Farmacia Hospitalaria
ISSN: 1130-6343
farmhosp@grupoaulamedica.com
Sociedad Española de Farmacia Hospitalaria
España

Tarinas, A.; Tápanes, R. D.; González, D.; Ferrer, G.; Abreu, D.; Pérez, J.
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Farmacia Hospitalaria, vol. 31, núm. 3, 2007, pp. 165-168
Sociedad Española de Farmacia Hospitalaria
Madrid, España

1130-6343/2007/31/3/165
FARMACIA HOSPITALARIA

FARM HOSP
Vol. 31. N.° 3, pp. 165-168, 2007
ORIGINAL BREVE

Bioequivalence study of two nevirapine tablet formulations in human immunodeficiency virus-infected patients

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Resumen

Objetivo: En el siguiente estudio se muestra la determinación de bioequivalencia de dos formulaciones diferentes de tabletas de nevirapina (NVP) –tabletas de NVP de 200 mg, de los laboratorios Novatec, como la formulación de prueba (P) *vs.* las tabletas de Viramune® de 200 mg, de Boehringer Ingelheim, como la formulación de referencia (R)–.

Método: Una dosis única de 200 mg de cada formulación fue administrada a 11 pacientes-voluntarios infectados con el VIH y se determinó la bioequivalencia entre ambas formulaciones por comparación de las curvas de concentración-tiempo y de otros parámetros farmacocinéticos medidos en plasma de estos pacientes para ambos productos.

Resultados: Los parámetros farmacocinéticos obtenidos para cada formulación fueron área bajo la curva de concentración en el tiempo de estudio e infinita (AUC_{0-12} y $AUC_{0-\infty}$), concentración máxima (C_{max}) y tiempo para alcanzar la concentración máxima (T_{max}). Estos parámetros fueron determinados por cromatografía líquida de alta resolución (HPLC). No se observaron diferencias significativas en los parámetros para ambas formulaciones. En el intervalo de confianza de 90% la razón de las medias de $lnAUC_{0-12\ P/R}$ (0,92-1,10), $lnAUC_{0-\infty\ P/R}$ (0,86-1,17) y $lnC_{max\ P/R}$ (0,71-1,38) están dentro de los rangos establecidos de bioequivalencia (0,80-1,25 ó 0,70-1,43). Para T_{max}, la media de la formulación de prueba se encuentra dentro del rango 2,64 ± 0,53 h.

Conclusiones: Los resultados muestran que ambas formulaciones son bioequivalentes en cuanto a la magnitud y velocidad de la absorción.

Palabras clave: Bioequivalencia. Formulaciones de nevirapina. Parámetros farmacocinéticos.

Summary

Objective: The present study describes the determination of the bioequivalence of two different nevirapine tablet formulations (nevirapine tablets 200 mg, Novatec, as the test formulation *vs.* viramune tablets 200 mg, Boehringer Ingelheim, as the reference formulation).

Method: A single 200 mg oral dose of each preparation was administered to 11 human immunodeficiency virus (HIV)-infected patients volunteers and their bioequivalence was assessed by comparing the both plasma nevirapine concentrations-time curves and others pharmacokinetic parameters.

Results: The pharmacokinetic parameters obtained for each formulation were the area under the time-concentration curve from 0 to 12 h (AUC_{0-12}) and from 0 to infinity ($AUC_{0-\infty}$), maximum concentration (C_{max}), and the time at which it occurred (T_{max}). These parameters were determined by high-performance liquid chromatography (HPLC). No significant differences were observed in these parameters. The 90% confident interval for the ratio of means for the $lnAUC_{0-12\ T/R}$ (0.92-1.10), $lnAUC_{0-\infty\ T/R}$ (0.86-1.17) and $lnC_{max\ T/R}$ (0.71-1.38) are within the guideline range of bioequivalence (0.80 to 1.25 and 0.70 to 1.43). For T_{max} the mean of test formulation is in the range 2.64 ± 0.53 h.

Conclusions: The results show that the formulations were bioequivalent in the extent and in the rate of absorption.

Key words: Bioequivalence. Nevirapine formulations. Pharmacokinetic parameters.

Tarinas A, Tápanes RD, González D, Ferrer G, Abreu D, Pérez J. Bioequivalence study of two nevirapine tablet formulations in human immunodeficiency virus-infected patients. Farm Hosp 2007; 31: 165-168.

Recibido: 27-11-2006
Aceptado: 03-04-2007

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INTRODUCTION

Nevirapine is an HIV-1 specific non-nucleoside reverse transcriptase inhibitor classified as a diazepine-type reverse transcriptase inhibitor[1] and binds directly to

the viral reverse transcriptase to block polymerase activity by causing a disruption of the enzymes catalytic site[2]. It is indicated for use in combination with nucleoside analogues for the treatment in both adults and children. In a twice daily dosing regimen has been proven safe effective[3].

Nevirapine is rapidly absorbed after oral administration, showing an absolute bioavailability of 93% for the oral tablet. Absorption is not affected by food or coadministration of antacids or didanosine (buffered formulation). It has been shown to be 50 to 60% protein bound. CSF concentrations are 45% of plasma concentrations, which are approximatively equal to the free fraction concentration in plasma[4]. Renal excretion of unchanged drug is minimal[5]. Eighty percent of a dose is recovered from the urine, mainly as the glucuronide conjugates; approximately 10% is found in the feces[4].

METHOD

Because of the potential toxicity and mutagenicity of antiretroviral agents, pharmacokinetic and bioequivalence studies have been conducted in HIV subjects[6].

Twelve subjects, male (11) and female (1) antiretroviral treatment-*naïve* patients, aged 20 to 43 years (mean ± SD; 36.45 ± 4.72), body weight 46 to 95 kg (mean ± SD; 70.45 ± 14.71) participated in the study. All subjects gave their written informed consent and the clinical protocol was approved by the ethics and research committee for human research at "Pedro Kouri" Institute.

The subjects were free from significant cardiac, gastrointestinal, and bleeding disorders, as determined by the individual history, physical examination and standard clinical laboratory tests. At the beginning of the study it was 12 patients; one exclusion was made (male, 37 years) for his owner wish.

The subjects breakfastless were hospitalized two hours prior to the study. They received a single oral dose of 200 mg nevirapine as either the test or the reference formulation, in a randomized cross-over manner. Drug administration was supervised by the staff nurses of the Hospital at "Pedro Kouri" Institute. With the oral dose of nevirapine the subjects were given 120 ml tap water, but were not allowed by food, a standard lunch and dinner were availables.

Commercially available 200 mg nevirapine tablets were obtained from Boehringer Ingelheim (Viramune®, lot numbers 257548A and 403227), Pharmaceuticals Inc., Ridgefield, CT, USA and from Novatec (nevirapine, lot numbers 20030001, 12540001, and 4750002), Cuba.

All used chemicals were HPLC-grade, from Merck (Darmstadt, Germany). The Marianao Blood Bank, Havanna City, Cuba, supplied drug-free human plasma.

The EuroChrom 2000 chromatography manager software was used to control the HPLC system witch consists of a K-1001 HPLC pump, a K-2.600 UV variable detector, and the automatic injector "Basic Marathon", all from Knauer (Berlin, Germany).

The analytical column was a LiChrospher RP-18 (250 mm x 4 mm i.d./particle size 5 μm) protected by a LiChroCART® 4-4 RP-18 guard column, both from Merck (Darmstadt, Germany).

Blood samples for plasma drug quantification were taken from a suitable forearm vein before dosing and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 12 hours after dosing. On each occasion, 10 mL sample was collected in heparinized tube from and indwelling venous catheter. Blood samples were allowed to clot at room temperature and the plasma were then separated by centrifugation (3,000 rpm, 10 min) and stored at -25 °C until assayed. The study was made until 12 hours because it has been reported that[7,8].

Nevirapine plasma concentrations were quantified using a modified version of a validated, sensitive, high-pressure liquid chromatography assay[9].

Briefly, sample pre-treatment consisted of protein precipitation (500 μl plasma sample) with 250 μl of tricloroacetic acid at 20%, mixed on a vortex mixer for 1min and then centrifuge at 10,000 rpm for 5 minutes. Subsequently, nevirapine was separated from endogenous compounds by isocratic ion-pair, reversed-phase high-performance liquid chromatography; buffer phosphate (pH 5.5) and acetonitrile (80:20, v/v) with 0.2% of triethylamine as mobile phase at the flow-rate of 1.2 ml/min and at the wave lengths of 265 nm.

Calibration plots for the analytes in plasma were prepared by spiking drug-free plasma with standard stock solutions to yield concentrations range between 0.1-10 μg/ml (0.1, 0.5, 1.0, 2.5, 5.0, 7.5, and 10.0 μg/ml). Five injections of each concentration were performed. The intra- and inter-day accuracy and precision of the assay in plasma were determined by assaying of quality control (QC) samples in different runs for each compound within the same day or on three different days respectively. Prior to the inter-assay, samples were stored frozen for three weeks. Determinations were carried out using one aliquot each time. The limit of quantitation (LOQ) was determined as the concentration for which the percentual deviation from the nominal concentration were both less than 20%. The recovery from plasma was determined by comparing the peak area of analyte after extraction with the respective non-extracted standard solution at the same concentration. This comparison was donts for three different runs of each concentration.

Plasma nevirapine concentrations were plotted as a function of time and the following pharmacokinetic parameters were obtained for each formulation from the curves: area under the concentration-time curve (AUC_{0-12} and $AUC_{0-\infty}$; calculated by the trapezoidal method plus extrapolation to infinity from the curve relating plasma concentration-time), the maximum achieved concentration (C_{max}) and the time of its occurrence (t_{max}). The pharmacokinetic parameters were determined by using the PKCALC and WinNonlin Professional Edition, version 2.1 programs (non compartmental method). The comparison of the pharmacokinetic parameters (ANOVA) was carried out at 95%

confidence interval using the NCSS 2000 and PASS 2000 trial software (paired t-test, null hypothesis).

The software Equiv Test, version 2.0, from statistical solutions made the bioequivalence test at 90% confidence interval for the ratio of central values of natural log-transformed AUC_{0-12}, $AUC_{0-\infty}$, and C_{max}. Bioequivalence of the two formulations was stablished when formulation or treatment effect of AUC_{0-12}, $AUC_{0-\infty}$ and C_{max} should not be different at alpha level of 0.05 and the 90% confidence interval of the mean ratio of these parameters between the tested product and the reference product should fell within the 0.80-1.25 or 0.70-1.43 for log-transformed data[10]. The evaluation for T_{max} was calculated using a non-parametric statistical method and to be applied to untransformed data[11].

RESULTS

Nevirapine was well tolerated in the administered dose, and no adverse effects were reported.

The HPLC method used in this study is simple and provides appropriated sensitivity for the pharmacokinetic study of nevirapine. Under chromatographic conditions, the retention times for nevirapine ranged between 8.5 and 11.3 min. Calibration curve was contructed using ratios of the observed analyte peak area *versus* concentration of analyte. Linear regression analysis of the data gave slope, intercept and correlation coefficient data (Table I) which were then used to calculate analyte concentration in each sample. Calibration curve was linear ($r^2 \geq 0.9799$) in the range 0.1-10 μg/ml with a coefficient of variation (CV) of 0.81%, and a mean intercept of -0.1366 ± 0.0422.

Within-day and day-to-day precision and accuracy data for plasma analysis of nevirapine were evaluated over the range 0.1-10 μg/ml. The results are presented in table I. The inter- and intra-assay coefficients of variation of precision of the QC samples were always less than 20% for all evaluated concentrations. LOQ level (Table I) were included in the calibration curve as the lowest concentration level. The LOQ for nevirapine had a CV of 15.3%. The efficiency of extraction procedure was determined by comparing the slopes of seven plasma calibration curves to the calibration curves of the pure working standards injected directly the same day. A mean nevirapine absolute recovery of 89.3% was found.

If nevirapine is used in patients with AIDS, then plasma samples must be treated at 57 °C for 40 min before analysis to inactivate the HIV. The effect of temperature on nevirapine concentrations was investigated with plasma samples ranging from 0.1-10 μg/ml. Each sample was divided into two parts: the first was heated at 57 °C for 40 min, and the second was kept for the same time at ambient temperature. Both parts were then assayed on the described conditions. Not statistical differences were found.

All of the pharmacokinetic parameters calculated for the test formulation were close to those of the reference formulation and there were no statistically significant differences between the two products (Table II).

The ratio of the log-transformed data of both formulations was calculated for each subject (Table III). The statistical analysis for bioequivalence assessment is shown in table III. The 90% confidence interval for log-transformed data for C_{max} of test product compared to that of the reference product (0.71-1.38) which are within the acceptable limits of 0.70-1.43 as per the criteria for evaluation. The 90% confidence interval for ln AUC_{0-12} ranged from 0.92 to

Table I. Validation data

t_R (min)	*Nevirapine 8.5-11.3*
r (n = 7)	0,9899
CV (%)	0,45
Intercept	-0,1366
r^2 (n = 7)	0,9799
CV (%)	0,81
Inter-day (n = 3)	0.1-10.0 (μg/ml)
Precision (%)	2.2-15.3
Accuracy (%)	80.1-119.7
Intra-day (n = 5)	0.1-10.0 (μg/ml)
Precision (%)	0.8-15.1
Accuracy (%)	84.9-100.5
LOQ	0.1 μg/ml
CV (%)	15.3
Absolute recovery (%)	89.3

t_R: retention time; r: regression slope; CV: coefficient of variation; r^2: correlation coefficient; LOQ: lower limit of quantitation (< 20%).

Table II. Mean pharmacokinetic parameters of nevirapine obtained after administration of either test or reference formulation

Pharmacokinetic	*Test product*	*Reference product*
C_{max} (μg/ml)	1.91 ± 0.54	1.93 ± 0.52
T_{max} (h)	3.14 ± 1.72	2.64 ± 1.55
AUC_{0-12} (μg • h/ml)	15.46 ± 5.50	15.00 ± 4.56
$AUC_{0-\infty}$ (μg • h/ml)	93.28 ± 145.22	55.73 ± 26.42

C_{max}: maximum plasma concentration; T_{max}: time to C_{max}; AUC_{0-12}: area under the drug concentration-time curve over the 12 h dosage interval; $AUC_{0-\infty}$: area under the drug concentration-time curve over the infinity.

Table III. Bioequivalence study for test *vs.* reference formulations neviparine

Patient	*C_{max} T/R*	*LN C_{max} T/R*	*$T_{max\ (T-R)}$ (hours)*	*AUC_{0-12} T/R*	*LN AUC_{0-12} T/R*	*$AUC_{0-\infty}$ T/R*	*LN $AUC_{0-\infty}$ T/R*
X	1.01	0.00	0.50	1.03	0.02	2.17	0.02
SD	0.15	0.16	1.45	0.19	0.20	4.12	1.11
GM	1.00	–	–	1.02	–	1.02	–
IC90%	–	0.71-1.38	–	–	0.92-1.10	–	0.86-1.17
BC mm	–	0.70-1.43	–	–	0.80-1.25	–	0.80-1.25
BC am	–	–	0.53	–	–	–	–

X: mean; SD: standard deviation; GM: geometric mean; IC90%: confidence interval 90%; BC_{mm}: bioequivalence criterium for the multiplicative model (relative values); BC_{am}: bioequivalence criterium for the aditive model (absolute values, "hours").

1.10 and for $AUC_{0-\infty}$ ranged from 0.86 to 1.17. It should also be noted that these values obtained for AUC_{0-12} and $AUC_{0-\infty}$ are within the range of 0.80-1.25. For T_{max} the mean of test product (3.14) are within the bioequivalence limits for this parameter 2.11 to 3.17 h (mean T_{max} reference product ± 20% of mean T_{max} test product; 2.64 ± 0.53 h).

DISCUSSION

A sensitive, accurate, and precise method based on HPLC has been re-validated for determination of nevirapine concentration in human plasma. The method was re-validated to meet the requirements of the pharmacokinetic research. Although the retention time resulted between 8.5 and 11.3 min for nevirapine. To eliminate the potential interference from a late eluting plasma peak in subsequent injections, the assay run time was extended to 20 min.

Two recognized organizations (U.S. Food and Drug Administration and European Agency for the Evaluation of Medical Products) have proposed that bioequivalence can only be assumed when the parameters of bioavailability show no more than a defined difference[12,13]. These differences depend on the nature of the drug, the patient population, and the clinical end point.

Therefore two medicinal products are bioequivalent if they are pharmaceutically equivalent or pharmaceuticals alternatives and if their bioavailabilities (bioavailability studies using pharmacokinetic end points to assess bioequivalence) after administration in the same molar dose are similar to such degree that their effects, with respect to both efficacy and safety, will be essentially the same[11].

Pharmaceutical equivalents are bioequivalent when they are absorbed to the same extent and rate.

Area under the concentration curve (AUC) is accepted as a good indicator of extent of absorption, whereas C_{max} and t_{max} are considered estimators of the rate of absorption.

For the pharmacokinetic parameters the differences were considered statistically significant when the probability of accepting the null hypothesis (Ho) was bellow 0.05. Results are expressed as mean ± standard deviation. The null hypothesis (t-test) was performed with terms of status of test, and reference formulation. In our study the AUC_{0-12}, $AUC_{0-\infty}$, Cmax and the tmax for the trade and generic formulations did not differ significantly (Ho > 0.05).

No important adverse experiences or drug-related changes in laboratory parameters were noted for either of two formulations of nevirapine.

The 90% confidence interval for ln-transformed data (AUC_{0-12}, $AUC_{0-\infty}$, and C_{max}) and for untransformed data (t_{max}) of test products compared to that of the reference products are within the acceptable limits for each pharmacokinetic parameter. This suggest that the results of the present bioequivalence study prove that test and reference formulations of nevirapine are bioequivalent when the same doses are administered under the experimental conditions designed for this study.

The results show that both formulations of nevirapine were bioequivalent in the extent and in the rate of absorption. We showed a HPLC-UV method for the quantitative determination of nevirapine in plasma, very easy to reproduce. In our hospital the assay has been in use for pharmacokinetic study and patient care for more than 20 months.

ACKNOWLEDGMENTS

We would like to thank the patients, nurses (in especial Margarita Silveira, Rodilcia Castillo, Ileana Santisteban, and Cristina Pérez), and all of the staff at Tropical Medicine "Pedro Kouri" Hospital, for their collaboration.

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