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Oral-intravenous Crossover Study of Fingolimod Pharmacokinetics, Lymphocyte Responses and Cardiac Effects

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ABSTRACT: *Objective.* The pharmacokinetics and lymphocyte responses to the immunomodulator fingolimod (FTY720) were characterized after oral and intravenous administration.

Methods. In this randomized, two-period crossover study 11 evaluable healthy subjects received single doses of fingolimod 1.25 mg orally and 1 mg intravenously infused over 2 h. The pharmacokinetics of fingolimod, blood lymphocyte counts and heart rate were characterized for 28 days after each dose.

Results. After oral administration, C_{max} was 1.1 ± 0.2 ng/ml occurring at 12 h postdose and the *AUC* was 201 ± 31 ng.h/ml. After intravenous infusion, C_{max} was 4.9 ± 0.8 ng/ml, *AUC* was 175 ± 50 ng. h/ml, clearance was 6.3 ± 2.3 l/h and distribution volume was 1199 ± 260 l. The oral/intravenous ratio of dose-normalized *AUC*s was 0.94 (95%CI: 0.78–1.12). The pharmacologically active metabolite fingolimod-phosphate was quantifiable near its peak after oral administration but not after intravenous administration. The mean lymphocyte nadir occurred on day 1 and was 35% lower after oral (0.74×10^9/l) than after intravenous (1.15×10^9/l) administration. Lymphocytes recovered to the normal range by day 15 for both treatments. The mean heart rate nadir occurred 3–4 h postdose and was 11% lower after oral administration (47 bpm) versus intravenous administration (53 bpm).

Conclusions. Average systemic exposure to fingolimod was similar after oral and intravenous administration. However, the acute decrease in lymphocyte counts was weaker after intravenous administration, likely because of lower blood levels of the active metabolite fingolimod-phosphate compared with oral administration.

Key words: fingolimod; immunosuppressants; absolute bioavailability; reversible metabolism

Introduction

Fingolimod (FTY720) is a synthetic sphingosine-1-phosphate receptor modulator in clinical development for the treatment of multiple sclerosis. It prevents the recirculation of effector T-lymphocytes from lymphatic tissue to susceptible target organs such as the central nervous system (CNS). After phosphorylation, fingolimod acts as an agonist at the G protein-coupled sphingosine-1-phosphate receptor-1 on thymocytes and lymphocytes and induces its internalization. The internalization of the receptor renders these cells unresponsive to sphingosine-1-phosphate, depriving them of an obligatory signal to egress from lymphoid organs and recirculate to other sites, including the CNS [1]. Known pharmacodynamic effects of fingolimod include a rapid and persistent concentration-dependent reduction of the blood lymphocyte count to 20%–40%

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of baseline values and a predictable reduction in heart rate that is maximal upon treatment initiation and attenuates over time, despite continued dosing [2]. The molecular basis of these effects are compatible with the known mode of action of fingolimod-phosphate via engagement of sphingosine-1-phosphate receptors. These effects have not been associated with any significant morbidity in healthy volunteers or patients [3–5].

After oral administration in man, fingolimod blood levels increase slowly to reach peak concentrations 12–36 h postdose. Steady state blood concentrations are reached after 2 months of daily dosing. The steady-state oral clearance of fingolimod averages 10.8 l/h and the elimination half-life averages 8.8 days. Fingolimod is reversibly phosphorylated by sphingosine kinase to form the active moiety fingolimod-phosphate. Fingolimod-phosphate is dephosphorylated back to fingolimod by sphingosine lyase and/or sphingosine phosphatase. During maintenance dosing, the interconversion between fingolimod and fingolimod-phosphate yields a stable blood level ratio of the two moieties. Fingolimod is also irreversibly metabolized by CYP4F2 to inactive carboxylic acid metabolites primarily excreted in the urine [2]. There is currently limited knowledge about this isozyme. The CYP4F family primarily metabolizes endogenous substances such as fatty acids, eicosinoids and leukotrienes [6].

The European Union note for guidance to industry on the investigation of bioavailability and bioequivalence encourages developers of a new chemical entity to determine its absolute bioavailability from the formulation intended for marketing [7]. The clinical utility of such a study is to provide definitive values for clearance and distribution volume and to serve as a basis for judging the pharmaceutical performance of oral formulations of the compound. In the case of a prodrug the intravenous reference solution should preferably be the therapeutic moiety [7]. While fingolimod is indeed a prodrug, it has the added feature of undergoing reversible metabolism to the active phosphate moiety. In 1991 a consensus workshop panel addressed the need for a more precise definition of bioavailability [8]. In their discussions, they considered drugs undergoing reversible metabolism among other cases but were unable to make any generally valid recommendations how to determine the absolute bioavailability under these conditions. One approach has been to administer the parent and metabolite separately by both oral and intravenous routes in a fourway crossover study [9]. However, based on the safety results of toxicology experiments in rats, intravenous administration of fingolimod-phosphate in man could not be justified even at low doses. We therefore conducted a conventional twoway crossover study to compare the pharmacokinetics of fingolimod after oral and intravenous administration. While the study could not provide an estimate of absolute bioavailability, it did yield the fingolimod oral/intravenous *AUC* ratio and some comparative insights into the lymphocyte and heart rate responses to orally and intravenously administered fingolimod.

Methods

Study design

This was a randomized, two-period, crossover study planned for 12 healthy subjects. There were 5 men and 7 women and all were white. They were 34.2 ± 6.6 years of age (range, 25–45) and weighed 67.8 ± 11.4 kg (range, 52–89). In each study period, the subjects were confined to the clinical center for at least 36 h before drug administration until 48 h thereafter (that is, day -2 to day 3). On day -1 of each study period baseline lymphocyte and cardiac assessments were performed over 24 h. On day 1 of period 1 the subject received either 1 mg fingolimod via intravenous infusion or 1.25 mg given orally. Pharmacokinetic, lymphocyte and cardiac assessments were performed to the morning of day 3 after which the subject was allowed to leave the site. Subjects returned to the study center for evaluations on study days 4, 5, 7, 9, 11, 15, 22 and 28. After a minimum of 30 days after the first dose, subjects returned for the same schedule of assessments in period 2 but with administration of fingolimod by the alternative route of administration. The study protocol was approved by an institutional review committee and subjects gave written informed consent to participate.

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Drug administration

In both periods subjects fasted for at least 10 h before starting the clinical assessments on day −1 (see below) and before dosing on day 1. They continued to fast for at least 4 h thereafter. The oral formulation was fingolimod 1.25 mg hard gelatin capsules consumed with 200 ml of water. For the intravenous administration, 1.5 ml of a 1 mg/ml solution for intravenous infusion (1.5 mg fingolimod) was transferred into a 250 ml dextrose infusion bag to yield a concentration of 0.006 mg/ml fingolimod. The administration set was attached to the bag and a total volume of 168 ml was infused intravenously over 120 min at an infusion rate of 1.4 ml/min using a calibrated infusion pump. A 1 mg intravenous dose of fingolimod was chosen, based on preclinical toxicology studies. Given the oral/intravenous *AUC* ratios measured in preclinical species and the available dose strengths of fingolimod hard gelatin capsules, an oral dose of 1.25 mg was chosen as likely to yield a similar *AUC* compared with the chosen intravenous dose. It was anticipated that the resulting *AUC*s from both administration routes would directly measure at least 80% of the full fingolimod *AUC* with an acceptable fraction for extrapolation to infinity. For both administration routes, the doses were given between 07:00 and 09:30 on day 1 of each period. Standard meals were served 4, 10 and 12 h postdose on days −1 and 1.

Clinical assessments

General safety and tolerability assessments included the monitoring and recording of all adverse events; determination of standard biochemistry, hematology and urinalysis laboratory parameters on days −1, 2–4 and 28; standard electrocardiography on days −1, 1, 2, 3, 15, 28; and measurement of vital signs on days −1, 1–3 and 15. Continuous 24 h electrocardiograms were recorded on days −1 and 1 via a Mortara H-12 digital Holter monitor and the recording media transferred to eResearch Technology (Philadelphia, PA, USA) for interpretation.

For the two pharmacodynamic responses to fingolimod, the following more intensive data collections were made. Absolute lymphocyte counts were obtained on days −1 and 1 at predose and 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12 h postdose and on days 2, 3, 4, 5, 7, 15, 22, 28 in the morning. Heart rate (pulse) was recorded as part of the vital signs assessments on day −1 and 1 at predose and 1, 2, 3, 4, 5, 6, 8, 12 h postdose; day 2 at 24 and 32 h postdose; and day 3 at 40 and 48 h postdose. For the lymphocyte and heart rate assessments on day −1, no dose was administered but measurements began in the morning at a timepoint approximately 24 h before the planned dose of study medication on day 1. 'Predose' and 'postdose' for these assessments on day −1 are relative to this timepoint.

Pharmacokinetic assessments

Venous blood samples (1.2 ml) were drawn into EDTA-containing vacuum tubes for analysis of fingolimod and into sodium citrate-containing tubes for analysis of fingolimod-phosphate. Blood samples were taken predose and then at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 144, 192, 240, 336, 504 and 648 h after administering the oral dose or after starting the intravenous infusion. Blood samples were inverted several times and frozen at −20°C.

Bioanalytics

The bioanalytical methods have been previously described [10]. As applied in this study for fingolimod, there were seven calibration concentrations (range, 0.08–30 ng/ml) and three quality control concentrations (0.24–25 ng/ml). Quality control bias ranged from 2.0% to 10.0% and precision from 104.2% to 104.8%. The lower quantification limit was 0.08 ng/ml. For fingolimod-phosphate there were six calibration concentrations (1.5–500 ng/ml) and three quality control concentrations (3.5–350 ng/ml). Quality control bias ranged from −2.1% to 6.9% and precision from 103.0% to 104.9%. The lower quantification limit was 1.5 ng/ml.

Pharmacokinetic evaluation

Standard noncompartmental pharmacokinetic parameters were derived including the lag time after oral dose administration until concentrations became quantifiable (t_{lag}), the peak concentration (C_{max}), the time of its occurrence (t_{max}),

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area under the concentration-time curve extrapolated to infinite time (*AUC*), clearance (*CL*), the distribution volume associated with the terminal phase (V_z), and the elimination half-life ($t_{1/2}$).

Lymphocyte and heart rate responses

Lymphocyte counts and heart rate were graphed with respect to time postdose and inspected for temporal patterns. Noncompartmental response parameters included the predose value, the minimal value (nadir), the time to nadir, and the area-under-the-effect curve over a given time period [*AUE*(0−t)].

Statistical evaluation

Fingolimod *AUC* was dose-normalized and log-transformed and compared between treatments in an ANOVA model with *treatment* and *period* as fixed factors and *subject* as a random factor. From this model the oral/intravenous ratio of geometric mean *AUC*s and its 95% confidence interval were derived. All other parameters are summarized as arithmetic mean ± standard deviation unless otherwise indicated.

Results

Tolerability and safety

One subject withdrew from the study after oral administration in period 1 due to ongoing nausea thought to be related to the study medication. This subject was not replaced; hence, 11 subjects completed the study. The duration of the study for each participant was 2 months. Over this time period, a total of 38 adverse events suspected by the investigator to be related to the study drug occurred in 9 subjects. These events were equally distributed between oral and intravenous administration. The most common adverse events were: nasopharyngitis, gastrointestinal symptoms (nausea, diarrhea, vomiting) and headache. There were no clinically relevant changes in laboratory parameters, electrocardiograms, or vital signs with the exception of lymphocyte counts and heart rate as described below. From Holter monitoring, no subject had atrial fibrillation or 'R on T' phenomena. Sinus pauses >2 s occurred in two subjects on day 1 after oral administration. One subject had a second degree type 1 atrioventricular block (Wenckebach) on day 1 after oral administration. No intervention was necessary for any of these events.

Fingolimod pharmacokinetics

As summarized in Table 1 and Figure 1A, mean fingolimod blood concentrations increased in a zero-order manner during the 2 h constant infusion to reach an end-of-infusion peak of 4.9 ng/ml. After oral administration there was a median 0.5 h time lag until fingolimod appeared in blood at quantifiable levels. An initial absorption 'shoulder' occurred to 4 h postdose and the subsequent peak of 1.1 ng/ml was reached by 12 h postdose. From 12 h onward, the mean concentration profiles from both routes of administration declined in parallel at very similar blood levels as shown in Figure 1B. Fingolimod was quantifiable up to week 3 for both formulations at the doses administered in this study. By week 4 blood levels of fingolimod were below the assay quantification limit; hence, there was no measurable drug carryover from period 1 to 2. The portion of the total *AUC* measured was 85 ± 6%. Fingolimod distributed into a very large volume and exhibited a relatively low clearance. Given the interconversion of fingolimod and fingolimod-phosphate, the calculated volume and clearance are not definitive values for fingolimod.

Across all 11 subjects the oral/intravenous ratio of dose-normalized *AUC*s was 0.94 with a

Table 1. Fingolimod pharmacokinetics

Parameter	Intravenous	Oral
Dose (mg)	1	1.25
t_{lag} (h)	—	0.5 (0–1)
t_{max} (h)	2 (1.5–2.0)	12 (8–36)
C_{max} (ng/ml)	4.9 ± 0.8	1.1 ± 0.2
AUC (ng.h/ml)	175 ± 50	201 ± 31
AUC/Dose (ng.h/ml/mg)	175 ± 50	161 ± 25
CL (l/h)	6.3 ± 2.3	—
V_z (l)	1199 ± 260	—
$t_{1/2}$ (days)	6.0 ± 1.9	6.1 ± 1.0

Values are mean ± SD except for temporal parameters which are median (range).
t_{lag} is lag time; t_{max} is time to peak concentration; C_{max} is peak concentration; *AUC* is area under the concentration-time curve; *CL* is clearance; V_z is distribution volume; $t_{1/2}$ is half-life.

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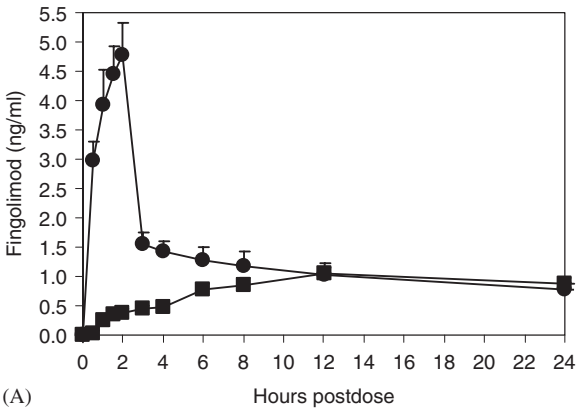


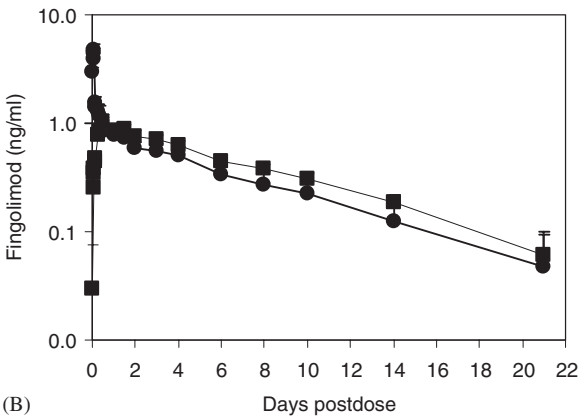
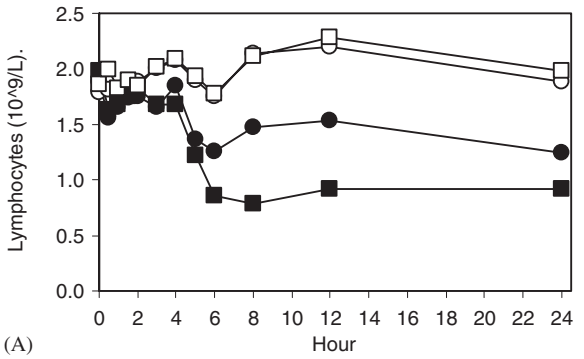


Figure 1. Fingolimod mean concentration-time profiles after intravenous administration (filled circles) and oral administration (filled squares). (A) curves to 24 h postdose on a linear concentration scale. (B) curves over the full study duration on a logarithmic concentration scale. Bars are the 95% confidence intervals



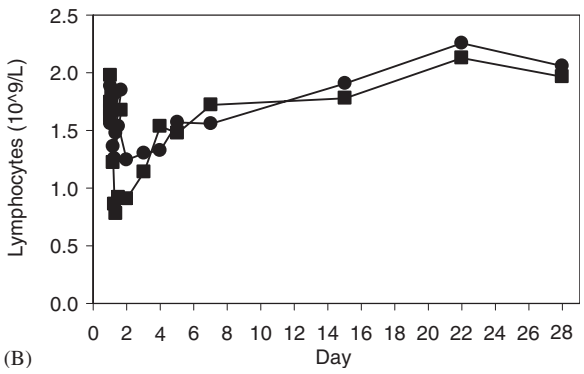


Figure 2. (A) Mean lymphocyte trajectories after fingolimod administration intravenously (filled circles) and orally (filled squares) on day 1. The corresponding open symbol curves are from the baseline measurements on day −1 in the absence of drug. (B) Mean lymphocyte trajectories over the full study course. Symbols as in (A)

95% confidence interval of 0.78–1.12. The inter-subject coefficient of variation was 30.6%.

Fingolimod-phosphate pharmacokinetics

Fingolimod-phosphate was not quantifiable in blood after intravenous administration with the exception of a single blood sample in one subject. After oral administration, fingolimod-phosphate was quantifiable in eight subjects between 6 and 12 h postdose. Peak concentrations ranged from 1.6 to 2.8 ng/ml occurring at 6 or 8 h postdose–hence preceding the peak of fingolimod. By 24 h postdose (the next blood sampling time after 12 h) fingolimod-phosphate blood levels fell below the quantification limit in these subjects.

Lymphocyte responses

Lymphocyte response parameters are summarized in Table 2. As shown in Figure 2A a normal circadian rhythm in lymphocyte counts was evident during the frequent blood sampling period in the first 12 h of the daytime on day −1 (08:00–20:00). Mean lymphocytes decreased from a morning predose count of about 1.8×10^9/l (08:00) to a morning nadir of about 1.6×10^9/12 h later (10:00). A similar night-time decline and increase is also known to occur but no lymphocyte counts were obtained in this period. This normal circadian pattern was highly consistent on day −1 for both treatments as seen in Figure 2A.

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Table 2. Lymphocyte and heart rate response parameters

Parameter	Intravenous fingolimod		Oral fingolimod	
	Day −1	Day 1	Day −1	Day 1
Lymphocyte responses				
Predose count (10^9/l)	1.79 ± 0.43	1.89 ± 0.30	1.86 ± 0.60	1.98 ± 0.41
Nadir time (h)	1.5 (0.5–12)	8 (5–24)	2 (0.5–6)	8 (6–24)
Nadir count (10^9/l)	1.57 ± 0.33	1.15 ± 0.25	1.62 ± 0.39	0.74 ± 0.19
Nadir ratio (day 1/−1)	—	0.73 ± 0.06	—	0.46 ± 0.10
AUE(0–24) (10^9/l × h)	49 ± 8	35 ± 6	50 ± 11	25 ± 5
AUE(0–24) ratio (day 1/−1)	—	0.73 ± 0.08	—	0.52 ± 0.08
AUE(0–28) (10^9/l × day)	—	1203 ± 283	—	1151 ± 299
Heart rate responses				
Rate predose (bpm)	63 ± 9	66 ± 13	62 ± 11	67 ± 10
Nadir time (h)	3 (1–4)	3 (2–8)	3 (1–4)	4 (2–8)
Nadir rate (bpm)	57 ± 10	53 ± 9	55 ± 9	47 ± 6
AUE(0–4) (bpm × h)	248 ± 52	235 ± 48	234 ± 32	224 ± 34
AUE(0–4) ratio (day 1/−1)	—	0.95 ± 0.05	—	0.96 ± 0.07
AUE(0–12) (bpm × h)	801 ± 150	714 ± 125	763 ± 84	643 ± 99
AUE(0–12) ratio (day 1/−1)	—	0.89 ± 0.05	—	0.84 ± 0.05

Values are mean ± SD except for nadir time which is median (range).
AUE is area under the effect-time curve.

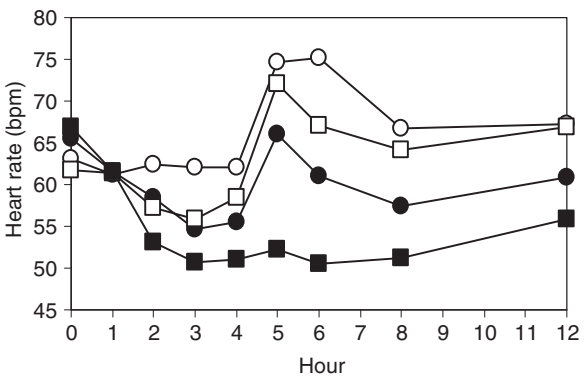

Figure 3. Mean heart rate trajectories after fingolimod administration intravenously (filled circles) and orally (filled squares) on day 1. The corresponding open symbol curves are from the baseline measurements on day −1 in the absence of drug

After intravenous administration of fingolimod the lymphocyte nadir occurred at a median 8 h postdose (16:00) and was reduced by 27% compared with the nadir on day −1. *AUE*(0–24) exhibited a similar 27% decrease compared with day −1. After oral dosing, a similar time shift but a stronger reduction in nadir was noted with a 54% decrease in nadir and 48% decrease in *AUE*(0–24) compared with day −1. Comparing the lymphocyte responses between treatments on day 1, oral administration elicited a 35% lower lymphocyte nadir and a 29% lower *AUE*(0–24) relative to the values after intravenous administration.

Mean lymphocyte counts were recovering already by day 3 (48 h postdose) and returned to predose values by day 15. As shown in Figure 2B, the full effect on lymphocytes over the 28-day study course based on *AUE*(0–28) was very similar after both treatments likely reflecting the fact that the acute between-treatment differences in lymphocyte response over the first 2 days were overridden by the nearly identical longterm return to baseline over 4 weeks.

Heart rate responses

Heart rate response parameters are summarized in Table 2. As shown in Figure 3 a normal daytime circadian rhythm in heart rate was evident over the first 12 h on day −1 of both treatments. Heart rate decreased from a morning mean predose rate of 62 or 63 bpm (08:00) to a morning mean nadir of 57 or 55 bpm generally 3–4 h later (11:00–12:00). This normal circadian pattern was highly consistent on day −1 for both

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treatments indicating that an appropriate washout period separated the two treatments without carryover of the heart rate effect from period 1 to 2.

On the two fingolimod dosing days, the mean predose heart rates were slightly elevated compared with those on day −1. After intravenous administration the heart rate nadir was 7% lower, the *AUE*(0–4) was 5% lower, and the *AUE*(0–12) was 11% lower compared with day −1. After oral administration the nadir was shifted slightly by 1 h and was 14% lower compared with day −1. While this reduction was not as apparent for *AUE*(0–4) with 4% decrease, *AUE*(0–12) showed a similar reduction as for the nadir with a 16% decrease. Comparing the nadir rates between treatments, oral administration elicited an 11% lower nadir relative to the nadir after intravenous administration. By day 2 the mean morning heart rates were recovering back to the day −1 baseline: 61 ± 9 bpm for intravenous and 62 ± 10 bpm for oral administration.

Discussion

This study sought to characterize the pharmacokinetics of the new chemical entity fingolimod after oral and intravenous administration. In selecting the doses the aim was to address the following considerations in order of priority: (1) to use a low intravenous dose inasmuch as this was the first study in man with this administration route; (2) to characterize fully both the input and elimination phases of the fingolimod concentration profiles by both routes of administration; (3) to achieve similar fingolimod *AUC*s for both routes of administration; and (4) to characterize fingolimod-phosphate concentration profiles. The pharmacokinetic results demonstrated that the first three goals were achieved. The fourth goal was not met at the selected doses due to the higher assay quantification limit and generally lower blood levels of fingolimod-phosphate relative to fingolimod. Although this imposed some limitations on the pharmacokinetic interpretation, it nonetheless provided some insights into this compound and its clinical pharmacology.

Fingolimod was generally well tolerated after oral and intravenous single-dose administration. Laboratory parameters and vital signs were not altered over the study course with the exception of decreased lymphocyte counts and decreased heart rate consistent with the pharmacology of fingolimod. The nadir lymphocyte count and heart rate and the area under the effect-time curves for these two responses were lower after oral administration compared with intravenous administration. It is hypothesized that these differences were due to lower systemic exposure to the pharmacologically active moiety fingolimod-phosphate after intravenous administration of fingolimod. Since it was not possible to directly quantify fingolimod-phosphate in blood for most subjects due to the low fingolimod doses administered, the hypothesis remains speculative but reasonable. Sinus pauses and second degree atrioventricular blocks (type 1 or Wenckebach) were observed in a few subjects and have been observed in other studies as well [5]. This type of block most likely reflects a vagal effect of fingolimod-phosphate on the atrioventricular node. Both of these cardiac responses were clinically benign and are occasionally observed in the Holter recordings of healthy subjects [5].

Although this study did not establish the absolute bioavailability of fingolimod because of its reversible metabolism, it did document the average oral/intravenous ratio of dose-normalized *AUC*s for fingolimod of 94%. This indicates that administration of a given dose of fingolimod by intravenous infusion or orally by hard gelatin capsule yields similar systemic exposure to fingolimod inasmuch as the 95% confidence interval of the *AUC* ratio included unity.

Systemic exposure to fingolimod-phosphate, however, appears to be administration route-dependent. In this context, the pharmacokinetic and response data collected in this study suggest that presystemic phosphorylation of fingolimod may be an important contributor to the formation of fingolimod-phosphate. Firstly, fingolimod-phosphate was quantifiable around its peak in some subjects after oral administration, but not after intravenous administration—a route that circumvents presystemic drug biotransformation. Despite the lower intravenous versus oral dose used in this study, fingolimod blood levels

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after intravenous administration between 6 and 12 h were higher than those after oral administration (Figure 1A), yet fingolimod-phosphate was not quantifiable after intravenous administration. Secondly, both the lymphocyte and heart rate responses were weaker after intravenous compared with oral dosing. Since these two effects are driven by fingolimod-phosphate, they indirectly support the hypothesis that fingolimod-phosphate blood levels after intravenous administration of fingolimod were likely lower than after oral administration. The hypothesis further suggests that sphingosine kinase (the enzyme responsible for the formation of fingolimod-phosphate) is active during the absorption process and/or first pass through the liver. Sphingosine kinase is expressed in the human liver [11].

Independent of the above hypothesis, this study demonstrated that there is good systemic availability to fingolimod from the hard gelatin capsule formulation yielding fingolimod exposure similar to that from intravenous administration. The immunomodulatory response to fingolimod-phosphate, however, may be influenced by the administration route with a moderately weaker acute lymphocyte decrease after intravenous administration.

Acknowledgements

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