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Bioequivalence between Two Capsules of Pirfenidonein Healthy Subjects under Fed Condition

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Abstract

Pirfenidone capsules are indicated for the treatment of patients with Idiopathic pulmonary fibrosis. The Bioavailability of two formulations containing pirfenidone 267 mg hard gelatin capsules was compared in a bioequivalence study under fed conditions. The study was single dose, randomized, open label, two-period crossover, with Brazilian healthy subjects, males and nonpregnant females. Blood samples were taken for12 hours after drug administration and plasmatic concentrations were determined using a validated HPLC-MS/MS method. Confidence intervals (CI90%) for the peak plasma concentration (C_{max}) and area under the concentration-time curve (AUC_{0-t}) were determined by calculating LN-transformed data. The ratios and 90% CI for the geometric mean test/reference were 99,01% (91,05 - 107,67) for C_{max} and 106,37% (99,16- 114,09) for AUC_{0-t}. Under fed condition, the test (pirfenidone267 mghard gelatin capsules, Asofarma S.A.I y C.) and reference (Esbriet®267mghard gelatin capsules, Produtos RocheQuímicos e Farmacêuticos S.A.) formulations were considered bioequivalent since the 90% CIs for the geometric mean test/reference ratios were within the ANVISA and FDA predetermined range of 80% to 125%.

Keywords: Pirfenidone; Bioequivalence; Mass Spectrometry

Introduction

Pirfenidone[5-Metyl-1-phenyl-2(1H)-pyridone], which belongs to the chemical class of pyridine, is indicated for the treatment of idiopathic pulmonary fibrosis (IPF). IPF is a disease with a poor prognosis [1,2]. Lung fibrosis and irreversible honeycomb lungs characterize the disease. Pirfenidone, a compound with anti-fibrotic, anti-inflammatory, and antioxidant properties [3,4], is currently a drug approved for clinical use in the treatment of IPF [1,2].

The mechanism of action of pirfenidonein the treatment of IPF has not been established. However, pirfenidone is capable of interfering with TNF alpha in human astrocytes and may be useful in the treatment of multiple sclerosis [1,2]. Data also demonstrate that pirfenidone can inhibit the proliferation of fibroblasts in the lung corroborating its function in the treatment of obliterative bronchiolitis [3-5]. A recent study suggests that inhibition of collagen fibril formation acts as a major mechanism of action for pirfenidone and puts forward extracellular collagen self-assembly as a druggable target in IPF [6].

After single oral-dose administration of 801 mg of Pirfenidone(three 267 mg capsules), the maximum observed plasma concentration (C_{max}) was achieved between 30 minutes and 4 hours (median time of 0.5 hours). Food decreased the rate and extent of absorption. Median T_{max} increased from0.5 hours to 3 hours with food. Maximum plasma concentrations and AUC_{0-inf} decreased by approximately 49% and 16% with food, respectively. A reduced incidence of adverse reactions was observed in the fed group when compared to the fasted group [1]. Pirfenidone binds to human plasma proteins, primarily to serum albumin, in a concentration in dependent manner over the range of concentrations observed in clinical trials. The overall mean binding was 58% at concentrations observed in clinical studies (1 to 10 µg/mL). Mean apparent oral volume of distribution is approximately 59 to 71 liters [1]. In vitro profiling studies in hepatocytes and liver microsomes have shown that Pirfenidone is primarily metabolized in the liver by CYP1A2 and multiple other CYPs (CYP2C9, 2C19, 2D6, and 2E1). Oral administration results in the formation of four metabolites. In humans, only pirfenidone and 5-carboxy-pirfenidone are present in plasma in significant quantities. The mean metabolite-to-parent ratio ranged from approximately 0.6 to 0.7. The mean terminal half-life is approximately 3 hours in healthy subjects. Pirfenidone is excreted predominantly as metabolite 5-carboxy-pirfenidone, mainly in the urine (approximately 80% of the dose). The majority was excreted as the 5-carboxymetabolite (approximately 99.6% of that recovered) [1,7].

Objective

The objective of this Study was to compare, in healthy subjects of both genders under fed conditions, the pharmacokinetic profiles of pirfenidone, to assess the bioequivalence between two formulations: 1) pirfenidone267 mg hard gelatin capsules, manufacturing by Asofarma S.A.I y C., imported by Zodiac Produtos Farmacêuticos S.A. (test drug) and2) Esbriet® 267mg hard gelatin capsules, Produtos RocheQuímicos e Farmacêuticos S.A. (reference drug).

The Study was conducted in compliance with guidelines and standards for researches involving human beings from Resolutions no. 466/12 and 251/97 by the National Health Council - Ministry of Health, Good Clinical Practices according to ICH, and the Document of the Americas and in compliance with the Declaration of Helsinki (adopted by the 18th WMA General Assembly in Helsinki/ Finland, 1964, and with the last amendment by the 64th WMA General Assembly in Fortaleza/ Brazil, 2013). The protocol was submitted and approved before study initiation by the Ethics Committee of Instituto de Ciências Farmacêuticas (Goiânia – BR) accredited by National Research Ethics Commission (CONEP). After explaining the nature and purpose of the study, all subjects provided their written informed consent for participation.

Material and Methods

Population

Thirty-six (36) subjects of both genders, 18 female and 18 male, aged 18 to50 years, with body mass index (BMI) between 18,5 and 30 Kg/m², were screened. All subjects were considered as being eligible to participate in this study based on the inclusion and exclusion criteria defined in the protocol. The subjects showed good health conditions or the absence of significant diseases after assessment of medical history, verification of vital signs, physical examination, electrocardiogram, and routine laboratory tests. They showed negative tests for hepatitis B (HBsAg and Anti-HBc IgM), hepatitis C, HIV and urine HCG (pregnancy test only for female subjects). The subjects also underwent a breathalyzer, rapid drug abuse test and cotinine test.

Study Treatments

The test formulation was pirfenidone 267 mg hard gelatin capsules, batch number 22813, manufacturing by Asofarma S.A.I y C., imported by Zodiac Produtos Farmacêuticos S.A. and the reference was Esbriet® 267mg hard gelatin capsules, batch number N0047B01, Produtos RocheQuímicos e Farmacêuticos S.A. Before starting the clinical study, test and reference formulations were evaluated *in vitro* to check if they could be considered pharmaceutical equivalents. Tests described in the products specifications/pharmacopeia. Test and reference drugs presented similar performance *in vitro* and were considered pharmaceutical equivalents.

Study Design

The study was conducted using an orally single dose, open-label, randomized, two-period, crossover, balanced design, in fed conditions with a washout period of 14 days between administrations. In each of the study periods, the subjects received one hard gelatin capsule containing 267 mg of pirfenidone from one of the two formulations mentioned above, with a 200-mLwater at room temperature. Test or reference drugs were administered in the morning at approximately 7 am, after a minimum fasting period of 8 hours, interrupted with a standardized breakfast with 800 Kcal approximately, 30 minutes before drug administration. To maintain the standardization, the diet (food and drink) followed the same for all subjects in both periods.

The intake of alcoholic beverages, food or beverages containing caffeine or xanthine (such as coffee, tea, chocolate and cola- or guarana-based soft drinks) and smoke cigarettes was not permitted. In addition, the use of nicotine was prohibited from 48 hours before hospitalizations until the last blood draw, as well as any regular drugs (for at least 14 days) or occasional drugs (up to 7 days) before study initiation.

Blood samples (7.5 mL) were collected in coated tubes, containing EDTA as anticoagulant. The schedule of collects included the pre-dose and00:30; 00:45; 00:30; 01:00; 01:15; 1:30; 1:45;2:00; 2:20: 2:40; 3:00; 3:20; 3:40; 4:00; 5:00;6:00; 8:00 and 12:00 hours after drug administration in each period. A total of 19 blood samples were collected from each subject in each period.

Immediately after collection, blood samples were centrifuged at 1,646g (3,500 rpm) for 10 minutes at approximately 4 °C. After centrifugation, the plasma was separated and transferred to two previously labeled cryotubes. The tubes were stored in freezer at -20 °C and were maintained at this temperature until the analysis.

Clinical, analytical, and statistical stages of the study were conducted by Centro Avançado de Estudos e Pesquisas - CAEP, located in city of Campinas/SP– Brazil.

Quantification of pirfenidone in human plasma

Plasma concentrations of pirfenidone were determined using reversed-phase high-performance liquid chromatography with tandem mass spectrometry (RP-HPLC-MS/MS). The analyte was extracted from plasma using precipitation extraction. Pirfenidone-d5 was used as the internal standard. To avoid inter-assay variations, all the samples from the same subject, in both periods, were assessed in the same analytical run.

The detection parameter used was the mass-to-charge ratio (m/z) between precursor ions and product, and the quantification parameter was the ratio of areas under chromatogram peak identified in the retention time between analyte and internal standard. Pirfenidone concentrations in subject samples were calculated using interpolation in the calibration curve.

The analysis was conducted in an UPLC Acquity Waters coupled to the mass spectrometer Quattro Premier XE with column X Bridge C18 2,1 x 150 mm, 5 μm, with a flow rate of 0.4 mL/min. The column was maintained at a temperature of 30 °C, while the auto injector was maintained at 10 °C. The mobile phase used was gradient elution of ammonium acetate 5mM and Acetonitrile. The injection volume was 2μLand the total run time set as 3 minutes. The mass spectrometry detection was conducted using electrospray ionization source in positive mode. The multiple reaction monitoring (MRM) method was used, and the transitions monitored were m/z 186,13 >91,97 and m/z 191,19 >91,97 for pirfenidone and the internal standard, respectively.

The method was validated in compliance with ANVISA guidance for bioanalytical method validation, RDC Resolution no. 27, dated May 17, 2012 [8].The validation parameters assessed were selectivity, linearity, intra- and inter-run precision, intra- and inter-run accuracy, matrix effect, residual effect, and stability of pirfenidone under different conditions.

Pharmacokinetic and Statistical Analysis

The pharmacokinetic parameters were obtained from the pirfenidone plasma concentration-time curves. These parameters were statistically assessed for bioequivalence determination using software Phoenix WinNon Lin version 6.4 and Microsoft Excel. The area under the plasma concentration-time curve was calculated using the linear trapezoidal method, from time zero to the last measurable concentration (AUC_{0-t}). The area under the plasma concentration-time curve was also calculated from time zero to infinity ($AUC_{0-\infty}$), where $AUC_{0-\infty} = AUC_{0-t} + Ct/z$, with Ct being the last drug concentration experimentally defined and z being the terminal phase elimination constant rate. The peak of maximum plasma concentration (C_{max}) of pirfenidone and the time to reach this peak (t_{max}) were obtained directly with no data interpolation. The elimination half-life (t½) was defined using the equation t½ = ln (2) /z.

For the bioequivalence assessment between the formulations, AUC and C_{max} were used. The model included a fixed effects term for sequence, period and treatment (ANOVA). Subjects (nested in sequence) were treated as a random effect. A 90% Confidence Interval (CI) was generated for the difference in averages of LN-transformed data from test and reference drugs. The antilog of obtained CI comprised the 90% CI for geometric mean ratio of primary parameters. The drug products are considered as bioequivalent if the bounds of the 90% CI generated for the geometric mean ratio for both primary parameters are equal or higher than 80% and equal or lower than 125%, as established by ANVISA and FDA [9-11].

Results

The validation method used allowed for the selective determination of pirfenidone in a linear range within 0,025 μg/mL. to 8,000 μg/mL. The method developed was robust, selectivity, accurate and precise. There was no carryover effect observed during the auto sampler carryover experiment. Further, the extent of the matrix effect in different lots of plasma was within the acceptable limit. The stability of the pirfenidone and its internal standard in human plasma and stock solutions were evaluated at different storage conditions.Pirfenidoneand pirfenidone-d5 were found to be stable in all tested conditions, including in plasma samples stored below -20 °C and -80 °C for 153 days.

Twenty-nine (29) subjects, fifteen females and fourteen males, completed the two study periods. The dropouts were either by simple abandonment for personal reasons or using medication prohibited by the protocol due to adverse events. The subjects had a mean age of 29.25 years, ranging from 18 to 48 years; a mean weight of 69.3 kg ranging from 46.0 to 92.0 kg; mean height of 1.67 m ranging from 1.51 to 1.94m and a mean BMI of 24.81 kg/m² ranging from 18.66 to 29.94 kg/m².

Pirfenidone was well tolerated at the administered dose in the study. No serious adverse events were seen or reported, and no pregnancies were detected. The most common adverse event was mild headache, reported by 38,9% of the subjects. This event was possibly related to the drugs in the study [1,2].

The mean plasma concentration-time curves for test and reference drugs are shown in Figure 1. The curves were shown to be overlapped, showing a similar pharmacokinetic profile between the drugs. None of the subjects had concentration on the pre-dose collection time in the second period of study, showing adequate washout period.
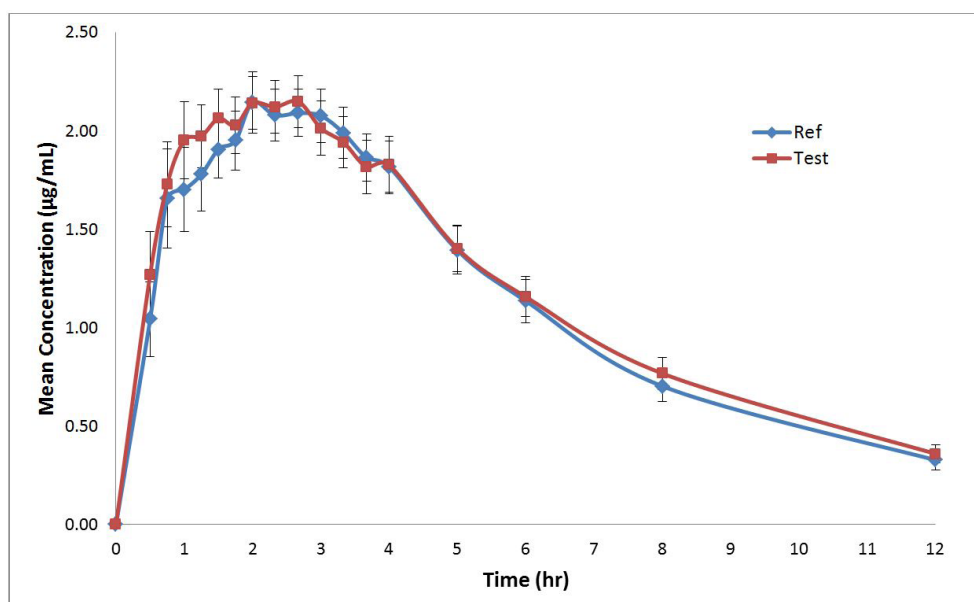
Figure 1: Mean curves of pirfenidone plasma concentration vs. time obtained for test and reference drugs under fed conditions

The measures of central location and dispersion for all pharmacokinetic parameters from both formulations are shown in Table 1.

Parameter	Test Drug	Reference Drug
C_{max} (µg/mL)	2.817 ± 0,146 (28.0)	2.828 ± 0.143 (27.3)
t_{max} (h)**	2.07 (0.5-6.0)	1.93 (0.5-5.0)
AUC_{0-t} (µg*h/mL)	14.259 ± 0,870 (32.9)	13.696 ± 0,920 (36.2)
$AUC_{0-\infty}$ (µg*h/mL)	16.418 ± 1,134 (36.5)	15.391 ± 1,197 (41.9)
t½ (h)	3.175 ± 0,158 (26.3)	2.986 ± 0,148 (26.8)

C_{max}: maximum plasma concentration; **t_{max}: time to reach the maximum plasma concentration (median and range); AUC_{0-t}: area under the plasma concentration vs. time curve from time 0 to t; $AUC_{0-\infty}$: area under the curve of plasma concentration vs. time from time 0 to infinity; t½: elimination half-life
Table 1: Pharmacokinetic parameters, [arithmetic mean ± SD (CV%)]of pirfenidone obtained after oral administration under fed conditions

No significant differences were observed in the parameters C_{max} and AUC after reference and test treatments. Thus, the treatments were considered bioequivalent under study conditions.

Table 2 show the test/reference geometric mean ratios for pharmacokinetic parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and the respective 90% CIs for the bioequivalence analysis. All 90% CIs were within the range of 80% to 125%.

Parameters	Geometric mean ratio(%)	Confidence interval (90%)	Intra-subject coefficient of variation (%)
$Ln(C_{max})$	99.01	91.05 – 107.67	18.81
$Ln(AUC_{0-12})$	106.37	99.16 – 114.09	15.69
$Ln(AUC_{0-\infty})$	110.48	104.53 – 116.76	12.16

Ln: natural logarithm; C_{max}: maximum plasma concentration; AUC_{0-t}: area under the plasma concentration vs. time curve from time 0 to t (12 hours); $AUC_{0-\infty}$: area under the curve of plasma concentration vs. time from time 0 to infinity.
Table 2: Test/reference geometric mean ratios for pharmacokinetic parameters C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ and the respective 90% CIs for the bioequivalence analysis

Discussion

Two drugs are considered bioequivalent if their rate and extent of absorption do not show statistically significant differences when administered at the same molar dose of the active ingredient, under the same experimental conditions [9-11]. In this paper, the relative bioavailability of two formulations of pirfenidone was assessed after single-dose administration under fed conditions.

With the purpose of obtaining a highly sensitive and rapid method for quantification of pirfenidonein plasma, a method by HPLC-MS/MS was developed and validated in this project. In the presented method the lower limit of quantification was 0.025 µg/mL, which allowed for an efficient and sensitive analysis of pirfenidoneplasma concentrations.

The pharmacokinetic results (C_{max}, AUC, t_{max} and t½) found in this study for pirfenidone (Table 1) were very similar to those reported on the literature [7,12,13] and similar between test and reference drug products. As shown in Table 2, 90% CIs obtained for pharmacokinetic parameters defining bioequivalence (C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$) of formulations of pirfenidone 267mg was shown to be within the bioequivalence limits defined by ANVISA (80%-125%) in RE Resolution no. 1170, dated April 19, 2006 [9].

Since the adverse events and laboratory test results were similar for both drugs in the evaluated condition (fed), it was also possible to verify that test and reference drugs have similar safety profile and are well tolerated by patients.

Conclusion

Based on the pharmacokinetic and statistical results obtained in this bioequivalence study conducted with administration of pirfenidone under fed conditions, we conclude that the test drug product (pirfenidone267, Asofarma S.A.I y C.) and the reference drug product (Esbriet® 267 mg, Produtos RocheQuímicos e Farmacêuticos S.A.) are bioequivalent. Thus, these drugs may be considered as being interchangeable in medical practice, i.e. having the same efficacy and safety profile.

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