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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 ($AUC_{0\text{-}12}$ y $AUC_{0\text{–}\infty}$), 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 $lnAUC_{0\text{-}12\ P/R}$ (0,92-1,10), $lnAUC_{0\text{–}\infty\ P/R}$ (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 ($AUC_{0\text{-}12}$) and from 0 to infinity ($AUC_{0\text{–}\infty}$), 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 $lnAUC_{0\text{-}12\ T/R}$ (0.92-1.10), $lnAUC_{0\text{–}\infty\ T/R}$ (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.

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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%