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First-in-Human Study to Assess the Safety, Tolerability, and Pharmacokinetics of Pirepemat, a Cortical Enhancer, in Healthy Volunteers

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Abstract

Pirepemat (IRL752) is a cortical enhancer being developed for the prevention of falls in patients with Parkinson disease. This first-in-human, randomized, double-blind, placebo-controlled phase 1 study evaluated safety, tolerability, and pharmacokinetics (PK) of pirepemat administered as oral single ascending doses (10, 35, 75, 175, 350 mg) and multiple ascending doses (100 and 250 mg 3 times daily) for 7 days to healthy male volunteers. Twenty and 24 subjects were randomly assigned in the single ascending dose and multiple ascending doses parts of the study, respectively. Pirepemat was generally well tolerated, although an increased frequency of adverse events of mild intensity within nervous system disorders (headache and dizziness) was seen after administration of 350 mg as a single dose and after multiple doses of 100 and 250 mg. PK of pirepemat showed a linear relationship over the dose range studied and exhibited dose proportionality after multiple-dose administration. Accumulation after 7 days of multiple dosing was minor. Absorption was rapid, with a median time to maximum concentration of 2.0 hours on day 1 and day 7 (100 and 250 mg) and a mean terminal half-life between 3.7 and 5.2 hours. Food intake had no (obvious) impact on PK. The results support 3-times-daily dosing and further clinical development.

Keywords

first-in-human, healthy volunteers, Parkinson disease, pharmacokinetics, pirepemat

The first symptoms of Parkinson disease (PD) are typically motor symptoms such as tremor, muscle rigidity, and slowness of movement. However, nonmotor features of PD are prominent in this complex neurologic disease, with symptoms worsening as the disease progresses.[1] In late stages of PD, nonmotor features include a range of symptoms from autonomic dysfunction and dysphagia to dementia and emotional problems.[1,2] With advancement of the disease, axial motor symptoms may also come to dominate the motor presentation.[3] While levodopa is the most widely used treatment for PD, late-stage axial motor symptoms and nonmotor symptoms are generally levodopa resistant and contribute significantly to disability and morbidity.[2]

Axial symptoms in the form of postural instability and freezing of gait are strongly associated with decreased mobility and frequent falls and correlate

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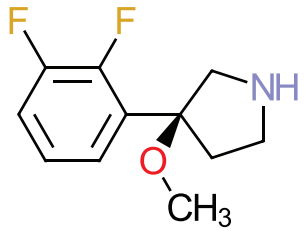

Figure 1. Chemical structure of pirepemat.

with the severity of the disease.[4,5] Correlation between other motor symptoms and nigral dopaminergic impairment has previously been shown but less so with postural instability.[6] Similarly to nonmotor features such as cognitive impairment, postural instability and falls are not reversible with dopaminergic therapy.[7] Axial motor deterioration and cognitive function are further interconnected, with a possible cause being PD patients' use of attentive strategies to compensate for postural dysfunction.[8]

Several nondopaminergic neuropathologic features that might account for these symptoms have been identified in PD, including loss of neurons causing norepinephrine and acetylcholine deficiency.[9,10] It is reasonable to think that symptoms resistant to dopaminergic treatment are associated with deficient cortical neurotransmission, albeit underlying pathologies are believed to be heterogeneous and related to complex interactions among cortical neurotransmitters.[11]

Pirepemat (Figure 1), (3S)-3-(2,3-difluorophenyl)-3-methoxypyrrolidine (also known as IRL752), is a cortical enhancer being developed to treat postural dysfunction and cognitive impairment in PD. Pirepemat was discovered by a process referred to as the integrative screening process and belongs to a class of compounds that elicit regional preference for increases in catecholamine (dopamine and norepinephrine) levels in the frontal cortex.[12] The pharmacologic profile of pirepemat has been found to be consistent with a cortically regioselective facilitatory impact on cortical neurotransmission, as well as cognitive impairment–reversing features.[13] In vitro neurotarget affinity and functional data suggest 5-hydroxytryptamine 7 receptor and α2C-adrenoceptor antagonism are key contributors to the in vivo efficacy profile of pirepemat.[13]

In vivo pharmacokinetics (PK) of pirepemat in rats and dogs is characterized by complete and rapid absorption, wide tissue distribution including the central nervous system, and log-linear elimination. Pirepemat is eliminated through a combination of renal excretion of the parent compound and metabolic transformation involving cytochrome P450 (CYP) oxidations and formation of glucuronide conjugates. In vitro CYP reaction phenotyping was done by established methods (Admescope Ltd, Oulu, Finland) using 8 recombinant human isozymes. The results revealed that CYP2A6, CYP2B6, and CYP2D6 metabolizes pirepemat by oxidations. However, only CYP2A6 and CYP2B6 gave rise to the main observed human plasma metabolites, M11b and M12, whereas CYP2D6 mainly metabolized pirepemat to M11a, which is found as a minor metabolite in human plasma (Integrative Research Laboratories Sweden AB, data on file). Uridine 5′-diphosphoglucuronosyltransferase reaction phenotyping has not been done.

The objective of this first-in-human study in healthy volunteers was to assess safety, tolerability, and PK of single (SADs) and multiple ascending doses (MADs) of pirepemat, to support further clinical development. Furthermore, this study aimed to use an adaptive design and integrated protocol to increase learning without undermining the safety or quality of the study.

Method

Study Design

The study (EudraCT 2015-004479-61) was approved by the Regional Ethics Review Board in Uppsala (as of 2019 part of the Swedish Ethical Review Authority) and was conducted at CTC Clinical Trial Consultants AB (Uppsala, Sweden). Informed consent was provided by all subjects before participation in any study-related procedures.

This was a first-in-human, randomized, double-blind, placebo-controlled, phase 1 study evaluating the safety, tolerability, and PK of SADs and MADs of pirepemat in healthy male volunteers. The study was designed and conducted per the Declaration of Helsinki and International Conference on Harmonization/Good Clinical Practice guidelines. The study consisted of SAD and MAD parts. Planned enrollment for both parts was 40 subjects (actual enrollment was 44 subjects due to withdrawals and replacements). Pirepemat (fumarate salt) and placebo were administered as oral capsules and were of identical appearance (capsule strengths 10, 25, and 50 mg pirepemat free base, corresponding to 15.4, 38.6, and 77.2 mg fumarate salt). Capsules were swallowed with 240 mL of tap water. All doses in the SAD part were administered in the fasted condition except for the third-dose period for cohort 2.

The SAD part consisted of 2 cohorts (1 and 2) of 8 subjects. An alternate panel design with within-subject dose escalation was used, where subjects received sequentially increasing oral doses. Within each cohort, subjects were randomized to receive pirepemat or placebo in different sequences (Table 1). At each dose level, 6 subjects received pirepemat and 2 subjects received placebo in a 3:1 ratio. A sentinel dosing strategy was used in all SAD cohorts, where 2 subjects were initially dosed (1 pirepemat, 1 placebo) and closely

Table 1. Dosing Schedule—SAD Part

Cohort/Dose	Sequence Number	Cohort 1 Dose 1	Cohort 2 Dose 1	Cohort 1 Dose 2	Cohort 2 Dose 2	Cohort 1 Dose 3	Cohort 2 Dose 3
Cohort 1 (n = 8)	1:1 (n = 2)	Placebo		75 mg		350 mg	
	1:2 (n = 2)	10 mg		75 mg		Placebo	
	1:3 (n = 2)	10 mg		75 mg		350 mg	
	1:4 (n = 2)	10 mg		Placebo		350 mg	
Cohort 2 (n = 8)	2:1 (n = 2)		Placebo		175 mg		175 mg[a]
	2:2 (n = 2)		35 mg		175 mg		175 mg[a]
	2:3 (n = 2)		35 mg		175 mg		Placebo[a]
	2:4 (n = 2)		35 mg		Placebo		175 mg[a]

SAD, single ascending dose.
[a]Standardized breakfast meal given within 60 minutes before pirepemat/placebo administration.

observed for 24 hours before proceeding to dose remaining subjects.

SAD cohort 1 received 10-, 75-, and 350-mg pirepemat or placebo. SAD cohort 2 received 35-, 175-(fasted), and 175-mg (after a standardized breakfast) pirepemat or placebo. The standardized high-fat, high-calorie breakfast consisted of eggs, bacon, rösti (potato pancake), 2 slices of bread with butter, 1 glass (2.3 dL) of whole milk, and coffee or tea. Single doses for individual subjects were separated by a washout period of at least 2 weeks. There was an interval of at least 1 week between each dose level to allow time for safety data and PK to be reviewed by a safety review committee. Subjects were confined to the research clinic from the evening before each dosing until 24 hours after dosing (8 hours for the food-effect period) and, with the exception of the 10-mg level, returned 48 hours after dosing for PK sampling. A follow-up visit was conducted for each cohort 5 to 10 days after dosing.

In the MAD part of the study, subjects were administered oral doses of pirepemat or placebo 3 times daily for 7 consecutive days. Two cohorts (1 and 2) of 12 subjects were included. Nine subjects in each cohort received pirepemat, and 3 subjects received placebo in a 3:1 ratio. MAD cohort 1 and cohort 2, respectively, received 100- or 250-mg pirepemat (or matching placebo) 3 times daily. At the 250-mg dose level, sentinel dosing was used. There was an interval of at least 1 week between cohorts to allow time for safety data and PK to be reviewed. Subjects participating in the MAD part were confined to the research clinic from the evening before the first dosing until 24 hours following the last dose on day 7. A follow-up visit was conducted 5 to 10 days after the last dose.

Dose Selection

The starting dose was based on allometric scaling/human equivalent dose calculations, as determined by toxicology studies in rats and dogs. A starting dose of 10 mg corresponded to <5% of the estimated no-observed-adverse-effect level in the most sensitive species and <25% of the predicted pharmacologically active dose.

Based on PK and safety data from the SAD part and exposures in preclinical studies, it was estimated that 100 and 250 mg administered 3 times daily would cover pharmacologically relevant plasma exposures within a safe range in terms of safety margins as determined in the toxicity studies.

Study Population

Healthy male volunteers aged 18 to 50 years and with a body mass index of 18 to 30 kg/m^2 were included. All subjects were in good health, as determined at the screening visit and with no history of clinically significant disease or disorder. Subjects were nonusers of nicotine products and with no use of concomitant medication. For the MAD part, 2 validated questionnaires (Generalized Anxiety Disorder 7-item scale[14] and Patient Health Questionnaire[15]) were used at screening so as not to include subjects with pronounced symptoms of anxiety or depression.

Safety Assessments

Safety assessments for both parts comprised recording adverse events (AEs), physical examinations, 12-lead electrocardiograms, vital signs (blood pressure, heart rate, body temperature), and clinical laboratory measurements. In the SAD part, continuous electrocardiogram telemetry was used for cardiac surveillance up to 24 hours after each dose. In the MAD part, vital signs also included respiratory rate and the Columbia-Suicide Severity Rating Scale[16] was used to assess suicidal thoughts and behaviors before the first dose and before leaving the clinic after the last dose. AEs were coded according to the Medical Dictionary for

Regulatory Activities (MedDRA) version 19.0, and the grading of AEs followed Common Terminology Criteria for Adverse Events version 4.03, also taking into account the safety grading system proposed by Sibille et al.[17]

Pharmacokinetic Assessments and Methods

Venous blood samples were collected for determination of pirepemat concentrations. In the SAD part, samples were collected before dosing and at multiple time points (20 minutes; 40 minutes; and 1, 2, 3, 4, 6, 8, 10, and 12 hours) during the day of dosing, as well as 24 (8 for food-effect period) and 48 (except for 10-mg period) hours after dosing. In the MAD part, PK samples were collected before the first dose and before the morning dose, respectively, on days 1 and 7 and at multiple time points (20 minutes; 40 minutes; and 1, 2, 3, 4, 5, 6, 8, 12 hours [day 7], and 14 hours) during days 1 and 7. The last PK sample was obtained 24 hours after the morning dose on day 7.

The PK assessments were performed using pirepemat (supplied by Syntagon AB, Södertälje, Sweden) as the reference material and as internal standard IRL921 x oxalate [pirepemat-d^3 x oxalate] (supplied by Integrative Research Laboratories Sweden AB, Gothenburg, Sweden). Samples for determination of pirepemat concentrations in plasma were analyzed by the Swedish National Veterinary Institute (Uppsala, Sweden), by means of a validated liquid chromatography–tandem mass spectrometry method. Samples were thawed, vortex mixed, and centrifuged. To 100 μL of study sample, 100 μL of acetonitrile:water (1:9) and 600 μL of precipitation solution containing internal standard were added, before being injected into a chromatographic system. Instrumentation consisted of an Acquity UPLC system coupled to a Xevo-TQ-S tandem quadrupole mass spectrometer (Waters Corp., Milford, Massachusetts). A positive electrospray ionization technique was used. The chromatographic column was Waters Acquity UPLC BEH C18 (50×2.1 mm length × inner diameter, particle diameter 1.7 μm). Mobile phases consisted of (1) 0.1% formic acid in water and (2) 0.1% formic acid in acetonitrile. The total run time was 2.0 minutes including washing and equilibration of the column. The auto-sampler was programmed to inject 1.0 μL of sample. Multiple reaction monitoring mass transitions were m/z 214/182 and m/z 217/182 for pirepemat and its internal standard, respectively. The molecular weight of pirepemat is 213.2 g/mol. Calibration curves for pirepemat ranged from 12.0 to 12 000 nM (2.6–2600 ng/mL) in human plasma, and the lower limit of quantitation in plasma was 12.0 nM using 100-μL sample volume.

A total of 1158 human plasma samples were analyzed in 33 accepted runs on 17 different days. All runs fulfilled the quality control (QC) criteria stating that at least two-thirds of the QC samples in each run must have determined concentration values within 15% of their respective nominal values and at least 1 QC sample for each level must have a determined value within this interval. In each run, at least three-quarters of the calibration samples had to be within 15% of their nominal values when back-calculated (except for the lowest point, which may differ 20%). Within-run mean accuracy (% bias) ranged from –1.9% to 2.8%, and precision (% coefficient of variation [CV]) was ≤4.71%. Between-run mean accuracy (% bias) ranged from –1.0% to 1.7% and precision (%CV) was ≤5.80%.

PK analyses were conducted using noncompartmental analysis with Phoenix WinNonlin version 6.3 or higher (Pharsight Corp., Certara Inc., Princeton, New Jersey). PK parameters included terminal half-life ($t_{1/2}$), maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), area under the plasma concentration–time curve (AUC) from time zero to infinity ($AUC_{0\text{-inf}}$), AUC from time 0 to last time with quantifiable concentration (AUC_t), and AUC during a dose interval at steady state (AUC_{ss} [$AUC_{0\text{-6h}}$ on day 7 MAD]), terminal elimination rate constant, and total apparent clearance of drug from plasma.

Statistical Analysis

Statistical calculations were performed using SAS Version 9.4 (SAS Institute Inc., Cary, North Carolina). The statistical analyses include descriptive statistics reflecting the explorative nature of the study. No formal sample size calculation was performed for this study. The size of the cohorts/dose groups was considered sufficient to provide adequate information on the safety and PK parameters for the purposes of this study.

The data for subjects receiving placebo are presented pooled across groups.

Continuous data were summarized by cohort/dose group using descriptive statistics. Relative bioavailability after fed and fasting conditions was determined from AUC from time 0 to 8 hours and C_{max} after the corresponding 175-mg doses in cohort 2. Values were log-transformed (natural logarithm).

Dose proportionality and the proportional constant was estimated using the random coefficients model: $\log(parameter) = \log(a) + S + (b + k) * \log(dose)$ where a is a constant, S is a random intercept, b is the proportional constant, and k is the random slope effect. The accumulation ratio for each subject was calculated by taking the AUC_{ss} on day 7 of dosing, divided by $AUC_{0\text{-6h}}$ for the first dose interval during day 1.

Results

Demographics

In total, 44 male subjects were enrolled in the study (Table 2). Twenty subjects were enrolled in the SAD part (2 subjects in each SAD cohort were withdrawn

Table 2. Demographics and Baseline Characteristics—SAD and MAD Parts

SAD		Cohort 1 (n = 10)	Cohort 2 (n = 10)	Total (n = 20)	
Age, y, mean (SD)		27.4 (6.1)	29.4 (8.7)	28.4 (7.4)	
Sex, n (%)					
	Male	10 (100)	10 (100)	20 (100)	
Ethnicity, n (%)					
	Hispanic or Latino	1 (10)	1 (10)	2 (10)	
	Not Hispanic or Latino	9 (90)	9 (90)	18 (90)	
Weight, kg, mean (SD)		75.12 (8.88)	75.16 (9.76)	75.14 (9.08)	
Height, cm, mean (SD)		176.3 (9.3)	176.3 (9.3)	177.6 (7.1)	
BMI, kg/m^2, mean (SD)		23.5 (2.9)	24.2 (2.7)	23.8 (2.7)	
MAD		100 mg (n = 9)	250 mg (n = 9)	Placebo (n = 6)	Total (n = 24)
Age, y, mean (SD)		33.4 (11.0)	32.9 (11.4)	31.5 (11.0)	32.8 (10.7)
Sex, n (%)					
	Male	9 (100)	9 (100)	6 (100)	24 (100)
Ethnicity, n (%)					
	Hispanic or Latino	0	1 (11.1)	0	1 (4.2)
	Not Hispanic or Latino	9 (100)	8 (88.9)	6 (100)	23 (95.8)
Weight, kg , mean (SD)		82.3 (11.3)	81.9 (8.6)	82.0 (15.3)	82.1 (11.0)
Height, cm, mean (SD)		182.4 (8.0)	182.9 (9.3)	179.4 (7.8)	181.8 (8.2)
BMI, kg/m^2, mean (SD)		24.8 (3.1)	24.5 (2.6)	25.3 (3.5)	24.8 (2.9)

BMI, body mass index; MAD, multiple ascending dose; SAD, single ascending dose; SD, standard deviation.

and replaced due to withdrawal of consent) and 24 subjects in the MAD part. One subject in SAD cohort 1 received only 2 doses before being withdrawn and not replaced due to an AE not related to study treatment. Mean age among subjects participating in the SAD part was 28.4 years and in the MAD part 32.8 years. No major differences across cohorts or dose groups were seen.

Safety and Tolerability

In the SAD part, a total of 22 treatment-emergent adverse events (TEAEs) were reported by 8 subjects (Table 3). The number of subjects experiencing any TEAE in relation to 10, 35, 75, 175, and 350 mg pirepemat and placebo was 2, 2, 2, 1, 4, and 2, respectively. Of the TEAEs reported, 12 of 22 (54.5%) occurred after administration of 350-mg pirepemat. Most events (16/22; 72.7%) were assessed as not related to study treatment, and 19 of 22 (86.4%) were of mild intensity. One subject experienced 2 serious adverse events (SAEs) assessed as not related to study treatment 6 days after administration with 350-mg pirepemat (see below). One AE assessed as not related to study treatment led to withdrawal.

In the MAD part, a total of 22 TEAEs were reported, and 20 of 22 (90.9%) were of mild intensity. No severe events were reported. No SAEs occurred, and no AEs led to withdrawal. TEAEs were experienced in all dose groups, including placebo. The proportion of subjects experiencing any TEAE at doses of 100 mg and 250 mg and placebo was 2 of 9 (22.2%), 6 of 9 (66.7%), and 2 of 6 (33.3%), respectively. Following administration of 100-mg pirepemat, 4 of 6 AEs reported (66.7%) were assessed as at least possibly related to study treatment, and 8 of 12 AEs (66.7%) following administration of 250 mg were assessed as at least possibly related. All events after placebo administration were assessed as not related.

During both SAD and MAD, the most frequently represented TEAEs were nervous system disorders (headache and dizziness being the most frequently reported events). One subject in SAD cohort 1 experienced 2 SAEs (concussion following syncope) assessed as not related to study treatment 6 days after the 350-mg dose. The subject presented with nasopharyngitis the day after the last dose (350 mg), followed by mild pyrexia. Six days after the last dose, he fainted while brushing his teeth at night and hit his head, developing a concussion. He was hospitalized for observation but did not require any treatment or intervention. It was revealed that he had once before experienced a similar episode of syncope while having a viral infection. One subject was withdrawn due to mild hypertension assessed as not related to study treatment before the planned third single dose. The last single dose administered before withdrawal was placebo.

In the MAD part of the study, there were no SAEs or withdrawals. One subject in the 100-mg dose group presented with transient moderate hypertension after

Table 3. Summary of number of subjects reporting TEAEs—SAD and MAD Parts

SAD	10 mg (n = 6)	35 mg (n = 6)	75 mg (n = 6)	175 mg (n = 6)	175 mg[a] (n = 6)	350 mg (n = 5)	Placebo (n = 12)	Total (n = 20)
Headache	0	1 (16.7)	1 (16.7)	0	0	3 (60.0)	0	4 (20.0)
Dizziness	0	0	0	0	0	2 (40.0)	0	2 (10.0)
Presyncope	0	1 (16.7)	0	0	0	0	0	1 (5.0)
Syncope	0	0	0	0	0	1 (20.0)	0	1 (5.0)
Nasopharyngitis	2 (33.3)	0	1 (16.7)	0	0	2 (40.0)	1 (8.3)	3 (15.0)
Pyrexia	0	0	0	0	0	1 (20.0)	0	1 (5.0)
Concussion	0	0	0	0	0	1 (20.0)	0	1 (5.0)
Nervousness	0	0	0	0	0	1 (20.0)	0	1 (5.0)
Epistaxis	0	0	0	1 (16.7)	0	0	0	1 (5.0)
Hypertension	0	0	0	0	0	0	1 (8.3)	1 (5.0)

MAD	100 mg (n = 9)	250 mg (n = 9)	Placebo (n = 6)	Total (n = 24)
Headache	1 (11.1)	0	1 (16.7)	2 (8.3)
Disturbance in attention	0	1 (11.1)	0	1 (4.2)
Dizziness	0	1 (11.1)	0	1 (4.2)
Diarrhea	1 (11.1)	0	0	1 (4.2)
Dyspepsia	0	1 (11.1)	0	1 (4.2)
Nausea	0	1 (11.1)	0	1 (4.2)
Nasopharyngitis	1 (11.1)	2 (22.2)	0	3 (12.5)
Hyperacusis	1 (11.1)	0	0	1 (4.2)
Fatigue	0	0	1 (16.7)	1 (4.2)
Hepatic enzyme increased	0	1 (11.1)	0	1 (4.2)
Hypertension	1 (11.1)	0	0	1 (4.2)

MAD, multiple ascending dose; SAD, single ascending dose.
Variants are presented as n (%).
[a]Standardized breakfast meal given within 60 minutes before pirepemat. Each subject can be counted more than once in this table.

the first dose on day 3. The study drug was interrupted and resumed the next morning on day 4 without reoccurrence of hypertension. Increase of hepatic enzyme values was reported at the follow-up visit for 1 subject treated with multiple doses of 250 mg. There were no other clinically significant abnormalities involving laboratory values, electrocardiograms, or physical examinations.

Pharmacokinetics

For the dose range 10- to 350-mg pirepemat (single dose, fasted) the mean $t_{1/2}$ ranged from 3.4 to 3.9 hours, and t_{max} was approximately 2 hours. The mean plasma concentration increased with increasing single doses (Figure 2; Table 4). When pirepemat was given in the fed condition, the median t_{max} was 3.0 hours. Four subjects received 175-mg pirepemat under both fasted and fed conditions. The geometric mean ratio (fed/fasted) was 99.8% (90% confidence interval [CI], 84.96-117.13) for AUC from time 0 to 8 hours and 99.7% (90%CI, 80.71-123.18) for C_{max}.

Analysis of dose linearity after single dosing for $AUC_{0\text{-inf}}$ and AUC_t showed a linear relationship with a proportionality constant of 1.13 (90%CI, 1.07-1.19) for $AUC_{0\text{-inf}}$ and 1.17 (90%CI, 1.11-1.22) for AUC_t, indicating exposure slightly greater than dose proportional, with 90%CIs above and not including 1.0. Dose linearity based on C_{max} after single dosing was not possible due to too many likelihood evaluations. Following multiple dosing, the proportionality constants for AUC_{ss} and C_{max} were 1.18 (90%CI, 0.97-1.40) and 1.11 (90%CI, 0.94-1.29), respectively, with 90%CIs including 1.0.

The mean accumulation ratio ($AUC_{0\text{-6h,Day 7}}$/$AUC_{0\text{-6h,Day 1}}$) after 7 days of dosing was 1.31 (standard deviation, 0.17) for 100 mg and 1.33 (standard deviation, 0.20) for 250 mg, suggesting only minor accumulation from first to last dose.

Discussion

There is an unmet medical need for PD patients with symptoms resistant to dopamine replacement therapy.[2] Pirepemat is a first-in-class, small-molecule cortical enhancer, increasing noradrenaline and dopamine as well as immediate early genes including activity-regulated cytoskeleton-related protein mRNA in the frontal

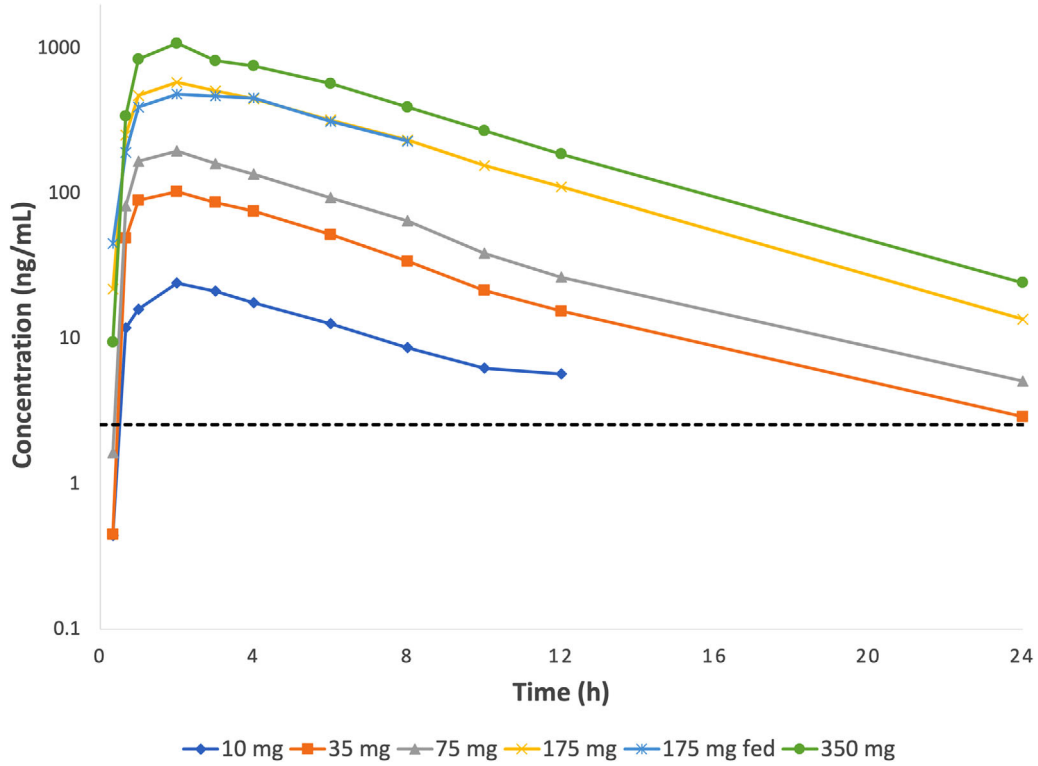

Figure 2. Mean plasma pirepemat concentration-time plots for the SAD part, by treatment. Semilogarithmic scale. Dotted line represents the lower limit of quantification of 2.6 ng/mL (12 nmol/L). For 10 mg, all 24-hour PK samples were below limit of quantification. For 175 mg fed (after standardized breakfast), last PK sample taken was 8 hours after dosing. PK, pharmacokinetic; SAD, single ascending dose.

cortex.[12] Arc is a key regulator of synaptic plasticity, triggered by synaptic N-methyl-D-aspartate receptor–related mechanisms[18] and implicated in memory-related processes and cognitive functions.[19,20] The results of this study enabled a recently published phase 2a study with pirepemat in patients, where exploratory analysis of efficacy outcomes indicated a possible effect on predominant symptoms in advanced stages of PD.[21]

Study Design

This first-in-human study was conducted with the primary aim to evaluate safety and tolerability of single and multiple ascending doses of pirepemat when administered to healthy male subjects. An integrated protocol was used, combining SAD and MAD as well as drug-food interaction. Protocols that combine more than 1 element in early clinical trials have now become common practice. Integrated protocols are considered to be time saving and cost efficient as compared to conducting separate trials. Generally, increased trial complexity might nevertheless increase the number of substantial amendments due to interim result requirements or other warranted substantial changes based on emerging data.[22,23]

This protocol allowed for an adaptive approach. Dose escalation between cohorts was guided by the recommendations from the safety review committee, based on available safety, tolerability, and PK data. A defined decision-making process outlined dose levels for the MAD part based on results from the SAD part. However, a substantial amendment between the SAD and MAD parts was required due to unexpected PK results. Initially, once-daily administration of pirepemat/placebo for 10 days was planned for the MAD part. Preliminary PK data from the SAD part showed that exposures achieved after single doses were lower than predicted from animal data, with $t_{1/2}$ being shorter than expected. Based on these findings and available safety data, the dosing regimen was revised, and a substantial amendment was submitted to allow for 3-times-daily dosing for 7 days.

In the SAD part, an alternate panel/crossover design was used where each of the 2 cohorts received every other dose. During the dosing of each cohort, the other was in washout. As a result, substantially fewer subjects were required. It has previously been concluded that an alternate panel design can be preferable over sequential panel designs in assessing dose proportionality and can increase first-in-human trial efficiency without

Table 4. Pharmacokinetic Parameters After SADs and MADs of Pirepemat

SAD

		10 mg (n = 6)	35 mg (n = 6)	75 mg (n = 6)	175 mg (n = 6)	175a mg (n = 6)	350 mg (n = 5)
$t_{1/2}$, h							
	Mean (SD)	3.7 (0.9)	3.6 (0.6)	3.4 (0.9)	3.9 (0.2)	3.8 (0.4)	3.9 (0.5)
t_{max}, h							
	Median (min-max)	2.0 (0.7-2.0)	2.0 (0.7-2.0)	2.0 (1.0-2.2)	1.5 (1.0-2.0)	3.0 (1.0-4.0)	2.0 (1.0-2.2)
C_{max}, ng/mL							
	Mean (SD)	25.5 (7.55)	108 (22.0)	224 (110)	620 (112)	568 (140)	1130 (248)
	Geometric mean (%CV)	24.3 (37.4)	106 (19.5)	206 (43.6)	612 (17.5)	554 (25.1)	1110 (25.6)
$AUC_{0\text{-}inf}$, ng • h/mL							
	Mean (SD)	172 (74.1)	705 (181)	1290 (610)	4510 (1060)	4400 (1290)	7750 (2340)
	Geometric mean (%CV)	160 (44.3)	687 (24.9)	1180 (49.3)	4420 (23.0)	4280 (28.6)	7380 (39.6)
AUC_t, ng • h/mL)							
	Mean (SD)	145 (53.2)	659 (197)	1250 (607)	4440 (1030)	2820 (661)	7610 (2270)
	Geometric mean (%CV)	136 (41.1)	636 (28.8)	1140 (50.2)	4340 (22.7)	2770 (21.3)	7250 (38.9)
CL/F, L/h							
	Mean (SD)	67.2 (28.1)	52.2 (12.3)	69.3 (30.7)	40.5 (8.7)	41.9 (11.0)	50.7 (23.4)
	Geometric mean (%CV)	62.5 (44.3)	51.0 (24.9)	63.5 (49.3)	39.6 (23.0)	40.9 (28.6)	47.5 (39.6)

MAD

		100 mg		250 mg	
		Day 1 (n = 9)	Day 7 (n = 9)	Day 1 (n = 9)	Day 7 (n = 9)
$t_{1/2}$, h					
	Mean (SD)	3.7 (0.9)	3.9 (1.0)	4.2 (0.7)	5.2 (1.9)
t_{max}, h					
	Median (min-max)	2.0 (0.4-4.0)	2.0 (0.7-4.0)	2.0 (0.7-5.0)	2.0 (1.0-4.0)
C_{max}, ng/mL					
	Mean (SD)	257 (43.0)	327 (58.8)	701 (144)	911 (194)
	Geometric mean (%CV)	254 (17.7)	322 (18.2)	688 (21.0)	893 (21.5)
$AUC_{0\text{-}6h}$, ng • h/mL					
	Mean (SD)	1050 (156)	1340 (245)	2930 (577)	4020 (1020)
	Geometric mean (%CV)	1040 (15.2)	1320 (18.8)	2870 (20.1)	3910 (25.7)
CL/F, L/h					
	Mean (SD)	57.9 (16.1)	76.7 (14.6)	49.2 (11.6)	65.7 (16.4)
	Geometric mean (%CV)	56.2 (27.3)	75.5 (18.8)	48.1 (23.2)	63.9 (25.7)

$AUC_{0\text{-}6h}$, area under the curve from time zero to 6 hours after dosing; $AUC_{0\text{-}inf}$, area under the plasma concentration-time curve from time zero to infinity; AUC_t, area under the curve from time zero to last time with quantifiable concentration; C_{max}, maximum plasma concentration; CL/F, total apparent clearance of drug from plasma; %CV, geometric coefficient of variation (%); MAD, multiple ascending dose; SAD, single ascending dose; SD, standard deviation; $t_{1/2}$, terminal half-life; t_{max}, time of occurrence of C_{max}.

[a]Standardized breakfast meal given within 60 minutes before pirepemat and last pharmacokinetic sample taken was 8 hours after dosing.

compromising on safety concerns.[24] We demonstrate that an alternate panel design where placebo administration rotates among subjects, demands fewer subjects while maintaining the benefits of including placebo treatment.[25] Even so, alternate panel designs have been less frequent in first-in-human studies in healthy volunteers,[26] and to our knowledge they still are.

Two subjects in each SAD cohort were replaced after the first treatment period due to withdrawal of consent, and 1 subject was withdrawn due to an AE and not replaced (AE was unrelated to study treatment). A total of 15 subjects completed the SAD part of the study. All 24 subjects randomized in the MAD part completed the study. Including delay due to submission and regulatory

approvals of the substantial amendment, time between first-subject-first-visit and last-subject-last-visit was 9 months and 5 days.

Since no data on reproductive toxicology was available, only males were recruited. Given the short duration of study participation, including women and women of childbearing potential could possibly have been considered, taking sufficient precautions to prevent pregnancy.

Safety and Tolerability

Overall, pirepemat was well tolerated at single doses up to 350 mg and multiple doses up to 250 mg 3 times daily for 7 days. An increased frequency of central nervous system–related AEs, headache, and dizziness, was seen at the highest SAD dose level (350 mg) and during treatment with multiple doses of pirepemat, as compared to placebo. These AEs were transient and mild in intensity.

The 2 associated SAEs reported during the follow-up period (syncope and concussion) occurred in the same subject in SAD cohort 1 and was not assessed as related to the study drug.

One single event of hypertension was reported for 1 subject receiving multiple doses of 100 mg. An ad hoc evaluation by the safety review committee was made, leading to a temporary interruption of treatment. The event was assessed as related to the study treatment, though the subject recovered; treatment was resumed the next day and hypertension did not reoccur for the remainder of the study. There were no other cardiovascular AEs in the study. Transient increase of hepatic enzymes was observed for 1 subject in the 250-mg MAD cohort at the follow-up visit with alanine aminotransferase 3.05 times the upper limit of normal (ULN) and aspartate aminotransferase 3.85 times the ULN. Subject's baseline values were also above the ULN.

In the recent phase 2a study in patients with PD, pirepemat was administered for 28 days. AEs were consistent with findings in this study, with predominantly mild and transient central nervous system–related AEs during the first 2 weeks and a few patients with transient and reversible mild to moderate increases in liver enzymes following discontinuation of treatment.[21]

Pharmacokinetics

For the dose range tested, pirepemat was rapidly absorbed and eliminated from plasma. For subjects receiving pirepemat both under fasted and fed conditions, no (obvious) food interaction was observed. Timing of drug intake in relation to food intake was concluded to be of less importance for the MAD part. Supraproportional exposure (AUC) was seen following single-dose administration based on 90%CI not including 1.0, though the proportional constant was 1.13, which is close to 1.0. Pirepemat did exhibit dose proportionality based on AUC_{ss} and C_{max} after multiple-dose administration. Accumulation after 7 days was minor.

Conclusions

Single and multiple doses of pirepemat were generally well tolerated in healthy male volunteers. The safety, tolerability, and PK profiles of this first-in-human study support 3-times-daily dosing and further clinical development.

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Conflicts of Interest

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Data-Sharing Statement

Data will be available from the corresponding author on reasonable request.

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