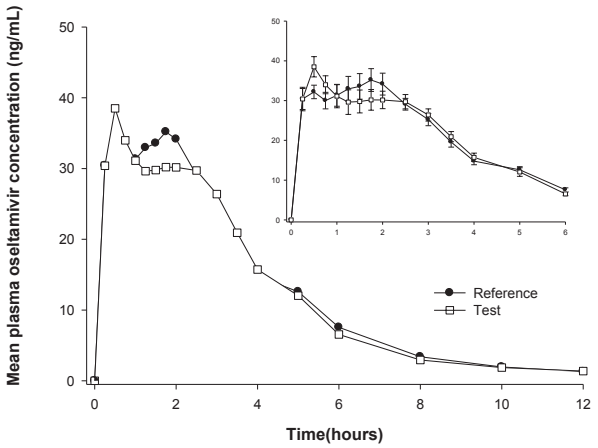

Figure 1: Mean plasma concentration-time curves after a single-dose administration of 75 mg of oseltamivir (in 12.5 mL of suspension) of a test (trademark: Seltaferon®) and a reference (trademark: Tamiflu®) oral-suspension formulations, containing oseltamivir at the concentration of 6 mg/mL (after constitution) in healthy Mexican adult subjects (n = 43). Inset: mean (±SE) concentrations over the first 6 hours after administration

Table 2: Pharmacokinetic parameters.

Parameter	Reference†	Test*
C_{max}, ng/mL	50.54 (18.46)	53.63 (18.40)
AUC_{0-t}, ngh /mL	152.95 (31.32)	150.12 (32.63)
$AUC_{0-\infty}$, ng•h /mL	157.29 (31.68)	155.35 (33.27)
T_{max}, h	1.29 (1.00)	1.30 (1.09)
$t_{1/2}$, h	2.33 (0.71)	2.62 (0.79)

Values are mean (SD)
C_{max} = Maximum plasma drug concentration
AUC_{0-t} = Area under the curve from time 0 (baseline) to the last measurable concentration
$AUC_{0-\infty}$ = Area under the curve from time 0 (baseline) to infinity
*Trademark: Seltaferon® powder for suspension formulation containing oseltamivir 6 mg/mL (after constitution)
†Trademark: Tamiflu® powder for suspension formulation containing oseltamivir 6 mg/mL (after constitution).

Table 3: Geometric means, geometric mean ratios, 90% CIs and the intra-subject %CV of the pharmacokinetic parameters.

Parameter	Geometric Means Test/ Reference	Geometric Mean Ratio (%)	90% CI	Intra-subject %CV
C_{max}, ng/mL	50.57/ 47.66	106.10	98.34, 114.47	21.16
AUC_{0-t}, ng•h/mL	146.64/ 149.87	97.85	95.27, 100.49	7.35
$AUC_{0-\infty}$, ng•h/mL	151.84/ 154.18	98.48	95.93, 101.10	7.24

C_{max} = Maximum plasma drug concentration
AUC_{0-t} = Area under the curve from time 0 (baseline) to the last measurable concentration
$AUC_{0-\infty}$ = Area under the curve from time 0 (baseline) to infinity.

Tolerability

Five subjects reported a total of six AEs. These events consisted

of five cases of nausea and one case of dental infection; three cases of nausea were reported after the administration of the reference formulation, while two cases of nausea were reported after the administration of the test formulation. The dental infection was considered unrelated to the administration of the studied medication.

None of the AEs was considered to be serious; rather, they were regarded as mild.

DISCUSSION

We found that all of the 90% CIs of the geometric mean ratios of the pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were within the predetermined bioequivalence range (80%-125%). These results show that the bioequivalence criteria were met.

Both formulations were well tolerated because the reported AEs were regarded to be mild and had spontaneously resolved under medical surveillance during the clinical stage, except for the dental infection that required pharmacological treatment.

This study has several limitations. First, it was an open label study. Only healthy adult subjects within a specific age range could participate, and these individuals, were received a single dose of the formulations. Moreover, because of the requirements for bioequivalence studies, pediatric patients or adult patients who cannot swallow a capsule could not be included in this study. Hence, the pharmacokinetic parameters of oseltamivir may differ among patient groups. In this way, the results of this research might not be generalizable to target populations.

In addition, we did not evaluate the effect of food on the bioavailability of oseltamivir because it has been reported that the concomitant food intake has little effect on its bioavailability [6].

Additional future studies are necessary to compare the test and reference formulations in different patient groups. We expect that the findings of this study may serve as a reference for future controlled studies of oseltamivir in a Hispanic population.

CONCLUSION

This study, which included healthy, fasting Mexican adult subjects of both genders, showed that the test formulation of oseltamivir powder for oral-suspension satisfied the Mexican regulatory requirements to assume bioequivalence.

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The authors declare that they have no conflicts of interest regarding the content of this article.

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