

## Bioavailability of Two Different Coated-Tablet Formulations of Valacyclovir of Two Different Strengths (500 mg and 1000 mg) in Healthy Mexican Adult Volunteers

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### Abstract

Valacyclovir is a prodrug of acyclovir. In Mexico, it is indicated for the treatment of herpes zoster and herpes simplex infections. The aims of these 2 studies were to compare the bioavailability and to determine the bioequivalence of 2- test formulations containing 500 mg and 1000 mg of oral valacyclovir. Two separate, single-dose, open-label, randomized, 2-period, crossover studies were conducted. For each study a different set of 26 subjects of both genders was enrolled, with a 7-day washout period. In both studies, the study formulations were administered after a 10-hour overnight fast. For pharmacokinetic analysis, blood samples were drawn at baseline, 0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75, 2, 3, 4, 6, 8, 12 and 24 hours after administration. Plasma concentrations of acyclovir were determined using HPLC coupled to a fluorescence detector. The test and reference formulations were to be considered bioequivalent if the 90% CI for the geometric mean test/reference ratios were within a predetermined range of 80% to 125%. In the study with valacyclovir 500 mg, the 90% CI were 95.24% - 115.33% for  $C_{max}$ , 96.20% - 103.55% for  $AUC_{0-t}$ , 97.12% - 104.34% for  $AUC_{0-\infty}$ . In the study with valacyclovir 1000 mg the 90% CI were 86.22% - 100.87% for  $C_{max}$ , 89.11% - 98.50% for  $AUC_{0-t}$ , 89.00% - 98.34% for  $AUC_{0-\infty}$ . In both studies, a single dose of the test formulation met the regulatory requirements to assume bioequivalence, based on the rate and extent of absorption.

**Keywords:** Valacyclovir; Acyclovir; Bioequivalence; Bioavailability; Pharmacokinetics; HPLC

### Introduction

Valacyclovir (2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl ester-L-valine) is a prodrug of acyclovir, a nucleoside analog with antiviral activity [1]. Valacyclovir is rapidly hydrolyzed both in the lumen of the intestine and in the cytoplasm of the enterocyte. Virtually no valacyclovir (<1%) is detected in the systemic circulation following oral administration [2,3]. Thus, the quantification of acyclovir in plasma was used as an endpoint for the purposes of the present studies.

The bioavailability of acyclovir after the administration of one gram of valacyclovir is 54% (3-5 times higher than the bioavailability obtained after the administration of acyclovir) and it is not altered by administration of food [4]. Its absorption is dependent on oligopeptide transporters. Thus, there is a lack of dose proportionality of the pharmacokinetics of acyclovir ( $C_{max}$  and AUC) with increasing doses of valacyclovir [5]. The plasma elimination half-life of acyclovir, after the administration of valacyclovir averaged from 2.5 to 3.3 hours [2,3,6].

In Mexico, valacyclovir is indicated for the treatment of herpes zoster and herpes simplex infections, the treatment and suppression of recurrent genital herpes and for the prophylaxis of cytomegalovirus infections [4].

The sponsor of these studies (Laboratorios Liomont, S. A. de C.V.) was interested in obtaining the marketing authorization for two dose-strengths of valacyclovir (500 mg and 1000 mg), as oral-tablet formulations (test formulations) in Mexico.

Because of the lack of dose proportionality in the pharmacokinetics of acyclovir, after the administration of valacyclovir, two separate studies were planned for each of the two valacyclovir strengths.

A search of PubMed, MEDLINE and Google data bases for literature published up to January of 2012, using the combination terms *valacyclovir*, *bioequivalence*, *bioavailability*, *pharmacokinetics*, *500 mg*, *1000 mg*, *Mexico*, *Mexican* and *population*, did not identify any published data concerning the bioavailability of both strengths of oral valacyclovir in the Mexican population.

Therefore, the aims of these studies were to compare the bioavailability and to determine the bioequivalence of 2 test formulations of oral valacyclovir (Valextra<sup>®</sup> 500 mg and 1000 mg coated-tablets, Laboratorios Liomont, S. A. de C.V, Mexico City, Mexico), containing valacyclovir hydrochloride (equivalent to 500 mg and 1000 mg of valacyclovir, respectively) with their corresponding 2 reference-drug formulations: Rapivir<sup>®</sup> coated-tablet (GlaxoSmithKline Mexico, S.A. de C. V, Mexico City, Mexico), containing valacyclovir hydrochloride (equivalent to 500 mg of valacyclovir) and Valtrex<sup>®</sup> caplets, herewith designated as coated-tablets (GlaxoSmithKline, Research Triangle Park, North Carolina), containing valacyclovir hydrochloride (equivalent to 1 g [1000 mg] of valacyclovir), for the purpose of obtaining marketing authorization of the two-test formulations in Mexico.

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## Subjects, Materials and Methods

The 2 protocols, P296S026V004 (valacyclovir 1000 mg study) and P370S026V004 (valacyclovir 500 mg study) and their corresponding informed-consent forms were reviewed and approved by an independent ethics and research committee of Medica Sur Hospital (Mexico City, Mexico) on February 24, 2010 (valacyclovir 1000 mg study) and on March 18, 2011 (valacyclovir 500 mg study). Both studies were conducted in accordance with the principles of Helsinki Declaration and its amendments and the International Conference on Harmonisation Guideline for Good Clinical Practice.

For each study, the principal investigator informed the subjects of all procedures, duration of the study, anticipated risks and discomfort it could entail, and an individual written informed-consent was obtained prior to the initiation of the study. The studies were conducted from February to April, 2010 (valacyclovir 1000 mg study) and from March to June, 2011 (valacyclovir 500 mg study).

### Inclusion/exclusion criteria

For each study, healthy Mexican adults aged 18 to 55 years and of either gender were eligible for inclusion. Subjects were recruited from the out-patient records retrieval database within the Pharmacological Research Unit of CIF-BIOTEC (clinical unit) at Medica Sur Hospital, Mexico City, Mexico.

A physical examination was conducted in each participant. Subject's health was based on unremarkable findings on a clinical health evaluation, which consisted on the following: a personal interview; complete physical examination (blood pressure [BP], heart rate, weight, height, temperature and respiratory rate); and diagnostic testing that included 12-lead ECG, chest radiography, and laboratory testing (complete blood cell count, metabolic and liver function tests [alanine and aspartate amino transferase], biochemistry [glucose, blood urea, nitrogen and creatinine, and serological tests for hepatitis B and C and HIV antibodies], urinalysis, and a pregnancy test in women. Systolic and diastolic BP was measured with a sphygmomanometer (Tycos; Welch Allyn, Skaneateles Falls, NY). The BP cuff was applied to the right arm and the reading was taken with the subject in a seated position. Candidates were excluded if laboratory values were significantly out of the reference range and/or if all tests had not been completed. In both studies, laboratory testing was performed at Medica Sur Hospital, which has been certified by the Mexican government and the College of American Pathologists. The scope of the certifications included the tests relevant to these studies. Before the enrollment of the participants, the laboratory data were reviewed by investigators at the clinical unit. Selected candidates were compensated for their participation.

### Study design and drug administration

In both studies, a single-dose randomized-sequence, open label, 2-period crossover design was used. The subjects for each study were admitted to the clinical site (CIF-BIOTEC) on the day before the study was begun, and were randomly assigned by the quality assurance personnel at the clinical unit, in a 1:1 ratio using a computer-generated table of random numbers, to 1 of the 2 sequences. For the valacyclovir 1000 mg study, the test formulation containing valacyclovir hydrochloride equivalent to 1000 mg of valacyclovir [lot

09-X-34; expiration date; October 31, 2011] was administered followed by the reference-drug formulation (Valtrex®) containing valacyclovir hydrochloride equivalent to 1000 mg of valacyclovir [lot 9ZP1864; expiration date August 31, 2012] or viceversa.

For the valacyclovir 500 mg study, the test formulation containing valacyclovir hydrochloride equivalent to 500 mg of valacyclovir [lot 140B0003; expiration date; March 31, 2013] was administered, followed by the reference-drug formulation (Rapivir®) containing valacyclovir hydrochloride equivalent to 500 mg of valacyclovir [lot 5509-1; expiration date October 31, 2012] or viceversa.

In both studies, randomization codes were concealed from all the investigators of the study.

To ensure reliable baseline plasma measurements, participants underwent a 10-hour overnight fast with a 7-day washout period, which exceeds the 7 half-lives required by the Federal Commission for the Protection of Sanitary Risks (COFEPRIS) [7].

Blood samples were drawn for baseline plasma determinations in the following way. An 18-G x 1.6 in (1.1 x 30 mm) indwelling angiocatheter (BD-InSyte, Becton, Dickinson and Co., Sao Paulo, Brazil) was inserted in suitable forearm vein and 7.5-mL blood sample was drawn into heparin-treated vacuum tube (S-Monovette, Sarstedt AG & Co., Nümbrecht, Germany).

Subjects were administered a single tablet (500 mg or 1000 mg) of the test or the reference formulation with 250 ml of water (whichever was applicable in the corresponding study). Additional blood samples were drawn at, and 0.25, 0.50, 0.75, 1, 1.25, 1.50, 1.75, 2, 3, 4, 6, 8, 12 and 24 hours after administration.

During hospitalization, the subjects were under medical surveillance, and during the washout period, participants maintained contact with the investigators to report any adverse event (AEs).

Plasma was obtained by centrifugation (1000 g for 15 minutes at 25°C) and stored at -75°C±5°C until analyzed using HPLC. After 7-day washout period, participants returned to the clinical unit, where the alternative formulation was administered as in the first treatment period.

Subjects were asked to refrain from water and food intake for 3 hours after study drug administration. Their diet, for each study and treatment period, consisted of 3 standardized meals (2146 kcal/d for the valacyclovir 1000 mg study and 2605 kcal/d for the valacyclovir 500 mg study), at 3, 7 and 10 hours after study drug administration.

### Determination of acyclovir plasma concentrations

**Chemicals:** Acyclovir (lot: QDM7012-2) reference standard obtained from Química Sintética (Madrid, Spain), and acyclovir (lot: J1G366) reference standard, obtained from the USP (Rockville, MD), were used for the 1000 mg and 500 mg studies, respectively. All solvents were HPLC grade (Avantor Performance Materials, Inc.) and all reagents were analytical grade (Mallinkrodt Baker, Inc, Phillipsburg, NJ).

**Method and sample preparation:** In both studies, acyclovir plasma levels were determined by using a HPLC method developed and validated by personnel of Biokinetics at Mexico City, Mexico. The method included the following: 0.2 ml of plasma and 0.2 ml of 7%

perchloric acid were vortexed in a test tube for one minute. The tube was then centrifuged at 1440 g for 10 minutes. The supernatant was filtered through a cellulose syringe filter (pore size, 0.45 micron) and injected (volume of injection = 50 µl) into the chromatographic system (HPLC, Agilent Technologies, model 1100, Palo Alto California), equipped with a fluorescence detector (G1321A, Agilent Technologies).

**Chromatographic conditions:** In both studies, acyclovir concentrations were determined with a 150 x 4.6-mm internal-diameter column of 5-µm particle size (Zorbax® SB -C8, Agilent Technologies, Palo Alto, California) equipped with a pre-column (12.5 x 4.6-mm internal-diameter column, 5-µm particle size, Zorbax® SB -C8, Agilent Technologies) and eluted with a mobile phase consisting of 0.02 M perchloric acid (pH 2 ± 0.1). The column temperature was 25°C. Flow rate was maintained at 1.5 ml/minute and acyclovir detection was carried out using fluorescence detector at 260 nm and 375 nm of excitation and emission wavelength, respectively. Typical retention time for acyclovir was around 11 minutes. The peak area was measured for the calculation of acyclovir plasma concentrations, with respect to the acyclovir calibration curve.

### Method validation

The method was validated according to Mexican [7] and international guidelines [8]. The selectivity of the method was tested by the analysis of: blank human plasma for 6 different subjects; blank human (hemolyzed and lipemic) plasma samples, as well as with regard to anticoagulants (heparin), xanthines (caffeine and theobromine), and another drug substance commonly used as analgesic (naproxen, ketorolac, paracetamol and acetylsalicylic acid). No interferences were observed in the resulting chromatograms. The range of the method was 0.035 to 1.5 µg/ml and with lower limits of quantification and detection of 0.035 and 0.015 µg/ml respectively. The method was found to be linear within this range of concentrations with a coefficient of determination of 0.9993. The intra-assay %CV and accuracy (relative error) for acyclovir were 3.6% to 6.7% and -2.5% to 5.9%, respectively, while inter-assay %CV and accuracy were 1.43% to 5.71% and -3.1% to 3.6%. The absolute recovery was above 90%.

Acyclovir in plasma was found to be stable after 48 hours at room temperature (25°C), after 3 freeze-thaw cycles and after 16 weeks at -75 ± 5°C. Quality control samples were prepared at 3 different concentration levels (designated as low, [0.1 µg/ml], medium [0.35 µg/ml] and high [0.625 µg/ml]) of acyclovir independent of the calibration curve. This method was considered suitable by the study investigators for both bioequivalence studies.

### Tolerability

In both studies, tolerability was determined using clinical assessment, monitoring of vital signs (BP, heart rate, armpit body temperature) at baseline, after the drug administration, during hospitalization, and at the end of the clinical stage of the studies. Laboratory results were also considered.

The subjects were interviewed (using open-ended questions) by the investigators during hospitalization and at the end of the clinical stage of the studies concerning the occurrence of AEs. Subjects were asked to spontaneously report any AE to the investigators at any time during the studies, including the washout periods. Data for all AEs were recorded on a case-report form.

AEs that were life-threatening, led to death; hospitalization,

disability, and/or medical intervention to prevent permanent impairment or damage were to be considered serious.

### Pharmacokinetic and statistical analyses

Mexican regulatory requirements [7] call for a minimum of sample size of 24 subjects for bioequivalence studies. In both studies, a sample size of 26 subjects was used, which included 2 additional subjects (with respect to the required sample size) considered in case of dropouts.

Individual plasma concentration-time curves were constructed;  $C_{max}$  (maximum plasma drug concentration) and  $T_{max}$  (time to reach  $C_{max}$  after the administration of the drug) were directly obtained from these curves, the area under the plasma concentration-time curve from baseline to the last measurable concentration ( $AUC_{0-t}$ ) was calculated according to the non-compartmental method using the trapezoidal rule. From the terminal log-decay phase, the elimination constant ( $k_e$ ) was estimated using linear regression, and  $t_{1/2}$  was estimated using the following equation [9].

$$t_{1/2} = \ln 2/k_e, \text{ where } \ln \text{ was defined as the natural logarithm.}$$

Extrapolation of AUC from baseline to infinity ( $AUC_{0-\infty}$ ) was calculated as follows:

$$AUC_{0-\infty} = AUC_{0-t} + (C_t/k_e), \text{ where } C_t \text{ was the last measurable plasma concentration.}$$

In both studies, to assess the bioequivalence between the test and reference formulations,  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  were considered as the primary variables. ANOVA for a 2 x 2 crossover design using log-transformed data for these parameters was carried out at the 5% significance level ( $\alpha = 0.05$ ).

The 90% CIs (confidence intervals) of the geometric means ratios (test/reference) of  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$  were calculated using log-transformed data. The test and the reference formulations were to be considered bioequivalent if the 90% CIs of these parameters fell within a predetermined range of 80% to 125% and if the probability of exceeding these limits was <0.05. The probability of exceeding the 80% to 125% range was obtained using the two 1-sided test described by Schuirmann [10]. All pharmacokinetic and statistical analyses were performed using WinNonlin version 5 (Pharsight, Mountain View, California).

### Results

A total of 26 subjects 13 men, 13 women; mean [SD] age, 30 [9] years [range, 19-49 years]; weight, 61.5 [8.2] kg [range, 47.5-80.5 kg]; height, 162 [9] cm [range, 145-176 cm]; and body mass index [BMI], 23.37 [2.02] kg/m<sup>2</sup> [range, 19.20-26.70 kg/m<sup>2</sup>] were enrolled and completed the clinical stage of the study for valacyclovir 1000 mg.

A total of 26 subjects 13 men, 13 women; mean [SD] age, 33 [8] years [range, 18-49 years]; weight, 62 [7.7] kg [range, 51-79 kg]; height, 164 [8] cm [range, 147-182 cm]; and body mass index [BMI], 23 [1.6] kg/m<sup>2</sup> [range, 19-26 kg/m<sup>2</sup>] were enrolled in the clinical stage of the study for valacyclovir 500 mg. Because of a protocol violation (alcohol consumption), one subject was withdrawn from the study at the second period of the clinical stage. Thus, the sample size for the evaluation of the PK parameters (valacyclovir 500 mg) was reduced from 26 subjects to 25 subjects, whereas the 26 subjects remained available for the evaluation of tolerability.

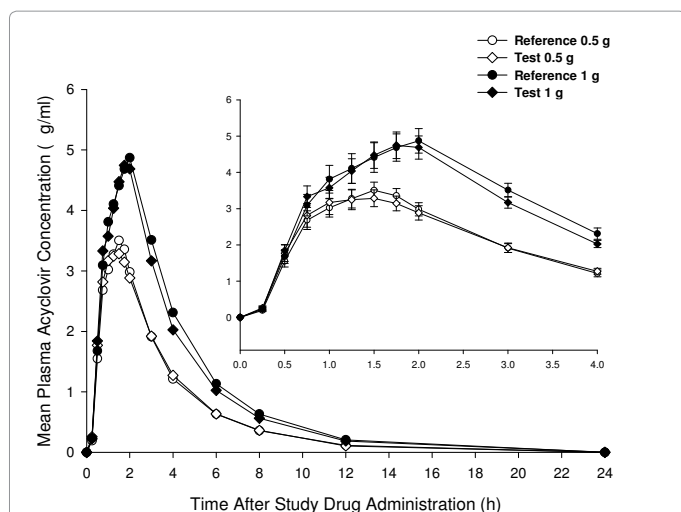


## Pharmacokinetic Parameters

Mean plasma concentration-time curves of the 4 valacyclovir formulations are shown in Figure 1. This figure suggests comparable mean plasma concentration-time curves for each pair of reference-test formulation corresponding to each study.

In addition, it suggests lack of dose proportionality in the pharmacokinetics of acyclovir after the administration of valacyclovir, because when increasing the dose of valacyclovir from 500 mg to 1000 mg, the mean plasma concentration-time curves for the valacyclovir 1000 mg formulations do not seem to have the expected proportional increments in  $C_{max}$  and AUC, that might have been expected by doubling the dose of valacyclovir.

The pharmacokinetic parameters ( $C_{max}$ ,  $T_{max}$ ,  $t_{1/2}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$ ) for the 4 valacyclovir formulations are shown on Table 1.



**Figure 1:** Mean plasma concentration-time curves of acyclovir after the administration of a single-dose of valacyclovir (500 or 1000 mg). Solid symbols represent the reference-test pair of formulations containing valacyclovir 1000 mg (n = 26). Open symbols represent the reference-test pair of formulations containing 500 mg of valacyclovir (n = 25). Right panel: mean ( $\pm$ SE) concentrations over the first 4 hours after administration. In the study with valacyclovir 500 mg, the trademarks were Rapivir® (GlaxoSmithKline Mexico, S. A. de C. V, Mexico City, Mexico) for the reference formulation and Valtrex® for the test formulation. In the study with valacyclovir 1000 mg, the trademarks were Valtrex® (GlaxoSmithKline, Research Triangle Park, North Carolina) for the reference formulation and Valtrex® for the test formulation.

Parameter	1000 mg		500 mg	
	Reference†	Test‡	Reference‡	Test*
No. of subjects	26	26	25	25
$C_{max}$ , $\mu$ g/ml	5.59 (1.76)	5.21 (1.60)	3.90 (1.25)	4.10 (1.31)
$AUC_{0-t}$ , h· $\mu$ g/ml	20.10 (4.82)	18.87 (4.66)	12.49 (2.89)	12.48 (2.97)
$AUC_{0-\infty}$ , h· $\mu$ g/ml	20.85 (4.96)	19.53 (4.74)	13.04 (2.88)	13.14 (2.94)
$T_{max}$ , h	2.15 (0.75)	2.09 (0.61)	1.60 (0.63)	1.58 (0.73)
$t_{1/2}$ , h	2.46 (0.31)	2.52 (0.49)	2.60 (0.50)	2.59 (0.45)

$AUC_{0-t}$  = AUC from time 0 (baseline) to the last measurable concentration  
 $AUC_{0-\infty}$  = AUC from baseline extrapolated to infinity

\*Trademark: Valtrex® (Laboratorios Liomont, S.A. de C.V., Mexico City, Mexico)

†Trademark: Valtrex® (GlaxoSmithKline, Research Triangle Park, North Carolina)

‡Trademark: Rapivir® (GlaxoSmithKline Mexico, S.A. de C.V., Mexico City, Mexico)

**Table 1:** Pharmacokinetic parameters of acyclovir after a single-dose administration of valacyclovir (500 mg or 1000 mg) in healthy Mexican adult subjects. Values are mean (SD).

Parameter	Geometric Mean Ratio (%)	90% CI	Probability of Exceeding Limits of Acceptance		Power
			P<80%	P>125%	
Valacyclovir 1000 mg					
$C_{max}$	93.26	86.22-100.87	<0.01	<0.01	1.00
$AUC_{0-t}$	93.69	89.11-98.50	<0.01	<0.01	1.00
$AUC_{0-\infty}$	93.55	89.00-98.34	<0.01	<0.01	1.00
Valacyclovir 500 mg					
$C_{max}$	104.8	95.24-115.33	<0.01	<0.01	0.98
$AUC_{0-t}$	99.81	96.20-103.55	<0.01	<0.01	1.00
$AUC_{0-\infty}$	100.66	97.12-104.34	<0.01	<0.01	1.00

$AUC_{0-t}$  = AUC from time 0 (baseline) to the last measurable concentration  
 $AUC_{0-\infty}$  = AUC from baseline extrapolated to infinity

**Table 2:** Geometric mean ratios, 90% CIs, the probabilities of exceeding the limits of acceptance (80%-125%) and the power test results of the pharmacokinetic parameter determined for acyclovir after a single-dose administration of valacyclovir (500 mg or 1000 mg) in healthy Mexican adult subjects.

In both studies, on ANOVA of  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$ , no significant period or sequence effects were detected for any of the PK parameters in either study (data not provided).

Table 2 shows the bioequivalence statistics (using the log-transformed data of  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$ ): geometric mean ratios (test/reference); 90% CI; the probabilities of exceeding the limits of acceptance for bioequivalence; and the power values of the test.

In both studies, all 90% CIs of the geometric mean ratios of the 3 parameters fell within the predetermined range of 80% to 125%. All probabilities values were <0.05. All the power values were >0.8 (power >80%), suggesting that the sample size was sufficient for the purposes of these studies.

These results suggested that the bioequivalence criteria were met in both studies.

## Tolerability

No serious adverse events were reported during these studies.

For the valacyclovir 1000 mg study, thirteen of the 26 subjects reported a total of 18 AEs. The AEs, regarded as possibly related to the study drug, were: headache (7) reported by seven subjects (26.9%), 5 after the administration of reference formulation, 2 after the administration of test formulation, dizziness (1, 3.8%) which was reported after reference formulation; diarrhea (1, 3.8%) which was reported after reference formulation; and abdominal pain (1, 3.8%).

Other AEs, regarded as unrelated to the study drug, were: nausea (1) and dizziness (1), which occurred during the washout period; cutaneous rash (1); local post-venipuncture ecchymosis (2); papulous lesion (1), common cold (1) and pharyngitis (1).

For the valacyclovir 500 mg study, six of the 26 subjects reported a total of 10 AEs. The AEs, regarded as possibly related to the study drug, were: headache (4) reported by 3 subjects (11.5%), 2 after reference formulation and 2 after test formulation; diarrhea (1) after reference formulation; and dizziness (1) and nausea (1) reported by the same subject (3.8%), both after reference formulation. Other AEs, which were regarded as unrelated to the study drug were: nasal bleeding (1); pain

of legs (1) and pharyngitis (1). All of the AEs resolved spontaneously. Three AEs were regarded as moderate in severity (one headache, pain of legs and pharyngitis), all others were regarded as mild.

Although it appears that the number of AEs was larger for the highest dose of valacyclovir (1000 mg), this finding should be interpreted as inconclusive, because the sample size for both studies was too small for detecting changes on the incidence of AEs associated to increasing doses of this drug.

## Discussion

The results of the two studies suggest that each pair of reference-test formulations (valacyclovir 500 or 1000 mg) was not statistically different in terms of their PK parameters ( $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$ ). In addition,  $T_{max}$  and  $t_{1/2}$  values were also found not to have clinically important differences between them (based on means and standard deviations).

Considering that all 90% CIs of the ratios of the pharmacokinetic parameters ( $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-\infty}$ ) were found to be within the predetermined range of bioequivalence (80%-125%) and that the Schuirmann two 1-sided *t* test (i.e., probability of exceeding limits of acceptance) found all of the probability values to be  $<0.05$ , the results of both studies satisfied the accepted regulatory requirements to assume bioequivalence.

In addition, the estimated PK parameters ( $C_{max}$  and AUC) for the 2 valacyclovir dose-strengths are consistent with the reported lack of dose proportionality of the pharmacokinetics of acyclovir after the administration of valacyclovir [4,5]. However, because only 2-dose levels were considered in these studies and different sets of subjects participated in each study, dose proportionality in the Mexican population should be confirmed in subsequent studies.

In both studies, none of the reported AEs were considered serious.

## Limitations

As with any clinical trial, and in particular for most bioavailability studies, the current studies had some limitations that should be considered. First, this is an open label study, so it might not objectively address the effectiveness and safety profiles of the formulations tested. The data were obtained from healthy adult subjects, in accordance with regulatory requirements [7], within a specific age range, who were administered a single dose; the PK parameters might differ in target populations. For example, differences in absorption, distribution, metabolism and excretion of both the prodrug and the drug might exist in patients, with respect to healthy subjects. Thus, the results of these studies might not be generalized to this population.

In addition, these studies were conducted under fasting conditions because the bioavailability of acyclovir after the administration of valacyclovir has been reported not to be affected by the concomitant intake of food [4]. However, further studies would be useful to assess the food effect on the bioavailability of this drug on the target population.

Because of the limited data (small sample size, single dose, healthy subjects, age range, and fasting conditions) in the present studies, we are unable to predict the response of the drug at any time following alternative doses and/or administration intervals with the present data set. Further studies are needed to compare the test formulations with the reference formulations in Mexican patient groups. The results of

these studies might serve as reference for future controlled studies of the drug on Hispanic population.

## Conclusions

In these two studies in healthy, fasting, Mexican adult subjects, following a single dose, the test formulations of valacyclovir 500 mg and 1000 mg met the Mexican [7] regulatory requirements to assume bioequivalence, based on the rate and extent of absorption. These formulations were also well tolerated.

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## References

1. Jacobson MA (1993) Valacyclovir (BW256U87): the L-valyl ester of acyclovir. *J Med Virol*: 150-153.
2. Perry CM, Faulds D (1996) Valacyclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in herpesvirus infections. *Drugs* 52: 754-772.
3. Pradeep B, Nagamadhu M, Banji D, Shekhar K, Bindu Madhavi B, et al. (2010) Valacyclovir: development, treatment and pharmacokinetics. *Int J App Biol Pharma Technol* 1: 1076-1083.
4. GlaxoSmithKline Mexico, SA de CV (2011) Prescribing information [in Spanish]. Rapivir® (valacyclovir hydrochloride equivalent to 500 mg of valacyclovir) coated tablets. *Diccionario de Especialidades Farmacéuticas (PLM®)*, 57<sup>th</sup> ed. Mexico City, Mexico 2935-2938.
5. Weller S, Blum MR, Doucette M, Burnette T, Cederberg DM, et al. (1993) Pharmacokinetics of the acyclovir pro-drug valacyclovir after escalating single- and multiple-dose administration to normal volunteers. *Clin Pharmacol Ther* 54: 595-605.
6. Soul-Lawton J, Seaber E, On N, Wootton R, Rolan P, et al. (1995) Absolute bioavailability and metabolic disposition of valacyclovir, the L-valyl ester of acyclovir, following oral administration to humans. *Antimicrob Agents Chemother* 39: 2759-2764.
7. COFEPRIS, Federal Commission for the protection of Sanitary Risks (1999) Mexican Official Standard NOM 177-SSA1-1998, Test and procedures to prove that a medication is interchangeable [in Spanish], General Standard Directorate, Mexico City, Mexico.
8. CDER, Center for Drug Evaluation and Research (2001) Guidance for Industry. Bioanalytical method validation.
9. Chow SC, Liu JP (2000) Design and Analysis of Bioavailability and Bioequivalence Studies. 2nd ed. New York, NY: M Dekker.
10. Schuirmann DJ (1987) A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J Pharmacokinet Biopharm* 15: 657-680.