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A randomized, open-label study assessing the bioequivalence of two formulations of Fingolimod 0.5 mg in healthy subjects

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Abstract

Fingolimod is an oral agent approved for the treatment of relapsing forms of multiple sclerosis (MS), which has demonstrated efficacy in Phase III trials in patients with relapsing-remitting MS (RRMS). The present study was designed to assess bioequivalence between a fingolimod Test capsule formulation (Teva Argentina, formerly IVAX Argentina S.A.) and a Reference capsule formulation (Novartis Pharma GmbH, Germany). In a single-center, randomized, single-dose, open-label, two-way crossover study under fed conditions, 16 healthy volunteers were randomized to receive a single oral dose of 0.5 mg of the Test and Reference formulations, with a 42-day washout period between administrations. The three pharmacokinetic (PK) parameters employed in the study to assess the bioequivalence between the Test and Reference formulations were maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), and area under the concentration–time curve from time zero to 72 h (AUC_{0-72}). No significant differences between the Test and Reference formulations for any of the three PK parameters were observed. Based on 90% geometric confidence intervals (CIs) within 80% to 125% for both AUC_{0-72} and C_{max}, the Test and Reference formulations were considered bioequivalent.

Keywords: Fingolimod, Bioequivalence, Pharmacokinetics, Multiple sclerosis

Background

Fingolimod is a sphingosine-1-phosphate receptor modulator initially approved in the US with an indication for RRMS to reduce the frequency and number of relapses and to delay the accumulation of physical disability (Gilenya Package Insert, Novartis Pharmaceuticals Corp., East Hanover, NJ). Administered orally, the recommended dose for fingolimod is 0.5 mg taken once daily with or without food (Gilenya Package Insert, Novartis Pharmaceuticals Corp., East Hanover, NJ). The efficacy of fingolimod was demonstrated in two pivotal Phase III clinical trials evaluating once-daily doses of fingolimod 0.5 mg and 1.25 mg in patients with RRMS, and a third Phase III trial that evaluated the same once-daily doses in patients with RRMS (Cohen et al. 2010; Kappos et al. 2010; Gilenya® [package insert] 2016; Calabresi et al. 2014).

The PK of fingolimod has been studied in patients with MS, renal transplant patients, and healthy volunteers (David et al. 2012). Fingolimod's PK profile has been well described, exhibiting a high degree of oral bioavailability (approximately 93%), with maximum plasma concentrations (C_{max}) usually reached 12–16 h after administration and a half-life of 6 to 9 days (David et al. 2012). Plasma concentrations achieve steady state after 1 to 2 months of once-daily dosing and are dose-proportional (David et al. 2012). Fingolimod's long half-life and its relatively slow rate of absorption confer a flat concentration profile after repeated dosing (David et al. 2012). The clinical pharmacology review of fingolimod by the Center for Drug Evaluation and Research of the Food and Drug Administration observed that intrasubject variability (ISCV) of the plasma drug concentration over time area-under-curve (AUC) is 16%, and that for the ISCV for C_{max} is 10% (FDA/CDER 2010).

The objective of this study was to assess the bioequivalence between a Test capsule formulation of fingolimod hydrochloride and a Reference capsule formulation of fingolimod hydrochloride (sold under the name Gilenya® [package insert], 2016) by determining the rate and extent of absorption of each compound after a single 0.5 mg dose in healthy volunteers under fed conditions.

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Methods

Study design

This was a single-center, randomized, single-dose, open-label, two-way crossover study. This study was carried out at inVentiv Health Clinique, Inc., Québec, Canada. The study consisted of two single-dose administrations of a Test fingolimod capsule (Teva Argentina, formerly IVAX Argentina S.A.) or Reference fingolimod capsule (Novartis Pharma GmbH, Germany). Each period was separated by a washout period of 42 days. Since bioequivalence studies involve a comparison of PK profiles, which are not subjective measurements, blinding was not deemed necessary for this study, and so the study was conducted in an open-label fashion.

All clinical work was conducted in compliance with Good Clinical Practices and Good Laboratory Practices as referenced in the International Conference for Harmonisation guidelines (ICH E6), local regulatory requirements, and the recommendations laid down in the Declaration of Helsinki (World Medical Association 2013). The clinical study protocol, all related associated documents, and informed consent forms were reviewed and approved by an independent ethics committee (Institutional Review Board Services [IRB Services], Aurora, Ontario, Canada), prior to beginning associated study procedures. All participants provided written informed consent prior to the start of any study procedure.

Study population

The study subjects were healthy male or non-childbearing female non-smokers aged 18 to 55 years, with a body mass index between 18.5 and 30.0 kg/m^2. Subjects enrolled in this study were members of the community at large. Subject screening procedures were performed within 28 days prior to first study drug administration and included informed consent, medical and medication histories, demographic data, body measurements, physical examinations, vital signs measurements, a 12-lead electrocardiogram (ECG), a urine drug screen, a serum pregnancy test (for female subjects), a urine cotinine test, and clinical laboratory measurements (biochemistry, hematology, serology, and urinalysis). In addition, a purified protein derivative skin test, or QuantiFERON® tuberculosis test was performed if no documented results were available within 2 months prior to dosing. All participating subjects were judged eligible for the study when assessed against the inclusion and exclusion criteria.

Study procedures

For each period, subjects were confined from at least 11 h before dosing until after the 36-h post-dose blood draw, and returned for all subsequent blood draws. In each period, according to the randomization scheme, subjects were administered a single 0.5 mg oral dose of either the Test or Reference products. This dose was considered sufficient to provide measurable levels of the study medication while allowing for adequate characterization of the concentration–time profiles of fingolimod. Subjects were supervised while undergoing an overnight fast of at least 10 h, followed by a high-fat, high-calorie breakfast 30 min before receiving the Test or Reference 0.5 mg capsule dose. Twenty-one K_2EDTA (dipotassium ethylenediaminetetraacetic acid) blood samples were taken for each dosing period: prior to drug administration and at hours 1, 2, 4, 6, 8, 10, 11, 12, 13, 14, 15, 16, 18, 20, 24, 28, 32, 36, 48, and 72, resulting in a total of 42 samples per subject. Pre-dose concentrations were measured to allow for exclusion of subjects who might be non-compliant with the study protocol or whose pre-dose fingolimod concentration was greater than 5% of the C_{max} value for that study period.

A urine pregnancy test was performed for all female subjects prior to drug administration in each period and at study exit. Clinical laboratory tests (biochemistry, hematology, and urinalysis) were performed for each subject at the time of the screening and study exit procedures. In addition, hematology and biochemistry tests were performed at check-in of each period, and approximately 14 days after the last dosing. ECG measurements and vital signs measurements were performed at the time of screening and study exit procedures. In addition, supine ECG measurements were performed at the time of the screening, prior to dosing, and at approximately 6 and 24 h post-dose. Seated blood pressure and heart rate measurements were also performed prior to dosing and at approximately 1, 2, 3, 4, 5, 6, 8, 10, 12, 14 and 24 h post-dose, in each period. Throughout the study, subjects were monitored for adverse events (AEs).

Bioanalytical methodology

Each 3-mL venous blood sample was in a K_2EDTA-containing vacuum tube. Sample tubes were inverted several times to mix the tube contents, and aliquots were frozen at − 80 °C. Whole-blood levels of fingolimod were determined by means of a validated high-performance liquid chromatography/ tandem mass spectrometric (LC–MS/MS) method. The analyte fingolimod and its internal standard, findgolimod-d_4, were extracted using an automated liquid-liquid extraction, and the extracted samples were loaded onto an ACE 3 C18 column (50 mm × 4.6 mm, 3 μm, Life Science, Peterborough, Canada) for separation. The high-performance liquid chromatography (HPLC) effluent was introduced into a Sciex API-5000 Tandem Mass Spectrometer equipped with Electrospray. The analytical range of 0.01–1 ng/mL had a within-run coefficient of variation (CV) ranging between 3.00% and 11.60%. Assay performance was judged based on three

quality control concentrations: 0.03, 0.5, and 0.75 ng/mL. Assay accuracy ranged from 99.98% to 113.45%, and precision CVs were from −0.2% to 13.45%.

Pharmacokinetic and statistical analyses

The following pharmacokinetic parameters were calculated by standard non-compartmental methods for fingolimod: C_{max}, time to C_{max} (T_{max}), and AUC from time zero to the time of the last measured concentration, which was hour 72 (AUC_{0-72}). An AUC truncated at 72 h was selected as an endpoint in light of fingolimod's relatively long terminal half-life and the common understanding that absorption of an immediate-release product is completed within 72 h. The use of AUC truncated at 72 h is also consistent with the European Medicines Agency (EMA) guidance for the conduct of bioequivalence studies (EMA 2010).

The PK population included all subjects completing the study for whom a PK profile could be adequately characterized. Using general linear model procedures in SAS®, analysis of variance (ANOVA) was performed on ln-transformed AUC_{0-72} and $C_{max.}$ Factors incorporated in the model included sequence, period, and treatment as fixed effects, and subject (sequence) as a random effect. Wilcoxon's signed-rank test was used to compare T_{max} between treatments, and ratios of least-squares means and 90% geometric CIs were calculated for ln-transformed AUC_{0-72} and C_{max}. Inter- and intra-subject CVs were also calculated.

The safety population included all subjects who had received at least one dose of the study medication. AEs were described using the Medical Dictionary for Regulatory Activities (MedDRA); treatment-emergent AEs (TEAEs) were summarized for the safety population. Each subject could contribute only once to each of the incidence rates, regardless of the number of occurrences. No statistical analysis of safety data was performed. Safety-related events were evaluated descriptively.

Results

Pharmacokinetic outcomes

Forty-two subjects were screened, and 16 (14 males, 2 females) were randomized and received treatment. Two subjects withdrew from the study after receiving just one of the study drugs, which made them ineligible for inclusion in the PK analysis, while both were included in the safety analysis. One subject withdrew voluntarily for personal reasons, and the other was withdrawn due to blood pressure exclusion criteria and abnormal biochemistry values. Baseline characteristics of the study subjects can be seen in Table 1. The PK population consisted of 12 subjects after two additional subjects were excluded: one due to pre-dose concentrations greater than 5% of C_{max} in both treatment periods, and one due to a pre-dose concentration greater than 5% of C_{max} during the first treatment period (both subjects had participated in an earlier fingolimod study which caused pre-dose values in period 1).

Table 1 Baseline Characteristics

Variable	PK Population ($n = 12$)
Age [years] (range)	41 (21–54)
BMI [kg/m^2] (range)	25.3 (20.1–29.0)
Height [cm] (range)	175 (160–187)
Weight [kg] (range)	77.5 (58.0–99.4)

BMI body mass index, *PK* pharmacokinetic

Mean concentration–time profiles for Test and Reference formulations can be seen both on a linear scale (Fig. 1a) and on a logarithmic scale (Fig. 1b). The two compounds show nearly identical concentration–time profiles on both scales. PK parameters for Test and Reference fingolimod are shown in Table 2.

There were no significant differences between the Test and Reference fingolimod formulations for ln-transformed AUC_{0-72} or C_{max} based on ANOVA, nor were any significant differences observed between treatments for T_{max} using Wilcoxon's test. As shown in Table 3, the 90% geometric CIs of the least-squares means (LSM) ratios were within 80.00% and 125.00%, indicating that the Test formulation was bioequivalent to the Reference product (FDA/CDER 2003). As also shown in Table 3, the ISCV for AUC_{0-72} and C_{max} was very low, with values of 4.53% and 5.56%, respectively.

Safety outcomes

Five of the 16 study subjects in the safety population reported a total of 14 TEAEs, with three TEAEs being reported by 2 of 15 subjects (13.3%) receiving the Test formulation, and 11 TEAEs being reported by 4 of 15 subjects receiving the Reference formulation. Headache was the most commonly reported TEAE. Eleven of the 14 TEAEs were graded as mild in severity, while three were graded as moderate. Investigators judged 9 of the 14 TEAEs as being possibly related to the study treatment and the other five as not being related to the study treatment. Three of the nine possibly treatment-related TEAEs occurred in subjects receiving the Test product, while six occurred in subjects receiving the Reference

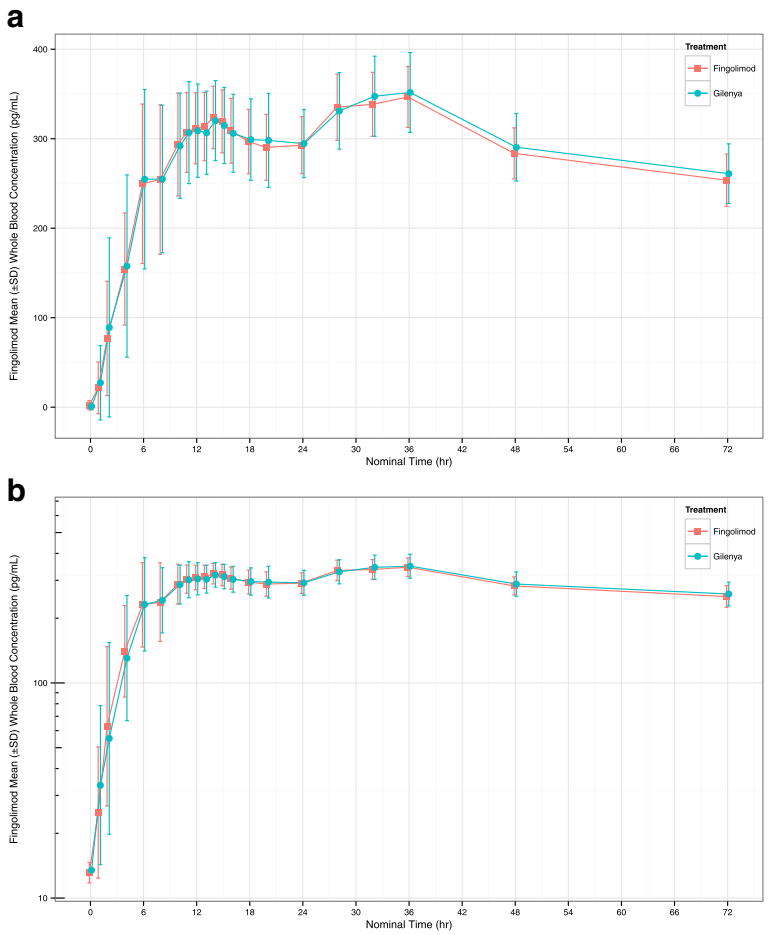

Fig. 1 Concentration–Time Profiles for Test and Reference Fingolimod on Linear (**a**) and Log (**b**) Scales

product. No deaths, serious AEs, or significant AEs were reported during the study.

Discussion

Two primary PK parameters were used to assess the bioequivalence of the Test versus the Reference formulations of fingolimod, and no significant differences were found between the two products in terms of C_{max} or AUC_{0-72}. Both the US Food and Drug Administration (FDA) and EMA have provided definitions of bioequivalence in comparing two products containing the same active compounds, and have also provided guidance regarding the parameters that should be employed in measuring bioequivalence. The FDA describes bioequivalence as the absence of a significant difference in the rate and extent to which the Test and Reference formulations, "become available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study" (FDA/CDER 2003). The EMA considers two comparator agents as bioequivalent, "if they are pharmaceutically equivalent or pharmaceutical alternatives and their bioavailabilities (rate and extent) after administration in the same molar dose

Table 2 AUC_{0-72} (±SD), C_{max} (±SD), and T_{max} values for Test and Reference Fingolimod

	Mean AUC_{0-72} [pg.hr/mL] ± SD (CV%)	Mean C_{max} [pg/mL] ± SD (CV%)	Median T_{max} [hr] (min–max)
Test (n = 12)	20,251 ± 1895 (9.36)	353 ± 33 (9.41)	34.0 (6.0–36.0)
Reference (*n* = 12)	20,487 ± 2552 (12.46)	360 ± 52 (14.50)	36.0 (6.0–36.1)

AUC_{0-72} area under the concentration–time curve from time 0 to hour 72, *C_{max}* maximum concentration, *CV* coefficient of variation, *SD* standard deviation, *T_{max}* time to C_{max}

Table 3 Ratios and 90% Geometric Confidence Intervals

Parameter	Treatment Comparisons	Ratio[a]	90% Geometric CI[b]		Intra-subject CV	Inter-subject CV
			Lower	Upper		
AUC_{0-72}	Test vs Reference	99.15%	95.88%	102.52%	4.53%	9.96%
C_{max}	Test vs Reference	98.58%	94.61%	102.72%	5.56%	11.35%

AUC_{0-72} area under the concentration–time curve from time 0 to hour 72, *C_{max}* maximum concentration, *CV* coefficient of variation
[a]Calculated using least-squares means according to the formula: $e^{(difference)} \times 100$
[b]90% geometric confidence interval (CI) using ln-transformed data

lie within acceptable predefined limits" (EMA 2010). Both the FDA and EMA recommend the use of a crossover study design (FDA/CDER 2003; EMA 2010), with the EMA specifying a randomized, two-period, two-sequence, single-dose design. The defined parameters for evaluation of bioequivalence are similar for both agencies. The EMA specifies the parameters as being AUC, C_{max}, and T_{max} (EMA 2010). The FDA also identifies AUC, C_{max}, and T_{max} as the relevant parameters, and further states that, for long half-life drugs that demonstrate low intra-subject variability in distribution and clearance, an "AUC truncated at 72 hours (AUC_{0-72}) can be used in place of AUC_{0-t} or $AUC0_{0-\infty}$" (FDA/CDER 2003). The present study has observed all of these criteria and used the recommended study design. In employing a 42-day washout period, the study has also observed the FDA recommendation that washout periods be at least 5 times the compounds' half-lives (FDA/CDER 2003; David et al. 2012). Although the number of subjects included in the study was low, the study nevertheless had > 99% power based on an observed ISCV of 6% even after several dropouts left only 12 subjects remaining.

Drug administration in the present study was conducted with subjects in fed state. The clinical pharmacology review of fingolimod by the Center for Drug Evaluation and Research of the Food and Drug Administration reports the results of a study (Study A2107) comparing fingolimod in fed and fasted healthy subjects. The results of that study demonstrated no significant differences in $AUC_{0-\infty}$, AUC_{0-t}, or C_{max} between fed and fasted subjects (FDA/CDER 2010). As previously noted, the same review also reported ISCV in subjects receiving fingolimod as 16% for AUC and 10% for C_{max} (FDA/CDER 2010). In the present study, ISCV values were notably lower than anticipated: 4.53% for AUC_{0-72} and 5.56% for C_{max}.

Conclusions

The results of this comparison of Test and Reference capsule formulations of fingolimod, following administration of single 0.5 mg doses under fed conditions, show that the two compounds may be regarded as bioequivalent based on 90% geometric CIs within 80% to 125% for both AUC and C_{max}. The intra-subject variability was low for both AUC and C_{max}, ranging from 4.5% to 5.6%. Both Test and Reference products were well tolerated, with no serious AEs and no relevant differences in safety profiles.

Abbreviations

AE: Adverse event; ANOVA: Analysis of variance; AUC: Area under the curve; CI: Confidence interval; C_{max}: Maximum plasma concentration; CV: Coefficient of variation; ECG: Electrocardiogram; EMA: European medicines agency; FDA: US Food and Drug Administration; HPLC: High-performance liquid chromatography; ICH E6: International conference for harmonisation guidelines; IRB: Institutional review board; ISCV: Intrasubject variability; K_2EDTA: Dipotassium ethylenediaminetetraacetic acid; LC-MS/MS: Liquid chromatography–tandem mass spectrometry; LSM: Least-squares mean; MedDRA: Medical dictionary for regulatory activities; MS: Multiple sclerosis; PK: Pharmacokinetics; RRMS: Relapsing-remitting multiple sclerosis; SD: Standard deviation; TEAE: Treatment-emergent adverse event; T_{max}: Time to maximum plasma concentration

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Availability of data and materials

Data on file, ratiopharm GmbH (Ulm, Germany).

Authors' contributions

All authors contributed to the conception, design, planning of the study, interpretation of the results, and critical review and revision of the manuscript for important intellectual content. All authors approved the final version for publication.

Competing interests

Dr. Tanguay reports that his employer, Syneos Health, was the contract research organization (CRO) contracted to conduct the present study.
Dr. Fröhlich reports that his employer, ratiopharm GmbH, part of Teva Pharmaceutical Industries Ltd., funded the present study.
Mr. Drouin reports that his employer, Syneos Health, was the contract research organization (CRO) contracted to conduct the present study.
Dr. Beuerle reports that his employer, ratiopharm GmbH, part of Teva Pharmaceutical Industries Ltd., funded the present study, and that he holds shares and share options in Teva Pharmaceutical Industries Ltd.
No other competing interests are reported.

Publisher's Note

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