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PHARMACODYNAMICS

The effect on heart rate of combining single-dose fingolimod with steady-state atenolol or diltiazem in healthy subjects

John M. Kovarik · Michael Lu · Gilles-Jacques Riviere · Irene Barbet · Steve Maton · D. Ronald Goldwater · Robert L. Schmouder

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Abstract
Objective The sphingosine-1-phosphate receptor modulator fingolimod (FTY720) is known to elicit a negative chronotropic effect at treatment initiation that attenuates over time with continued dosing. The authors determined the effect of combining a single dose of fingolimod with steady-state atenolol or diltiazem on heart rate and mean arterial pressure.
Methods In a partially randomized, single-blind, placebo-controlled, three-period, crossover study, 25 healthy subjects received (1) a single oral 5-mg dose of fingolimod, (2) either 50 mg atenolol or 240 mg diltiazem once daily for 5 days, and (3) the antihypertensive for 5 days and a single dose of fingolimod on day 5. Telemetry and pharmacokinetic data were collected.
Results The daytime mean heart rate nadir was 15% lower when fingolimod was combined with atenolol (42±7 bpm) compared with fingolimod alone (51±9 bpm) yielding a combination/monotherapy ratio of 0.85 (90%CI, 0.79–0.92). The daytime mean heart rate nadir from fingolimod alone (55±5 bpm) was not altered when combined with diltiazem (56±8 bpm) yielding a ratio of 0.99 (0.94–1.05). There was no clinically relevant change in mean arterial pressure when fingolimod was administered with atenolol or diltiazem compared with administration of the drugs alone in normotensive subjects. The pharmacokinetics of the drugs were not altered during coadministration.
Conclusion Adding fingolimod to a beta-blocker such as atenolol resulted in a moderately lower mean heart rate nadir compared with fingolimod alone. However, subjects who had a stronger negative chronotropic response to fingolimod alone (nadir < 50 bpm) had minimal or no further reduction in heart rate with the drug combination. Adding fingolimod to a calcium channel blocker such as diltiazem did not further lower the heart rate compared to fingolimod alone.

Keywords Antihypertensives · Heart rate · Blood pressure · Immunomodulators · Pharmacokinetics

J. M. Kovarik (✉) · M. Lu · G.-J. Riviere · I. Barbet · S. Maton · R. L. Schmouder
Novartis Pharma,
Building WSJ 210.423,
4002 Basel, Switzerland
e-mail: john.kovarik@novartis.com

D. R. Goldwater
PAREXEL Clinical Pharmacology Research,
Baltimore, MD, USA

Introduction

Fingolimod (development code, FTY720) is a synthetic sphingosine-1-phosphate receptor modulator that prevents the recirculation of effector T-lymphocytes from lymphatic tissue to susceptible target organs such as the central nervous system [1]. It is in clinical development for the treatment of multiple sclerosis based on a positive proof-of-concept study in which fingolimod reduced the number of lesions in the central nervous system and reduced clinical disease activity compared with placebo [2]. Fingolimod is administered orally once daily in a capsule formulation. It is reversibly phosphorylated in vivo by sphingosine kinase to form the active moiety fingolimod-phosphate. Both fingolimod and fingolimod-phosphate are quantifiable in blood during treatment, whereby fingolimod-phosphate levels are around half those of the parent. Fingolimod is also irreversibly metabolized by the CYP4F isozyme with metabolites eliminated in the urine and feces. Fingolimod's

oral clearance averages 10.8 L/h, and its half-life averages 8.8 days. Fingolimod-phosphate blood levels in the elimination phase decline in parallel with those of fingolimod with a similar half-life to that of the parent compound [3].

In addition to their expression on lymphocytes, sphingosine-1-phosphate receptors are also expressed on atrial myocardial cells. Treatment with fingolimod in clinical trials was associated with a transient and dose-dependent reduction in heart rate that was maximum within 4–6 h after the first dose and then attenuated over time with continued treatment. This effect has been asymptomatic in the vast majority of patients [2, 4]. Preclinical experiments indicated that the mechanism of reduced heart rate involves activation of G-protein-regulated, inward-rectifying potassium (GIRK) channels in atrial myocytes, most likely via sphingosine-1-phosphate receptors [5]. Using continuous telemetry, a dedicated clinical pharmacology study has characterized the effect of fingolimod on heart rate and rhythm in healthy subjects who received 1.25 or 5 mg fingolimod or placebo once daily for 7 days. Fingolimod caused an acute, dose-dependent decrease in mean nadir heart rate up to 10 bpm after the first dose compared with placebo. Although a persistent fingolimod-related decrease in heart rate was measured from day 2 to 7, additional doses of fingolimod after day 1 resulted in no further incremental decreases in heart rate. Mean PR interval increased by approximately 8–10 ms in fingolimod-treated subjects but no changes in QRS or QT intervals occurred [6].

Antihypertensives are widely used medications and may be coadministered with fingolimod in multiple sclerosis patients. In addition to their ability to lower blood pressure, some antihypertensive classes are associated with decreases in heart rate. Given the acute negative chronotropic effect of fingolimod described above, we were interested to see if combination with an antihypertensive would potentiate fingolimod's effect on heart rate. We selected atenolol from the class of beta-blockers and diltiazem from the class of calcium channel blockers based on their known negative chronotropic effects and high frequency of use. We also included measurement of drug levels in our study to assess whether there are pharmacokinetic drug interactions when these agents are combined.

Methods

Study population and disposition Potential subjects were informed about the study and signed a consent form to participate. We enrolled a total of 36 subjects in the study. There were 25 men and 11 women, 29.5±7.2 years of age (range 18–49) and 70.8±11.5 kg (range 50.6–94.0). There were 20 blacks, 12 whites, 3 Asians, and 1 of other ethnicity. Three subjects were withdrawn from the study: one for an adverse event (see below), one for frequent premature ventricular contractions noted in period 1 when receiving diltiazem alone, and one for elevated creatine kinase at baseline of period 1. None of these three subjects received fingolimod. Consequently, 33 subjects completed the study: 8 subjects in part 1, 12 subjects in part 2, and 13 subjects in part 3.

Study design The study protocol was reviewed and approved by Chesapeake Research Review (Columbia, MD, USA). The study consisted of three separate parts: a test dose administration of low-dose fingolimod with atenolol or diltiazem in part 1, higher-dose fingolimod with atenolol in part 2, and higher-dose fingolimod with diltiazem in part 3.

Because we had no prior clinical experience with these drug combinations, we gave test administrations in a randomized, open-label design to eight healthy subjects in part 1. Four subjects received 50 mg atenolol once daily for 5 days and four subjects received 240 mg diltiazem once daily for 5 days. Both groups received a single 0.5-mg dose of fingolimod on day 5. After the safety and tolerability of these combined regimens were demonstrated, the study proceeded to parts 2 and 3 with higher-dose fingolimod.

Study parts 2 and 3 were conducted in parallel and were identical in design except for the antihypertensive used: part 2 used atenolol 50 mg once daily and part 3 used diltiazem 240 mg once daily. The design was a partially randomized, single-blind, placebo-controlled, three-period, crossover study planned for 12 evaluable subjects in each part. Each subject received three treatments. In period 1, the treatment was fixed inasmuch as all subjects received their assigned antihypertensive (atenolol or diltiazem) once daily for 5 days with a fingolimod placebo on day 5. This was followed by a 7-day washout phase to allow elimination of drug and its effect on heart rate. In periods 2 and 3 the treatments were randomized. Subjects received either their assigned antihypertensive or antihypertensive placebo once daily for 5 days and 5 mg fingolimod on day 5 in period 2. Period 3 was the same as period 2 but subjects received the alternate treatment (antihypertensive placebo or antihypertensive) and 5 mg fingolimod on day 5. Periods 2 and 3 were separated by a 33-day washout phase to allow elimination of fingolimod (half-life 8.8 days) and its effects on heart rate.

We chose a single 5-mg dose of fingolimod for study parts 2 and 3 because it elicits a near-maximum effect on heart rate [6]. The dose regimens of atenolol and diltiazem are within the typical dose ranges when initiating treatment for hypertension and were given for 5 days in order to reach a pharmacokinetic steady state. Given the long half-life of fingolimod and fingolimod-phosphate, a fully randomized study design would have required a 33-day washout phase after periods 1 and 2, resulting in a long commitment for the

participants. We therefore chose a partially randomized design in which treatment with the antihypertensive alone was conducted in period 1 requiring a 1-week washout followed by randomized treatments with antihypertensive or antihypertensive placebo (both with fingolimod) requiring a single 33-day washout between period 2 and 3. The study sample size of 12 subjects for each of parts 2 and 3 was chosen to detect at least a 10% difference in heart rate nadir between fingolimod alone versus fingolimod plus an antihypertensive with 80% power. This determination was based on an intrasubject coefficient of variation in heart rate nadir after fingolimod alone of 9% in previous healthy-subject clinical pharmacology studies.

Domiciling and drug administration Subjects were present at the clinical site from the night before the start of each study period until the morning of day 6. Study medication consisted of 0.5- and 5-mg fingolimod capsules and matching fingolimod-placebo capsules (Novartis Pharmaceuticals), atenolol 50-mg tablets (AstraZeneca), diltiazem 240-mg extended-release tablets (Biovail Pharmaceuticals), and a generic placebo for the antihypertensives (Forest Pharmaceuticals). Study drugs were administered with 240 ml of water in the morning after a light breakfast on all 5 days of each period. Standard meals were served 4, 9, and 13 h postdose on all treatment days.

Clinical assessments Standard biochemistry, hematology and urinalysis laboratory parameters and 12-lead electrocardiography were performed at baseline before each period and at the end of the study. Blood pressure and pulse rate were recorded at baseline before each period, on day 1 (0, 1, 2, 3, 4 h postdose), on day 5 (0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 20, 24 h postdose), and at the end of the study. Continuous telemetry was recorded on day 5 from the time of dosing to 24 h postdose and the recording media transferred to eResearch Technology (Philadelphia, PA, USA) for an independent interpretation.

Cardiovascular data evaluation Mean hourly heart rate was calculated for each subject from the 24-h telemetry recordings on day 5 of each period in study parts 2 and 3. Mean arterial blood pressure (MAP) was calculated for each subject at each timepoint that systolic/diastolic blood pressure (SBP/DBP) was recorded on day 5 of each period: MAP=1/3SDP+2/3DBP. Two cardiovascular response parameters were subsequently derived for heart rate and for MAP: the daytime nadir effect taken as the minimum value over 0–12 h postdose and the daytime area under the effect-time curve from 0–12 h postdose [AUEC(0–12)] calculated by trapezoidal summation.

To compare the cardiovascular responses of fingolimod to those of atenolol or diltiazem, response parameters were log-transformed and compared between fingolimod with placebo in period 1 (reference) and atenolol/diltiazem with placebo in period 2 or 3 (test) in a linear mixed-effects model with *sequence* and *treatment* as fixed factors and *subject-within-sequence* as a random factor. Test/reference point estimates and 90% confidence intervals were generated. To compare the negative chronotropic effect of fingolimod (reference) to that of fingolimod with atenolol or diltiazem (test), response parameters from periods 2 and 3 were log-transformed and compared in the model described above. Parameter ratios and 90% confidence intervals which transgressed the 0.80–1.25 bounds were considered of possible clinical relevance.

Pharmacokinetic assessments Blood samples for the determination of fingolimod (EDTA tubes) and fingolimod-phosphate (sodium citrate tubes) were collected predose and then 2, 4, 6, 8, 10, 12, and 24 h postdose in study parts 2 and 3. Samples were inverted several times, the contents transferred to polypropylene vials and frozen at −18°C. Blood samples for the determination of atenolol and diltiazem (heparin tubes) were collected predose and then 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h postdose on day 5 of each period. Samples were centrifuged to yield plasma, which was frozen at −18°C.

Bioanalytics Blood concentrations of fingolimod and fingolimod-phosphate were determined separately by validated liquid chromatography methods and tandem mass spectrometry as previously described [7]. As applied in this study for fingolimod, there were seven calibration concentrations (range 0.08–25 ng/ml) and three quality control concentrations (0.24, 5, 25 ng/ml). Quality control accuracy ranged from 100.8 to 111.7% and precision from 4.6 to 16.6%. 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 (3.75, 37.5, 375 ng/ml). Quality control accuracy ranged from 93.6 to 103.7% and precision from 3.2 to 5.2%. The lower quantification limit was 1.5 ng/ml.

Plasma concentrations of atenolol were determined after solid-phase extraction by high-performance liquid chromatography with fluorescence detection. There were seven calibration concentrations (range 25–1,000 ng/ml) and three quality control concentrations (50, 500, 900 ng/ml). Quality control accuracy ranged from 92.6 to 96.6% and precision from 1.5 to 4.4%. The assay limit of quantification was 25 ng/ml.

Plasma diltiazem concentrations were determined after liquid-liquid extraction by high-performance liquid chromatography with ultraviolet detection. There were seven calibration concentrations (range 2–250 ng/ml) and three quality control concentrations (5, 125, 200 ng/ml). Quality control

Table 1 Cardiovascular responses to atenolol and fingolimod

Response	Atenolol	Fingolimod	Fingolimod + atenolol
Heart rate			
Predose (bpm)	60±8	73±9	60±7
Nadir (bpm)	55±7	51±9	42±7
Time of nadir (h)	4 (3–11)	4.5 (3–8)	4 (3–8)
AUEC(0–12) (bpm·h)	687±81	674±120	561±82
Mean arterial pressure			
Nadir (mmHg)	77±5	79±6	74±4
AUEC(0–12) (mmHg·h)	934±51	953±68	881±57

Values are mean ± sd except for nadir time which is median (range)
AUEC(0–12) Area under the response effect-time curve to 12 h postdose

accuracy ranged from 96.8 to 102.4% and precision from 3.7 to 9.3%. The assay limit of quantification was 2.5 ng/ml.

Pharmacokinetic data evaluation Standard noncompartmental pharmacokinetic parameters were calculated from the drug concentration-time data. These included the peak concentration Cmax, the time of its occurrence tmax, and the area under the concentration-time curve to 24 h postdose by trapezoidal summation [AUC(0–24)]. We did not sample blood for fingolimod beyond 24 h in order to reduce the participation burden on the subjects of repeat clinical visits. We reasoned that any clinically relevant pharmacokinetic influence of the antihypertensives on fingolimod would manifest over the 24-h dose interval during coadministration.

Fingolimod and fingolimod-phosphate Cmax and AUC (0–24) were log-transformed and compared between fingolimod with placebo (reference) and fingolimod with atenolol/diltiazem (test) in a linear mixed-effects model with *sequence, treatment*, and *period* as fixed factors and *subject-within-sequence* as a random factor. Test/reference ratio of geometric means and 90% confidence intervals were generated. Atenolol and diltiazem Cmax and AUC(0–24) were log-transformed and compared between atenolol/diltiazem with placebo (reference, period 1) and atenolol/diltiazem with fingolimod (test, period 2 or 3) in the model mentioned above for cardiovascular responses. Parameter ratios and 90% confidence intervals that transgressed the 0.80–1.25 bounds were considered a possible drug interaction.

Results

Test dose administration (part 1) In this study part, a low dose of 0.5 mg fingolimod was given during steady-state atenolol or diltiazem. Cardiovascular assessments focused on the first 4 h postdose, the period within which the nadir heart rate occurs for fingolimod. Mean hourly heart rate to 4 h postdose (08:00–12:00) exhibited a conventional circadian decline in the morning when both antihypertensives were given alone on day 1: for atenolol from 60±2 to 55±5 bpm and for diltiazem from 63±6 to 61±7 bpm. After the addition of low-dose fingolimod on day 5, the mean heart rate decrease between 0 and 4 h was from 68±5 to 49±2 bpm for atenolol and from 67±7 to 66±12 bpm for diltiazem. The addition of low-dose fingolimod did not change the mean arterial pressure for either antihypertensive (data not shown). These cardiovascular response data allowed the study to progress to parts 2 and 3 with higher-dose fingolimod.

Fingolimod with atenolol (part 2) Heart rate and mean arterial blood pressure responses over 12 h postdose are summarized in Table 1, and mean heart rate trajectories for 24 h are shown in Fig. 1. The mean heart rate nadir was significantly lower by 8% for 5 mg fingolimod alone compared with atenolol alone ($P=0.03$), but the fingolimod/atenolol ratio and 90% confidence interval remained in the equivalence bounds: 0.92 (0.86–0.98). None of the other cardiovascular responses were different between treatments when the drugs were given alone with fingolimod/atenolol ratios for heart rate AUEC(0–12) of 0.97 (0.92–1.03), for mean arterial pressure nadir of 1.02 (1.00–1.04), and for mean arterial pressure AUEC(0–12) of 1.02 (1.00–1.04).

Fingolimod combined with atenolol decreased heart rate responses by 15% and mean arterial pressure responses by 7–8% compared with fingolimod alone. With the single exception of heart rate nadir, however, all other ratios and 90% confidence intervals remained in the standard equivalence bounds as follows: heart rate nadir 0.85 (0.79–0.92), heart rate AUEC(0–12) 0.85 (0.81–0.89), mean arterial pressure nadir 0.93 (0.91–0.96), and mean arterial pressure AUEC(0–12) 0.92 (0.90–0.95). As seen in Fig. 1, the circadian heart rate trajectory with fingolimod alone was nearly superimposable on that of atenolol alone, indicating

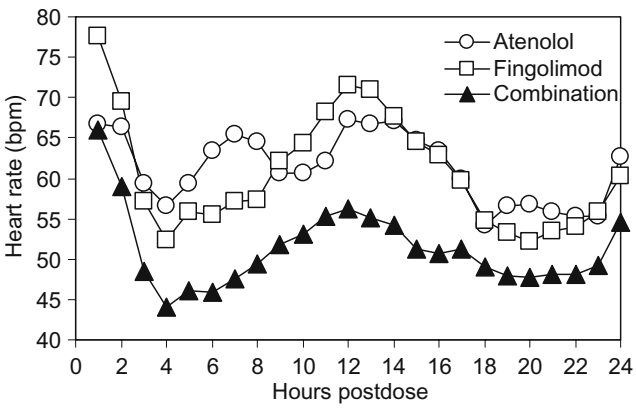

Fig. 1 Mean hourly heart rate trajectories from telemetry for atenolol, fingolimod, and the drug combination

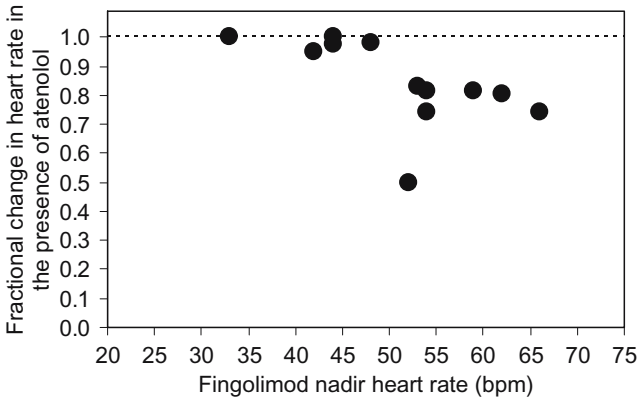

Fig. 2 Relationship of heart rate nadir on fingolimod alone versus the fractional change in nadir when fingolimod was added to atenolol in 12 subjects. Values near the*dashed unity line*indicate no additional decrease in heart rate nadir in the presence of atenolol

a similar magnitude of effect with both agents. The heart rate trajectory was shifted downward on the measurement scale after fingolimod with atenolol but retained its normal circadian pattern. Even though the population average heart rate nadir in the presence of fingolimod with atenolol was 15% lower than with fingolimod alone, Fig. 2 shows that subjects who had a more robust lowering of heart rate from fingolimod alone (nadir<50 bpm) did not have an additive decrease when fingolimod was administered with atenolol. Rather the stronger decrease in heart rate nadir after fingolimod with atenolol occurred in subjects who had a mild to moderate heart rate response to fingolimod alone (nadir>50 bpm).

Table 2 shows that addition of single-dose fingolimod to steady-state atenolol did not change atenolol Cmax with a test/reference ratio 0.92 (0.81–1.05) or AUC(0–24) [0.93 (0.85–1.02)]. Likewise, the presence of steady-state atenolol did not change fingolimod Cmax [1.02 (0.96–1.08)] or AUC(0–24) [1.00 (0.96–1.05)] or fingolimod-phosphate Cmax [0.98 (0.87–1.10)] or AUC(0–24) [1.05 (0.99–1.11)].

Fingolimod with diltiazem (part 3) Heart rate and mean arterial blood pressure responses are summarized in Table 3 and mean heart rate trajectories are shown in Fig. 3. The mean heart rate nadir and the AUEC(0–12) were significantly lower by 18 and 13%, respectively, for 5 mg fingolimod alone compared with diltiazem alone ($P<$ 0.001). The corresponding fingolimod/diltiazem ratios were 0.82 (0.78–0.85) and 0.87 (0.84–0.90). Mean arterial pressure nadir and AUEC(0–12) were similar between treatments with ratios of 1.00 (0.97–1.04) and 0.98 (0.96–1.01).

Heart rate and mean arterial pressure responses were not different for fingolimod alone compared with fingolimod with diltiazem. The ratios were 0.99 (0.94–1.05) for heart rate nadir, 0.98 (0.95–1.00) for heart rate AUEC(0–12), 1.00 (0.96–1.03) for mean arterial pressure nadir, and 0.98 (0.96–1.01) for mean arterial pressure AUEC(0–12). As seen in Fig. 3, the heart rate trajectory after fingolimod with diltiazem retained a normal circadian pattern. The heart rate curve was lower on fingolimod than on diltiazem.

Table 2 summarizes the pharmacokinetic data. Addition of single-dose fingolimod to steady-state diltiazem did not change diltiazem Cmax [0.99 (0.83–1.19)] or AUC(0–24) [0.99 (0.87–1.13)]. Likewise, the presence of steady-state diltiazem did not change fingolimod Cmax [1.05 (0.97–1.13)] or AUC(0–24) [1.02 (0.95–1.10)] or fingolimod-phosphate Cmax [1.05 (0.96–1.15)] or AUC(0–24) [1.02 (0.94–1.09)]. Diltiazem AUC(0–24) ranged fourfold across subjects. A plot of diltiazem AUC versus the heart rate nadir in the presence of coadministered fingolimod did not indicate that increasing exposure to diltiazem led to a lower heart rate nadir (data not shown).

Safety and tolerability There was one serious adverse event of syncope in a subject who received atenolol alone in period 1. He was subsequently diagnosed with pinhole ventricular septal defect and patent foramen ovale, which predated enrollment in the study. The subject was with-

Table 2 Pharmacokinetic parameters

Parameter	Antihypertensive		Fingolimod		Fingolimod-phosphate	
	Alone	Combined	Alone	Combined	Alone	Combined
Atenolol + fingolimod						
tmax (h)	2 (1.5–4)	3 (1.5–6)	12 (8–24)	12 (8–24)	6 (6–12)	6 (6–10)
Cmax (ng/ml)	315±70	291±65	4.7±1.0	4.8±1.1	5.4±1.1	5.3±1.4
AUC (ng·h/ml)	3023±485	2834±554	90±17	92±21	72±14	75±16
Diltiazem + fingolimod						
tmax (h)	12 (8–24)	12 (8–24)	12 (8–24)	12 (10–24)	6 (6–8)	6 (6–10)
Cmax (ng/ml)	144±51	142±51	4.6±0.9	4.8±1.0	5.3±1.2	5.7±1.8
AUC (ng·h/ml)	2491±944	2465±972	91±15	93±16	77±14	80±21

Values are mean ± sd except for tmax which is median (range)
Tmax Time to peak concentration, *Cmax* peak concentration, *AUC* area under the concentration-time curve to 24 h postdose

Table 3 Cardiovascular responses to diltiazem and fingolimod

Response	Diltiazem	Fingolimod	Fingolimod + diltiazem
Heart rate			
Predose (bpm)	70±9	72±10	69±9
Nadir (bpm)	67±7	55±5	56±8
Time of nadir (h)	5 (2–11)	5 (3–10)	5 (3–7)
AUEC(0–12) (bpm·h)	828±86	724±77	710±84
Mean arterial pressure			
Nadir (mmHg)	78±7	79±9	78±7
AUEC(0–12) (mmHg·h)	949±80	935±95	918±80

Values are mean ± sd except for nadir time which is median (range)
AUEC(0–12) Area under the response effect-time curve to 12 h postdose

drawn from the study in period 1 and did not receive fingolimod. There were 39 adverse events in 19 subjects, primarily dizziness and headache of mild or moderate severity and resolved with intervention. There were no clinically relevant changes in laboratory parameters, vital signs, or electrocardiograms over the study course with the exception of fingolimod-related decrease in heart rate described above.

Discussion

In this study, we sought primarily to characterize the effect on heart rate of combining a single dose of fingolimod with representative agents of two antihypertensive classes—beta-blockers and calcium channel inhibitors. The intent was to anticipate the situation that is likely to occur in clinical practice whereby a patient being treated with an antihypertensive agent that has negative chronotropic properties would subsequently start treatment with fingolimod.

The heart rate trajectories indicated that a 5-mg single dose of fingolimod alone elicited a negative chronotropic effect similar to that elicited by atenolol alone. Adding fingolimod to atenolol resulted in a further lowering of heart rate. Nonetheless, the magnitude of this additional decrease with the drug combination was generally moderate (15% lower) and the associated 90% confidence interval around the test/reference ratio of nadirs was only marginally outside the lower equivalence bound. Reassuringly, subjects who had a stronger negative chronotropic response to fingolimod alone (nadir<50 bpm) had minimal or no further reduction in heart rate when combined with atenolol. Rather, the lowering of the mean heart rate nadir in this treatment group on the drug combination was attributable to subjects who had only a mild to moderate negative chronotropic effect from fingolimod alone (nadir> 50 bpm). Recognizing that the healthy subjects in this study were normotensive, there was no evidence that fingolimod altered the antihypertensive effects of atenolol to a clinically relevant extent as determined from mean arterial pressure measurements.

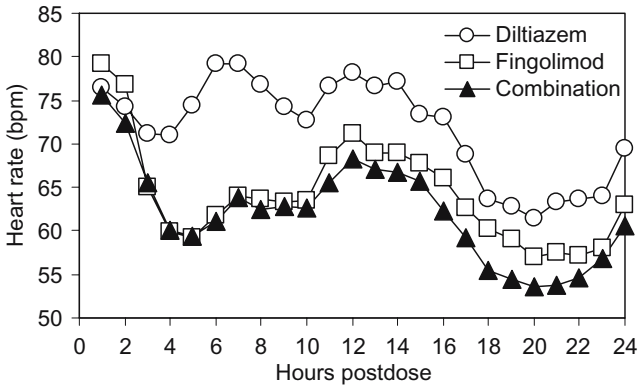

Fig. 3 Mean hourly heart rate trajectories from telemetry for diltiazem, fingolimod, and the drug combination

Fingolimod alone had a stronger negative chronotropic effect compared with diltiazem alone. Combining the drugs did not further lower the heart rate compared to fingolimod alone nor did the combination influence mean arterial pressure compared with diltiazem alone. Although we studied diltiazem at a single dose level, the corresponding AUCs ranged fourfold across subjects. There was no indication that increasing exposure to diltiazem over this range led to a lower heart rate nadir in the presence of fingolimod. We chose to study diltiazem because it is typically associated with the strongest negative chronotropic effect among calcium channel inhibitors. We infer from these results that adding fingolimod to other calcium channel inhibitors—whose intrinsic negative chronotropic effects are less than those of diltiazem–is unlikely to exacerbate the heart-rate-lowering effect of fingolimod alone.

With regard to the pharmacokinetics of these drugs, atenolol is not metabolized but rather excreted unchanged about half in urine and half in the feces [8]. Diltiazem is extensively metabolized by deacetylation in the liver with about one-third of the total dose eliminated in urine and two-thirds in feces, primarily as metabolites with minor amounts of unchanged drug [9]. Fingolimod, on the other hand, is primarily metabolized via CYP4F and excreted in

the urine and feces as metabolites [3]. Given the different routes of metabolism and elimination of these drugs, we did not expect pharmacokinetic interactions between them and the pharmacokinetic data collected in this study confirmed our hypothesis. Hence, any differences in cardiovascular responses among the treatments tested were not confounded by altered exposure to the study drugs.

Our study has some limitations. For example, we studied normotensive subjects over a short time period and this may have compromised our ability to find changes in mean arterial pressure or to address longterm cardiovascular implications of these drug combinations in patients. Nonetheless, we interpret the lack of change in mean arterial pressure under these circumstances as a reassurance that a dramatic change in this parameter is unlikely in hypertensive patients treated with these drug combinations. While the once-daily, steady-state regimens of the two antihypertensives used in this study were within the ranges used to treat hypertension, the fingolimod treatment was single dose. We chose a single dose because our primary interest in this study was the acute cardiovascular responses to fingolimod in the presence of antihypertensives. Our previous clinical pharmacology study addressing the cardiovascular responses to multiple-dose fingolimod alone indicated that a near maximum negative chronotropic effect is exerted shortly after the first dose of 5 mg and attenuates with continued treatment [6]. Hence, using a single dose of fingolimod with multiple-dose antihypertensives in the present study likely captured the maximum effects of these drug combinations on heart rate. Pharmacokinetics was a secondary objective in this study. We acknowledge that we did not fully characterize the concentration-time profile of fingolimod or fingolimod-phosphate into the elimination phase in order not to overburden the participants with several outpatient visits to the clinic in an already long total study duration. In our opinion, the lack of any alteration in drug levels over the first 24 h of coadministration with antihypertensives is a strong signal that pharmacokinetic interactions between the study drugs are unlikely.

We conclude from this study that adding fingolimod to a beta-blocker such as atenolol results in a moderately lower mean heart rate nadir compared with fingolimod alone. However, subjects who had a stronger negative chronotropic response to fingolimod alone (nadir<50 bpm) had minimal or no further reduction in heart rate when combined with atenolol. Adding fingolimod to a calcium channel blocker such as diltiazem did not further lower the heart rate compared to fingolimod alone.

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References

1. Brinkmann V, Lynch KR (2002) FTY720: targeting G-protein-coupled receptors for sphingosine 1-phosphate in transplantation and autoimmunity. Current Opin Immunol 14:569–575
2. Kappos L, Antel J, Comi G et al (2006) Oral fingolimod (FTY720) for relapsing multiple sclerosis. N Engl J Med 355:1124–1140
3. Kovarik JM, Schmouder RL, Slade AJ (2004) Overview of FTY720 clinical pharmacokinetics and pharmacology. Ther Drug Monit 26:585–587
4. Tedesco-Silva H, Pescovitz MD, Cibrik D et al (2006) Randomized controlled trial of FTY720 versus MMF in de novo renal transplantation. Transplantation 82:1689–1697
5. Koyrakh L, Lujan R, Colon J et al (2005) Molecular and cellular diversity of neuronal G-protein-gated potassium channels. J Neurosci 25:11468–11478
6. Schmouder RL, Barilla D, Wang Y et al (2006) FTY720: placebo-controlled study of the effect on cardiac rate and rhythm in healthy subjects. J Clin Pharmacol 46:895–904
7. Kovarik JM, Schmouder RL, Barilla D et al (2004) Single-dose FTY720 pharmacokinetics, food effect, and pharmacological responses in healthy subjects. Br J Clin Pharmacol 57:586–591
8. Heel RC, Brogden RN, Speight TM et al (1979) Atenolol: a review of its pharmacological properties and therapeutic efficacy in angina pectoris and hypertension. Drugs 17:425–460
9. McAuley BJ, Schroeder JS (1982) The use of diltiazem hydrochloride in cardiovascular disorders. Pharmacotherapy 2:121–133