

Determination of Seminal Concentration of Fingolimod and Fingolimod-Phosphate in Multiple Sclerosis Patients Receiving Chronic Treatment With Fingolimod

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Abstract

The safety profile of fingolimod 0.5 mg, approved therapy for relapsing multiple sclerosis, is well established in clinical and real-world studies. As fingolimod is teratogenic in rats, it was considered important to assess the concentrations of fingolimod and its active metabolite, fingolimod-phosphate, in the semen of male patients on treatment and the risk of harming a fetus in a pregnant partner. In this multicenter open-label study, 13 male patients receiving fingolimod for at least 6 months provided 1 semen and 1 blood sample for analyte concentration measurements. The steady-state seminal concentrations of fingolimod and fingolimod-phosphate were close to those simultaneously observed in blood. The amount of fingolimod-related material in 10 mL of ejaculate was estimated to be 47.5 ng. The estimated fingolimod and fingolimod-phosphate blood C_{max} values in a woman having regular sexual intercourse with a male patient treated with fingolimod 0.5 mg were approximately 400 and 2400 times smaller than the estimated values in the embryo-fetal development study in rats at the no-observed-adverse-event level. Consequently, the risk of harming a fetus in a pregnant woman is considered extremely unlikely.

Keywords

fingolimod, fingolimod-phosphate, semen, blood

Introduction

It is well known that drugs can be transported to semen, and the presence of chemicals and drugs in semen has been documented in many studies.^{1,2} The concentration of drugs in semen is governed by their physicochemical properties that regulate its distribution into the physiological compartment of interest, such as protein binding, lipid solubility, and ionization constant.² Few studies have studied the concentration of chemicals in the semen and blood of the same individuals, and results suggested that for most compounds seminal concentrations are lower than blood/serum concentrations.^{1,2}

Fingolimod 0.5 mg once daily (Gilenya, Novartis Pharma AG, Basel, Switzerland) is approved as an oral therapy for the treatment of relapsing forms of multiple sclerosis (MS). Fingolimod is a sphingosine 1-phosphate (S1P) receptor modulator and exerts its therapeutic effects by modulating the S1P receptors expressed on peripheral lymphocytes.^{3,4} The efficacy

and safety profile of fingolimod has been well demonstrated in clinical trial programs and in real-world settings.^{5–10}

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Fingolimod is a structural analogue of sphingosine and is phosphorylated to form its active metabolite, fingolimod-phosphate. Pharmacokinetic (PK) profiling studies have shown that both fingolimod and fingolimod-phosphate have a half-life of 6–9 days. Steady-state blood concentrations were reached within 1–2 months following once-daily oral administration and were 10-fold higher compared with the initial dose.^{11–13}

In animal studies, fingolimod was found to be teratogenic in rats at doses of 0.1 mg/kg or higher. Fingolimod had no effect on sperm count/motility or on fertility in male or female rats up to the highest tested dose (10 mg/kg).¹² In whole-body autoradiography studies, fingolimod-related radioactivity was observed in rat seminal glands, indicating transfer of fingolimod-related radioactivity into the seminal fluid. Therefore, it is conceivable for a male MS patient treated with fingolimod to transfer fingolimod via the semen to a pregnant partner during sexual intercourse. Hence, it was considered important to assess fingolimod and fingolimod-phosphate concentrations in semen samples of male patients with relapsing-remitting MS (RRMS). The present study aimed to measure the seminal concentration of fingolimod and fingolimod-phosphate in RRMS patients on chronic treatment with fingolimod 0.5 mg. The secondary objective of the study was to measure the concentration of fingolimod and fingolimod-phosphate in blood samples in the same patients.

Methods

Study Design

This was a multicenter open-label exploratory sub-study comprising a single visit and included patients from TRANSFORMS extension (NCT00340834) or the LONGTERMS study (NCT01281657). LONGTERMS is an open-label, single-arm extension study of phase 2, 3, and 3b core and extension studies, evaluating the long-term safety and tolerability of fingolimod. The protocol was reviewed and approved by the Independent Ethics Committee of Technische Universität Dresden. Three centers (Praxis, Ulm; Neurologisches Facharztzentrum, Berlin; Technische Universität, Dresden) from Germany were involved in the study. The study was conducted according to the principles of the Declaration of Helsinki. All patients provided written informed consent.

Only patients who were on fingolimod treatment for at least 6 months were enrolled to ensure adequate time for the semen compartment to have reached a kinetic steady state after initiation of fingolimod treatment. During the study visit, patients provided 1 semen sample and 1 blood sample for fingolimod and fingolimod-phosphate determination.

Subjects

Male patients with RRMS who were participating in the TRANSFORMS extension study or LONGTERMS study were included. Accordingly, the eligibility criteria were treatment with fingolimod 0.5 mg for at least 6 months and the ability to produce a semen sample. The detailed inclusion and exclusion criteria of TRANSFORMS have been published previously.⁵

Sample Collection

On the day of the study visit, blood samples were collected before administration of the study drug to assess patient exposure to both analytes. Patients were instructed to refrain from taking their regular doses of medication until after the visit was completed. Semen samples were collected at any time during the visit.

Analytical Methods

Concentrations of fingolimod and fingolimod-phosphate in human semen and in blood samples were measured using a validated high-performance liquid chromatography method with tandem mass spectrometry (API 5000, Applied Biosystems/MDS SCIEX) with a C18 column (Gemini C18 5 μ m [2.0 mm I.D. \times 50 mm] from Phenomenex) using gradient separation by aqueous 2 mM ammonium acetate with 0.1% formic acid and 0.01% 1,2-dimethylhydrazine and 95% acetonitrile with 2 mM ammonium acetate and 0.1% formic acid. CHAPS and sodium vanadate were added to semen samples to make the final concentration 10 and 2 mg/mL, respectively. Because semen was a rare matrix, plasma with 10 mg/mL CHAPS and 2 mg/mL sodium vanadate was used as a substitute matrix for semen sample analysis. The analytes as well as their internal standards (D4-fingolimod and D4-fingolimod-phosphate) were extracted by protein precipitation with methanol followed by solid-phase extraction plates. The extracted supernatant was evaporated by nitrogen stream and reconstituted with 30% acetonitrile containing ammonium acetate buffer with 0.1% formic acid. In both semen and blood, the lower limit of quantification was 0.080 and 0.1 ng/mL for fingolimod and fingolimod-phosphate, respectively. The mass spectrometric transitions monitored were: fingolimod, precursor ion m/z 308.50, product ion m/z 255.50; D4-fingolimod, 312.50, 259.50; fingolimod-phosphate, 386.27, 78.89; D4-fingolimod-phosphate, 390.43, 78.89, respectively. The method was specific for fingolimod and fingolimod-phosphate, and there was no interference observed. The method validation with plasma for calibrators and with semen for quality checks (QCs; 0.08, 0.24, 2.4, and 12 ng/mL for fingolimod and 0.1, 0.3, 3, and 15 ng/mL for fingolimod-phosphate) showed good agreement with

the matrix. For fingolimod the accuracy (% bias) of mean values was from -3.8% to -1.7% , and precision (% coefficient of variation [CV]) was from 3.3% to 6.6% . For fingolimod-phosphate, the accuracy (% bias) of mean values was from -4.3% to 0.7% , and precision (% CV) was from 1.8% to 10.2% .

The assay performance during the study was also evaluated with QC samples, 0.24, 2.4, and 12 ng/mL for fingolimod. The accuracy (% bias) of means ranged from 0.4% to 6.3% in blood and -1.3% to 0.0% in semen, and precision (% CV) ranged from 1.6% to 2.5% in blood and 2.1% to 6.0% in semen. The assay performance for fingolimod-phosphate was evaluated with QC samples, 0.3, 3, and 15 ng/mL. The accuracy (% bias) of means ranged from -2.3% to 5.0% in blood and -3.7% to -1.3% in semen, and precision (% CV) ranged from 1.6% to 3.1% in blood and 1.6% to 4.5% in semen. All samples were analyzed within the known stability periods of fingolimod and fingolimod-phosphate in semen and in blood.

Statistical Analysis

All patients who received the study drug were included in the PK analysis set. Descriptive statistics, including arithmetic mean, standard deviation (SD), geometric mean (geo-mean), CV for arithmetic mean and geo-mean, median, and minimum and maximum, were used to report the concentrations in semen and blood and the ratio of concentrations in semen over that in blood.

Results

A total of 13 patients who had received fingolimod for at least 6 months were enrolled in this substudy and included in the PK analysis data set. Mean \pm SD age was 40.5 ± 6.51 years.

Pharmacokinetic Data

Semen and blood samples were collected between 11.3 and 29.3 hours and between 10.1 and 28.5 hours after dosing, respectively. Fingolimod and fingolimod-phosphate concentrations were quantifiable in blood and semen samples from all patients. For both analytes, the variability, as measured by the coefficient of variation of the geometric mean, was moderate to high in blood, semen, and ratios (31% – 45%). Fingolimod concentrations in semen were higher than those in blood (geometric mean ratio, 1.47), whereas fingolimod-phosphate concentrations in semen and blood were in the same range (geometric mean ratio, 0.89). Safety evaluations were performed but not reported because this study was an exploratory substudy contained within the TRANSFORMS extension study or LONGTERMS study. The summary of concentrations in semen and blood, as well as the ratio

of semen concentrations over blood concentrations for fingolimod and fingolimod-phosphate, is presented in Table 1.

Discussion

This study provided a more precise assessment of the risk for a male patient with MS receiving fingolimod treatment to transfer fingolimod and/or fingolimod-phosphate to a pregnant partner via semen during sexual intercourse.

The blood concentrations of fingolimod and fingolimod-phosphate observed in these patients were comparable to those observed at the steady state in patients treated with once-daily fingolimod 0.5 mg for 12–24 months in pivotal studies. In the phase 3 FREEDOMS study, concentrations of fingolimod and fingolimod-phosphate in terms of geo-mean (CV%) were 2.51 ng/mL (56%) and 1.35 ng/mL (53%), respectively, and in the TRANSFORMS¹⁴ study in terms of mean \pm SD were 2.31 ± 1.35 and 1.28 ± 0.706 ng/mL, respectively. In this exploratory study, only patients who were on fingolimod treatment for more than 6 months were included, as the semen compartment may take several months to reach kinetic steady state after initiation of treatment. The semen concentrations of fingolimod and fingolimod-phosphate were at quantifiable levels in all patients. They were low and close to those observed in blood. The ratio of semen concentrations over blood concentrations was greater for fingolimod than for fingolimod-phosphate. This may be because of a difference in lipophilicity between the 2 analytes and greater diffusion of fingolimod into tissue.

The amount of fingolimod-related material (ie, fingolimod and fingolimod-phosphate) present in 10 mL of ejaculate was estimated to be 47.5 ng, which is 10 000 times smaller than the approved oral dose of 0.5 mg. During pregnancy, there is no access to the uterus from the vagina, and the dense mucus at the cervical canal is impervious to sperm and microorganisms and likely to drugs. Therefore, local toxicity to the embryo from direct delivery of seminal fingolimod or fingolimod-phosphate into the uterine cavity is considered extremely unlikely. Moreover, embryonic exposure through absorption of the drug into the vaginal vein followed by distribution into the uterine artery and eventually diffusion into the placenta is also unlikely. As fingolimod would have to pass multiple biological barriers, diffusion would likely occur in blood. Thus, a risk of high embryonic or fetal exposure to fingolimod from semen via vaginal absorption and distribution through female systemic circulation is considered implausible.

In an embryo-fetal development study in rats, the no-observed-adverse-event level (NOAEL) dose was

Table 1. Summary Statistics of Semen and Blood Concentrations and Ratio of Semen Concentrations Over Blood Concentrations for Fingolimod and Fingolimod-Phosphate

Compound	Statistic	Semen Concentration (ng/mL)	Blood Concentration (ng/mL)	Ratio ^a
	n	13	13	13
Fingolimod	Mean (SD)	4.23 (1.81)	2.80 (0.964)	1.59 (0.598)
	CV% mean	42.9	34.4	37.7
	Geo-mean	3.91	2.65	1.47
	CV% geo-mean	43.0	36.2	44.5
	Median (Min; Max)	3.69 (2.02; 8.07)	2.59 (1.35; 4.77)	1.59 (0.600; 2.67)
Fingolimod-phosphate	Mean (SD)	1.10 (0.331)	1.25 (0.372)	0.925 (0.253)
	CV% mean	30.0	29.9	27.3
	Geo-mean	1.06	1.19	0.889
	CV% geo-mean	30.5	34.4	31.1
	Median (Min; Max)	0.997 (0.692; 1.61)	1.23 (0.543; 1.77)	0.954 (0.513; 1.27)

CV%, coefficient of variation (%) = SD/mean × 100.

CV% geo-mean = (sqrt [exp {variance for log transformed data} - 1]) × 100.

^aSummary statistics on ratio was calculated using the individual ratio of semen concentrations/blood concentrations.

found to be 0.03 mg/kg/day, and the estimated fingolimod and fingolimod-phosphate mean C_{max} values were 1.63 and 4.82 ng/mL, respectively. The estimated fingolimod and fingolimod-phosphate blood C_{max} values in a woman having regular sexual intercourse (once daily) with a male MS patient treated with fingolimod 0.5 mg, assuming the same bioavailability as the oral route (93%), would be 0.343 and 0.169 pg/mL, respectively, which is approximately 400 and 2400 times smaller than the estimated values in rats at the NOAEL (data on file; Novartis Pharma AG, Basel, Switzerland).

In conclusion, the results from this study showed that steady-state seminal concentrations of fingolimod and fingolimod-phosphate were close to those simultaneously observed in blood. However, the amount of these substances that would be transferred to a pregnant woman during an act of intercourse is low, and the consequent risk of harming the fetus via this route is considered extremely unlikely.^{1,15}

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Declaration of Conflicting Interests

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