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RESEARCH ARTICLE

Isradipine plasma pharmacokinetics and exposure–response in early Parkinson's disease

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Abstract

Objectives: Isradipine is a dihydropyridine calcium channel inhibitor that has demonstrated concentration-dependent neuroprotective effects in animal models of Parkinson's disease (PD) but failed to show efficacy in a phase 3 clinical trial. The objectives of this study were to model the plasma pharmacokinetics of isradipine in study participants from the phase 3 trial; and, to investigate associations between drug exposure and longitudinal clinical outcome measures of PD progression. **Methods**: Plasma samples from nearly all study participants randomized to immediate-release isradipine 5-mg twice daily (166 of 170) were collected for population pharmacokinetic modeling. Estimates of isradipine exposure included apparent oral clearance and area under the concentration-time curve. Isradipine exposure parameters were tested for correlations with 36-month changes in disease severity clinical assessment scores, and time-to-event analyses for initiation of antiparkinson therapy. **Results**: Isradipine exposures did not correlate with the primary clinical outcome, changes in the antiparkinson therapy-adjusted Unified Parkinson's Disease Rating Scale parts I–III score over 36 months (Spearman rank correlation coefficient, r_s: 0.09, $P = 0.23$). Cumulative levodopa equivalent dose at month 36 was weakly correlated with isradipine plasma clearance (r_s: 0.18, $P = 0.035$). This correlation was sex dependent and significant in males, but not females. Those with higher isradipine exposure had decreased risk of needing antiparkinson treatment over 36 months compared with placebo (hazard ratio: 0.87, 95% CI: 0.78–0.98, $P = 0.02$). **Interpretation**: In this clinical trial, higher isradipine plasma exposure did not affect clinical assessment measures of PD severity but modestly decreased cumulative levodopa equivalent dose and the time needed for antiparkinson treatment initiation. **Trial Registration**: ClinicalTrials.gov NCT02168842.

Introduction

Parkinson's disease (PD) is the world's fastest-growing neurodegenerative disease, and will affect as many as 12 million people worldwide by 2040.[1] The major pathologic feature of PD is the degeneration of dopaminergic neurons in the substantia nigra (SN).[2] Striatal dopamine replacement with pharmacologic interventions is the mainstay of therapy, however, as the disease progresses, motor and nonmotor symptoms intensify, and function and quality of life decrease.[3] There are no therapeutic strategies at present to slow the progression of PD.

Brain-permeable dihydropyridine (DHP) calcium channel inhibitors demonstrate neuroprotective effects in neurotoxin and synuclein animal models of PD with sustained systemic drug delivery,[4] and are also associated with a reduced risk of developing PD in epidemiological studies.[5–7] Isradipine is a DHP calcium channel inhibitor

that is highly brain penetrant.[8] However, an immediate-release (IR) formulation of isradipine in a phase 3 clinical trial (STEADY-PD III) demonstrated no difference from placebo in slowing the clinical progression of PD.[9] Despite a single dose level tested in STEADY-PD III, the interindividual variability associated with isradipine pharmacokinetics presented the opportunity to test potential exposure–response relations, similar to those observed in preclinical studies.[10]

The objectives here were to develop a pharmacokinetic model describing isradipine plasma pharmacokinetics in STEADY-PD III trial participants; and, to perform exposure–response analyses between estimated isradipine plasma exposures with longitudinal efficacy and safety outcomes.

Methods

STEADY-PD III was a phase 3, multi-center, randomized, double-blind, placebo-controlled trial (NCT02168842) to determine the effects of isradipine on slowing the clinical progression of PD.[9] Study participants were randomized 1:1 to receive either IR isradipine 5-mg twice daily or placebo for a treatment duration of 36 months. Isradipine was supplied as 2.5-mg over-encapsulated capsules. Participants initiated study drug at 2.5-mg twice daily, and were titrated up to a 5-mg twice daily regimen (every 12 h) over 4–12 weeks. Dosage reductions were allowed for intolerability but participants were required to be on their final stabilized dose by the end of the titration period. Concomitant use of cytochrome P450 isoenzyme 3A inhibitors or inducers was not permitted. The trial protocol was approved by the ethics committee at the University of Rochester, and at each participating site. All participants provided written informed consent. The trial was performed in accordance with the principles of the Declaration of Helsinki.

Plasma samples for measurement of isradipine concentrations were collected from all randomized study participants. Samples were collected at screening to ensure the absence of isradipine administration outside of the trial, and at study months 3 and 6 following baseline visit. On the day of the 3-month visit, participants withheld their morning dose of study drug in order to collect a postdose sample approximately 12 h since last dose. At the same visit, a second plasma sample was collected approximately 2–3 h following an in-clinic dose of study drug. For the 6-month study visit, a plasma sample was collected 4–8 h after study drug intake.

Plasma concentrations of isradipine were measured using a validated ultra-performance liquid chromatography method with tandem-mass spectrometric detection (AIT Bioscience, LLC, Indianapolis, IN). The assay lower limit of quantitation was 0.100 ng/mL (Appendix S1).

Population pharmacokinetic modeling of plasma isradipine concentrations was performed using nonlinear mixed-effects modeling (NONMEM v7.4.0; ICON Development Solutions, Ellicott City, MD). One- and two-compartment models with linear absorption and elimination were evaluated with up to three transit compartments and a lag time for describing drug absorption. Interindividual variability was assumed log-normally distributed. Additive, proportional, and mixed residual variability functions were tested for quantifying unexplained variability. The final model was evaluated for goodness-of-fit, stability, and predictive performance (Appendix S1).

The pharmacokinetic model was used to estimate individual Bayesian estimates of apparent oral clearance (CL/F) for an individual. CL/F is the measure of the ability of the body to eliminate drug via metabolism and elimination following an oral dose with a bioavailability of *F*. CL/F is inversely related to the area under the concentration-time curve (AUC). Isradipine has linear pharmacokinetics in the dose-range examined, so CL/F can be considered to be constant for an individual; meaning that the clearance of drug does not change with the dose of drug.[11] Thus, the CL/F parameter is a reasonable indicator of one's overall elimination of isradipine during the course of the trial. The protocol allowed for participants to be on a dosage less than 10-mg daily for tolerability concerns. Thus, as a secondary exposure parameter of interest, AUC_{24} was calculated for each participant by dividing total daily dose of isradipine over CL/F.

Analyses of the relationships between isradipine plasma exposure with safety and efficacy outcomes included study participants who had an estimated plasma isradipine exposure and had the safety or efficacy measurements assessed at baseline and month 36. Spearman rank tests were performed to determine the correlation (r_s) between isradipine plasma exposure parameters and changes in efficacy outcomes from baseline visit to month 36, including: Unified Parkinson's Disease Rating Scale (UPDRS) parts I–III in the ON state (unadjusted and adjusted for current and cumulative use of antiparkinson therapy); UPDRS parts I–III at month 12 or when there was determination of need for antiparkinson therapy (whichever came sooner); UPDRS parts I, II, III, and IV; Movement Disorders Society UPDRS (MDS-UPDRS) nonmotor effects on daily living (EDL) scale; MDS-UPDRS motor EDL scale; ambulatory capacity scale; Schwab and England Activities of Daily Living; modified Rankin; 39-Item Parkinson's disease Questionnaire; and, differences in usage and cumulative use of antiparkinson medications

calculated as the levodopa equivalent dosages (LED). Separate analyses were also performed by sex.

Time to need antiparkinson medication in relation to isradipine exposure was assessed using Cox proportional hazards models stratified by clinical trial site, with low enrolling sites ($n < 4$) grouped together as previously described.[9] Values of CL/F and AUC_{24} were evaluated as evenly divided tertile groups: fast, middle, and slow for CL/F; and, low, middle, and high for AUC_{24}. The tertile groups were evaluated as a continuous parameter and as a nominal variable. The placebo arm participants ($n = 166$) were defined as the reference group. Separate proportional hazards models were also performed to adjust for age and sex.

Chi-square tests were used to study the difference in occurrences of dizziness and peripheral edema by CL/F and AUC_{24} tertile. Spearman tests assessed correlations between isradipine plasma exposures and changes in systolic and diastolic blood pressure readings. Statistical analyses were performed using SAS software (SAS Institute Inc. 2013. SAS/ACCESS® 9.4 Interface to ADABAS: Cary, NC).

Results

Plasma isradipine concentrations at screening were undetectable in all 336 randomized study participants. There were no detectable isradipine concentrations among the 166 participants randomized to placebo. There were 166 study participants from the assigned isradipine treatment group contributing 484 isradipine concentrations for pharmacokinetic modeling (Fig. A1). Baseline characteristics are in Table 1.

A two-compartment disposition model with first-order absorption and one transit compartment provided an optimal fit to the isradipine concentration-time data. Inclusion of age as a covariate on CL/F significantly improved model fit (objective function value change of −17.6, $P < 0.001$), and corresponded to a decrease in isradipine CL/F by ~11% for every 10-year increase in age (Fig. 1). Final pharmacokinetic parameter estimates are in Table 2. Goodness-of-fit plots and visual predictive check showed the model adequately described the observed concentrations (Fig. A2).

Table 1. Baseline demographic and disease characteristics overall and by CL/F tertile among participants randomized to isradipine treatment arm with pharmacokinetic data.

Characteristic	Overall ($n = 166$)	CL/F tertile groups		
		Fast ($n = 55$)	Middle ($n = 55$)	Slow ($n = 56$)
Mean age (SD), years	62.3 (8.7)	59.2 (9.9)	62.9 (6.5)	64.7 (8.5)
Men, *n* (%)	118 (71%)	45 (82%)	37 (67%)	36 (64%)
Non-Hispanic Ethnicity, *n* (%)	162 (98%)	53 (96%)	53 (96%)	56 (100%)
Race, *n* (%)				
White	156 (94%)	52 (94%)	53 (96%)	51 (91%)
Asian	5 (3%)	1 (2%)	–	4 (7%)
Black	3 (2%)	1 (2%)	1 (2%)	1 (2%)
American Indian	1 (1%)	1 (2%)	–	–
Unknown	1 (1%)	–	1 (2%)	–
Mean disease duration from diagnosis (SD), years	0.8 (0.7)	0.9 (0.6)	0.8 (0.7)	0.9 (0.8)
Receiving amantadine, *n* (%)	15 (9%)	3 (5%)	8 (15%)	4 (7%)
Receiving anticholinergic(s), *n* (%)	3 (2%)	2 (4%)	0	1 (2%)
Mean UPDRS score (SD)				
Total	23.5 (8.7)	24.8 (8.8)	21.7 (7.9)	24.2 (9.3)
Part I (mental)	0.6 (0.8)	0.4 (0.6)	0.7 (0.9)	0.7 (0.9)
Part II (ADL)	5.0 (2.9)	5.4 (3.0)	5.1 (3.0)	4.4 (2.6)
Part III (motor)	18.0 (7.3)	19.0 (7.1)	15.9 (6.8)	19.0 (7.8)
PIGD	0.2 (0.2)	0.2 (0.2)	0.2 (0.1)	0.2 (0.2)
Tremor	0.5 (0.3)	0.5 (0.3)	0.5 (0.3)	0.5 (0.3)
Mean Hoehn and Yahr stage (SD)	1.7 (0.5)	1.7 (0.5)	1.6 (0.5)	1.8 (0.4)
Mean Schwab and England ADL scale score (SD)	94.4 (5.2)	94.3 (5.1)	94.9 (4.9)	94.0 (5.8)
Mean modified Rankin score (SD)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)	1.1 (0.3)
Mean PDQ-39 total score (SD)	7.1 (6.2)	6.5 (5.5)	7.4 (6.0)	7.5 (6.9)
Mean systolic blood pressure (SD), mm Hg	127.8 (16.9)	126.7 (16.3)	129.1 (17.5)	127.7 (17.0)
Mean diastolic blood pressure (SD), mm Hg	76.5 (9.8)	76.9 (9.4)	78.3 (9.4)	74.5 (10.3)

ADL, activities of daily living; CL/F, apparent oral clearance; PDQ-39, 39-item Parkinson's Disease Questionnaire; PIGD, postural instability and gait disorder; UPDRS, Unified Parkinson's Disease Rating Scale.

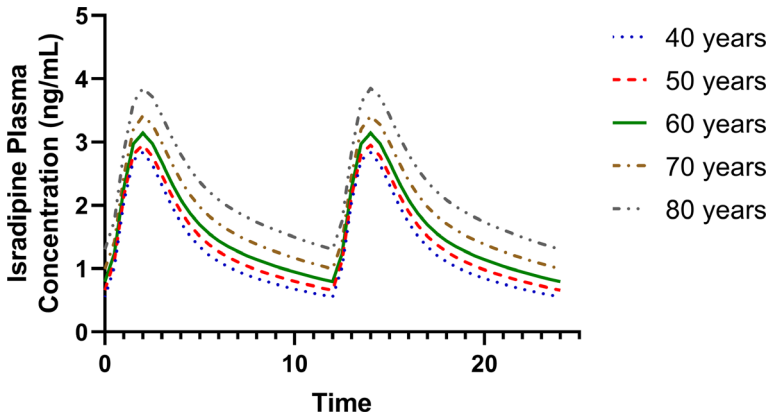

Figure 1. Simulated mean isradipine plasma concentration versus time curves at steady state by age. Simulations for each age decade were performed 1000 times. According to pharmacokinetic modeling simulations, average steady-state plasma maximum concentration (C_{max}) for a 60-year-old person would have been 3.24 ng/mL, while a 40-year-old and 80-year-old would have had values of 2.94 and 3.95 ng/mL, respectively.

Among the 166 participants with an estimated isradipine CL/F, there were up to 162 participants with clinical outcomes data at baseline and month 36. The primary outcome measure in the trial, change in UPDRS parts I–III from baseline to month 36 in the defined ON state, was not correlated with plasma isradipine CL/F or AUC_{24} (r_s = 0.005, P = 0.95) (Table 3). Similarly, there were no significant correlations between isradipine exposure parameters and changes in UPDRS parts I–III adjusted for antiparkinson therapy. Other secondary clinical scale outcome measures of disease severity were not correlated with exposures, except for an overall weak correlation between MDS-UPDRS nonmotor EDL scores and AUC_{24} (score worsened as AUC_{24} increased), and a correlation with CL/F among men (score worsened as CL/F decreased).

Table 2. Population pharmacokinetic model parameter estimates of isradipine from final model and bootstrap analysis.

Parameter	Population estimate (% relative standard error)	Bootstrap estimate [95% confidence interval]
K_{tr} (1/h)	1.68 (18%)	1.71 [1.01–2.36]
CL/F (L/h)	292 (5%)	290 [266–318]
V_c (L)	895 (22%)	824 [535–1255]
Q/F (L/h)	354 (18%)	347 [246–462]
V_p (L)	1320 (19%)	1401 [739–1905]
Age on CL/F (linear function)[1]	−4.15 (24%)	−4.18 [−5.94 to −2.35]
IIV on CL/F	0.207	0.203
IIV on V_c	1.45	1.69
Additive error	0.21	0.20

K_{tr}, transit compartment rate; CL/F, apparent oral clearance; V_c, apparent central volume of distribution; Q/F, apparent intercompartmental clearance; V_p, apparent volume of distribution; IIV, interindividual variability.

[1]The effects of different participant-level factors on isradipine PK were tested as covariates on model parameters that had shrinkage of no more than 30%. For continuous covariates, linear, power, and exponential functional forms were evaluated. Other covariates tested included sex, race, ethnicity, weight, body mass index, alkaline phosphatase, total bilirubin, and uric acid.

Among men, there was a modest statistically significant correlation between cumulative levodopa equivalent dose (milligram-years) required through the 36-month visit and isradipine CL/F (r_s = 0.18, P = 0.035). This correlation was driven by the values in men (r_s = 0.24, P = 0.015), and the correlation among women was in the opposite direction and not statistically significant (r_s = −0.24, P = 0.16). A linear regression model also revealed CL/F of isradipine to be an independent predictor of cumulative LED over 36 months (β coefficient = 0.47; P = 0.01); that is, cumulative LED would increase by approximately 5 mg for every 10 L/h increase in plasma CL/F of isradipine.

Time to first initiation of antiparkinson therapy was evaluated in relation to isradipine exposure, with the placebo group (n = 166) defined as the reference group. Isradipine exposure parameter values were divided into tertile groups resulting in fast, middle, and slow CL/F groups; or, low, middle, and high AUC_{24} groups (Table 4). Compared to the placebo group, when testing for a trend for each CL/F tertile decrement from fast to middle to slow, or AUC_{24} increment from low to middle to high, the risk of needing antiparkinson treatment was decreased by 13% (HR: 0.87, 95% CI: 0.78–0.98, P-value for trend = 0.02). When evaluating tertile groups as a nominal variable, the association between isradipine CL/F and time to need antiparkinson therapy did not reach statistical significance in CL/F tertile groups (Fig. 2). However, when considering AUC_{24}, which accounts for the

Table 3. Correlations between outcomes measures and isradipine plasma exposures among participants randomized to isradipine treatment arm.

	Spearman's rho (*P*-value)						Number of participants (*N*)		
	CL/F			AUC_{24}					
Change from baseline to specified time point	CL/F, overall	CL/F, women	CL/F, men	AUC_{24}, overall	AUC_{24}, women	AUC_{24}, men	*N* overall	*N* women	*N* men
UPDRS parts I–III ON, month 36	0.005 (0.95)	0.023 (0.88)	−0.046 (0.63)	0.005 (0.95)	0.12 (0.42)	−0.004 (0.96)	162	48	114
Adjusted UPDRS I–III ON[1], month 36	0.094 (0.23)	0.11 (0.44)	0.019 (0.84)	−0.063 (0.43)	0.010 (0.95)	−0.063 (0.51)	162	48	114
UPDRS parts I–III, month 12 or need antiparkinson therapy	0.058 (0.46)	0.25 (0.085)	−0.021 (0.82)	−0.078 (0.32)	−0.27 (0.057)	0.003 (0.97)	166	49	117
UPDRS part I, month 36	−0.027 (0.73)	0.043 (0.77)	−0.087 (0.36)	0.060 (0.45)	0.022 (0.88)	0.094 (0.32)	162	48	114
UPDRS part II, month 36	−0.038 (0.63)	0.092 (0.54)	−0.11 (0.24)	0.061 (0.44)	0.13 (0.37)	0.056 (0.56)	162	48	114
UPDRS part III OFF[2], month 36	0.011 (0.90)	0.19 (0.27)	−0.091 (0.39)	0.024 (0.79)	−0.028 (0.87)	0.053 (0.61)	129	36	93
Adjusted UPDRS part III ON[1], month 36	0.12 (0.14)	0.11 (0.46)	0.065 (0.49)	−0.10 (0.19)	−0.028 (0.85)	−0.11 (0.25)	162	48	114
UPDRS part IV[3], month 36	0.042 (0.61)	0.043 (0.79)	0.056 (0.58)	−0.057 (0.49)	−0.065 (0.69)	−0.063 (0.53)	144	41	103
MDS-UPDRS nonmotor EDL, month 36	−0.14 (0.076)	−0.10 (0.50)	**−0.19 (0.045)**	**0.17 (0.028)**	0.21 (0.15)	0.18 (0.054)	162	48	114
MDS-UPDRS motor EDL score ON, month 36	−0.013 (0.89)	0.12 (0.50)	−0.09 (0.39)	0.070 (0.43)	0.13 (0.45)	0.058 (0.58)	128	35	93
Ambulatory Capacity score ON, month 36	0.015 (0.85)	0.20 (0.17)	−0.082 (0.39)	0.018 (0.52)	−0.0053 (0.97)	0.044 (0.65)	162	48	114
Schwab and England ADL, month 36	−0.071 (0.37)	−0.19 (0.19)	−0.029 (0.76)	−0.007 (0.93)	−0.071 (0.63)	0.005 (0.96)	162	48	114
Modified Rankin, month 36	0.064 (0.42)	0.031 (0.83)	0.077 (0.41)	−0.088 (0.26)	−0.019 (0.90)	−0.11 (0.25)	162	48	114
PD Questionnaire-39, month 36	0.015 (0.85)	0.18 (0.21)	−0.082 (0.40)	−0.012 (0.89)	−0.17 (0.25)	0.084 (0.38)	157	47	110
LED at month 36[4]	0.15 (0.055)	−0.002 (0.99)	0.17 (0.072)	−0.12 (0.11)	−0.089 (0.55)	−0.13 (0.18)	162	48	114
Cumulative LED through 36 months[5]	**0.18 (0.035)**	−0.24 (0.16)	**0.24 (0.015)**	−0.16 (0.056)	0.081 (0.63)	**−0.20 (0.045)**	139	37	102
Adjusted UPDRS part III nontremor items (ON)[1,6]	**0.181 (0.02)**	0.195 (0.19)	0.131 (0.17)	−0.152 (0.055)	−0.053 (0.72)	−0.173 (0.07)	159	47	112

Bolding indicates $P < 0.05$. CL/F, apparent oral clearance; AUC_{24}, area under the concentration-time curve over 24 h; UPDRS, Unified Parkinson's Disease Rating Scale; MDS, Movement Disorders Society; LED, levodopa equivalent dosages.

[1]Adjusted for current and cumulative use of antiparkinson therapy.

[2]Participants with separate pre- and postdose forms at 36-month visit.

[3]Sum of parts A and B (dyskinesia and clinical fluctuations).

[4]LED (levodopa equivalent dose) in mg at the 36-month visit.

[5]Cumulative LED in mg-years through the 36-month visit.

[6]Post-hoc analysis of UPDRS part III nontremor items: speech, facial expression, rigidity, finger taps, hand movements, rapid alternating movement of hands, leg agility, arising from chair, posture, gait, postural stability, body bradykinesia and hypokinesia.

Table 4. Isradipine pharmacokinetic exposures by tertile exposure groups.

Mean (standard deviation) exposure parameter	Isradipine exposure group		
	Fast CL/F, low AUC_{24}	Middle CL/F, middle AUC_{24}	Slow CL/F, high AUC_{24}
CL/F, L/h	477 (173)	296 (27)	195 (41)
AUC_{24}, ng×h/mL	21.6 (4.4)	32.9 (2.9)	49.8 (11.2)
C_{max}, ng/mL[1]	1.86 (1.22)	3.28 (1.78)	4.90 (2.28)
C_{min}, ng/mL[1]	0.56 (0.25)	0.86 (0.48)	1.40 (0.85)

CL/F, apparent oral clearance; AUC_{24}, area under the concentration-time curve over 24 h; C_{max}, maximum concentration; C_{min}, minimum concentration.
[1]Derived from pharmacokinetic modeling simulations.

dose of isradipine administered, the highest exposure isradipine group was associated with a reduced HR for time to need therapy compared to the placebo group (HR: 0.66, 95% CI: 0.45–0.97).

Results were similar when adjusting the Cox proportional hazards models for sex or age (Table A1). For example, when adjusted for age, the highest AUC_{24} isradipine group had a slightly attenuated but still significantly reduced HR for time to need therapy compared to the placebo group (HR: 0.68, 95% CI: 0.46–0.99). The dependency of age was also assessed by a proportional hazards model testing the relation between time to treatment initiation and age within the highest AUC_{24} isradipine group. Age was not a significant risk factor to treatment initiation among the highest exposure group (HR: 1.022, 95% CI: 0.94–1.1; $P = 0.61$).

Upon further examination of the reasons for initiating antiparkinson therapy, nontremor-related symptoms (bradykinesia, rigidity, gait, and postural imbalance) were less commonly reported in the high exposure group (47%) compared to the middle (51%) and low (67%) groups, and the placebo group (57%). Therefore, as a post-hoc analysis, we evaluated the correlations between the antiparkinson therapy-adjusted UPDRS part III nontremor items and isradipine exposures. There was a modest, but significant correlation with CL/F, suggesting that as isradipine CL/F increased, antiparkinson treatment-adjusted UPDRS part III nontremor scores worsened ($r_s = 0.18$, $P = 0.02$). A similar trend was observed for AUC_{24} but did not reach statistical significance ($r_s = -0.15$, $P = 0.055$) (Table 3).

The occurrence of dizziness did not differ by exposure tertile groups (Table A2). Frequency of edema was 9% in the fast CL/F tertile, 30% in the middle tertile, and 19% in the slow tertile ($P = 0.03$). Changes in systolic and diastolic blood pressure measurements from baseline to month 36 were not significantly correlated with isradipine plasma exposures (Table A3).

Discussion

The phase 3 STEADY-PD III clinical trial provided definite evidence that treatment with 5-mg twice-daily IR isradipine in early PD did not significantly alter progression of UPDRS scores over 36 months compared with placebo. While disappointing, the trial allowed us to investigate the relationship between plasma exposure to a DHP Ca^{2+} channel inhibitor with PD progression outcomes.[10,12–14] Such analysis is specifically relevant as the dose of isradipine was selected based on the maximal tolerable dose observed in the preceding phase 2 study, which was primarily powered to assess tolerability and

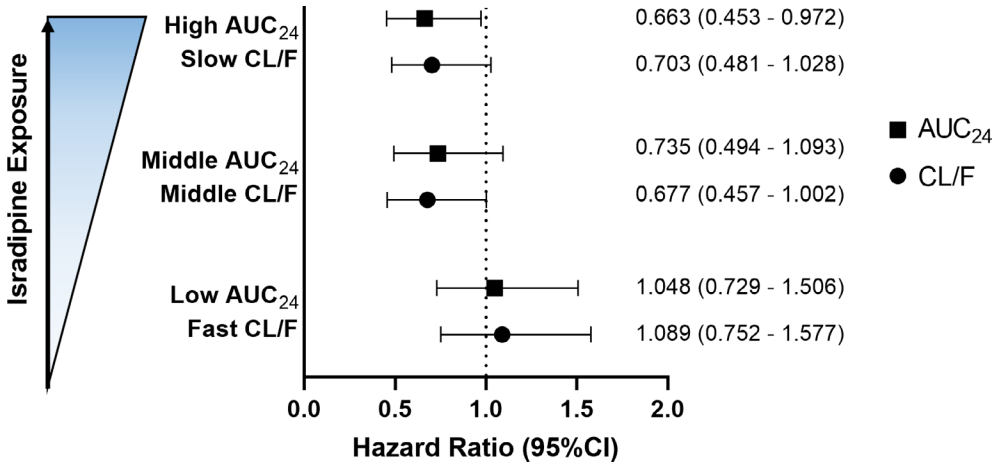

Figure 2. Proportional hazard model hazard ratios (95% confidence intervals) of time to need antiparkinson therapy by isradipine plasma exposure tertile groups (nominal variable) compared to placebo ($n = 166$).

not disease-modifying efficacy.[15] Thus, it is plausible that negative results might be related to insufficient target engagement.[16] While there is no way to validate target engagement of Ca_v1 (L-type) channels, preclinical data suggested that with sustained systemic drug delivery, there was a threshold exposure necessary to achieve protection of SN dopaminergic neurons.[10] The pharmacokinetic modeling conducted in this study suggests that although plasma isradipine concentrations varied considerably between individuals,[17–19] that threshold may not have been achieved or was exceeded for only a relatively short period of the dosing interval.

Within the plasma concentration range achieved by twice-daily dosing with an IR formulation, isradipine exposures were not associated with 36-month changes in most clinical scale assessment measures of PD severity, function, disability, and quality of life. There was a significant association with the MDS-UPDRS EDL score, which actually worsened with increased exposure. The explanation for this correlation in a single measure is not clear and is of questionable clinical significance. There was a statistically significant positive exposure–response relation between isradipine and the time to initiation of symptomatic treatment (with higher isradipine exposures being associated with a decreased hazard in time to need symptomatic treatment); there was also a significant, but weak, positive correlation between isradipine clearance and mean cumulative LED after 36 months in the overall population and in men. In addition, there was a modest correlation between isradipine clearance and nontremor UPDRS part III items, with less worsening with slower isradipine clearance.

In this clinical trial cohort, those in the highest isradipine AUC_{24} tertile had an estimated 34% reduced risk of needing antiparkinson therapy compared to the placebo group over 36 months of follow-up. The decreased risk of treatment initiation remained significant after adjustment for age (32% reduced risk). There was also no dependency of age on risk of needing antiparkinson therapy in the highest exposure group. Therefore, the observed relationship between higher exposure and reduced risk of treatment initiation was not driven by a confounding interrelationship between age and exposure. In the efficacy analysis of the STEADY-PD III trial, time-to-initiation of antiparkinson therapy favored the pooled isradipine treatment group compared to placebo group, but this trend was not statistically significant (HR: 0.79, 95% CI: 0.61–1.03; $P = 0.077$).[9] Clinical significance of this finding in absence of an impact on measures of motor and nonmotor disability remains to be determined. Time-to-initiation of antiparkinson therapy is a measure of increasing disease burden, and is manifested by a level of disability sufficient to require initiation of therapy.[20–25] In STEADY-PD III, individual need for symptomatic treatment was assessed through standard questionnaire by the site investigator that assessed one's function or current daily activities. There are some neuroimaging data to suggest this milestone-based outcome measure relates to underlying pathophysiologic changes of disease progression. In the PRECEPT clinical trial, striatal dopamine transporter levels, measured by β-CIT single-photon emission computed tomography, were inversely related to the need for dopaminergic therapy.[20]

It is important that any interpretation of potential beneficial effects of higher isradipine exposures in delaying time for initiation of antiparkinson therapy be done so with caution since there were not exposure–response relations observed with most changes in the clinical assessments. Furthermore, the dosage of isradipine used (10-mg/day) was selected on the basis of the tolerability threshold established in a dose-ranging phase 2 study using a controlled-release (CR) formulation.[15] In that study, 10-mg/day was the highest tolerable dosage compared to 5-mg and 20-mg daily dosages; the latter considered intolerable. Thus, although it is reasonable to ask whether higher doses of isradipine or an increased daily exposure might have produced more robust effects, there are real dose-limiting intolerabilities with this compound.

L-type channels are distributed widely in the cardiovascular, musculoskeletal, and nervous systems and have a Cav1.2 pore-forming subunit.[26] In contrast, L-type Ca^{2+} channels with the $Ca_v1.3$ pore-forming subunit are thought to be the key target for neuroprotection in SN dopaminergic neurons.[12,13,27–29] Unlike most DHPs, isradipine has nearly equal affinities for Cav1.2 and Cav1.3 channels, making it the DHP of choice for disease modification trials in PD.[4] However, this also means that $Ca_v1.2$-mediated side effects will parallel any PD-protective effects with increasing isradipine concentrations. The occurrence of peripheral edema with DHPs increases with increasing doses and duration.[30] Treatment with isradipine in PD participants demonstrated a clear dosage relationship of this side effect in the preceding phase 2 study and was more common in the phase 3 isradipine treatment arm versus placebo.[9,15] Participants in the middle and slow CL/F tertile groups made up 84% of the peripheral edema occurrences among those randomized to isradipine.

Preclinical data demonstrated that with continuous systemic drug delivery, neuroprotection could be achieved at plasma isradipine concentrations that were within concentration ranges observed in humans. In the 6-hydroxydopamine (6-OHDA) neurotoxin mouse model, protection of dopaminergic axon terminals and cell bodies by systemic isradipine was dose dependent, having plasma IC50s of 7.2 and 5.0 ng/mL for neuroprotection

(not channel inhibition), respectively.[10] Although the preclinical studies used a sustained drug delivery format, the phase 3 trial was constrained to use twice-daily dosing of IR isradipine because of commercial discontinuation of the CR formulation. Both IR and CR formulations are considered equivalent with regard to overall plasma exposure based on AUC_{24}, but the trough-to-peak fluctuations are more prominent with the IR formulation.[31–33] The mean ($\pm$ standard deviation) observed peak-to-trough ratio of IR isradipine in our study was approximately 5.3 ($\pm$4.3). In contrast, the apparent peak-to-trough ratio for CR isradipine is approximately 2.5, due to an attenuated peak concentration and a more sustained trough with the CR formulation.[32] Because the isradipine binding to Cav1 channels is sigmoidal for sustained periods of the day, individuals may have had little or no channel inhibition with use of the IR formulation.[34] Although our analysis suggests higher isradipine exposures are associated with delaying antiparkinson treatment initiation, further work is required to determine whether higher peak plasma isradipine concentrations, or more sustained concentrations above a threshold, would achieve disease modification.

It is worth noting that the neuroprotection studies implicating Cav1 channels in PD have largely been limited to an examination of SN dopaminergic neurons. However, the pathology in PD patients is not limited to the SN and this broader deficit undoubtedly contributes to symptoms.[35] Although Cav1 Ca^{2+} channels have been shown to contribute to stress in several of these populations,[36,37] the extent to which isradipine inhibition of Cav1 Ca^{2+} channels might slow the loss of these other neuronal populations in PD remains to be determined. Nevertheless, it is of interest that the nontremor motor disability of PD patients, which can readily be linked to degeneration of SN dopaminergic neurons, appeared to be reduced (albeit modestly) in PD patients with slower isradipine clearance.

One other notable finding that merits further consideration if DHPs continue to be studied for PD, was the effect of age on isradipine pharmacokinetics. As previously reported for DHP calcium channel inhibitors, age-related decreases in the clearance of drug were observed.[18,38,39] The isradipine package insert states that AUC is increased by 40% in elderly patients.[40] Our model showed that for every decade increase in age, isradipine CL/F decreased by 11%. Advancing age produces anatomical and physiological changes affecting pharmacokinetic processes, thus prolonging the elimination half-life of isradipine.[41,42]

A limitation to these results is that isradipine exposure quantification was restricted to the plasma compartment and was not assessed in the brain or cerebrospinal fluid, which would have given more proximal proof of target engagement (or lack thereof). While the investigators considered integrating collection of CSF samples, the scientific benefits were outweighed by the costs and invasiveness of measuring such samples. Additionally, isradipine is highly lipophilic, and is able to cross the blood–brain barrier to achieve concentrations that exceed those in plasma.[8,43,44] Thus, it is reasonable to assume that isradipine plasma exposures reflected similar or higher concentrations in the brain.[45]

As some of our data suggest, it is possible that the dose of isradipine used in STEADY-PD III did not achieve sufficient sustained concentrations to inhibit $Ca_v1.3$ channels in the brain. These pharmacokinetic data are the closest measure to understanding the potential relationship between isradipine exposure and target engagement in people living with PD. However, as pointed out by others, more direct in vivo measures of target engagement are essential for the decisions to proceed with clinical development of putative neuroprotective agents. Also, more sensitive measures of disease progression for PD would improve the readout of drug effects for future disease modification clinical trials in PD.[16] Furthermore, if $Ca_v1.3$ channel inhibition remains a drug target of interest for PD, more selective, brain-penetrant calcium channel inhibitors would insure better tolerability which would allow for higher dose exposure and target engagement. It will also be helpful to understand whether disruption of calcium homeostasis as an underlying pathogenic mechanism is ubiquitous across all individuals living with PD, or if it is only disrupted in certain subtypes of PD and at certain stages of pathogenic process.

In conclusion, isradipine plasma concentrations measured from STEADY-PD III participants were not correlated with most changes in PD severity, function, disability, and quality of life. However, higher isradipine exposures were modestly but significantly associated with a delay in the need for symptomatic therapy and a lower cumulative LED over 36 months. While these results raise the possibility that the study failed because of insufficient drug exposures, dose-limiting side effects deterred increasing isradipine dose with the IR formulation. Thus, more targeted and sustained delivery of L-type calcium channel inhibitors to the brain would provide a better test of their disease-modifying potential. Most importantly, better strategies for determining target engagement and biological efficacy need to be developed.

Author Contributions

CSV and TS conceptualized the study. CSV, LY, MJ, and DO performed modeling and statistical analyses. CSV wrote the original draft manuscript. CSV, LY, MJ, DO,

DJS, and TS provided interpretation of the data, and reviewed and edited the manuscript. TS acquired funding.

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Conflict of Interest

Dr. Surmeier reports financial support from Surculus Therapeutics, outside the submitted work. In addition, Dr. Surmeier has a patent US20180280371 issued.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Additional details on the isradipine bioanalytical assay and pharmacokinetic modeling procedures.
Figure A1. Isradipine plasma pharmacokinetic samples available for modeling analysis from the STEADY-PD III clinical trial.
Figure A2. Goodness-of-fit diagnostic plots of the final population pharmacokinetic model for isradipine. (A) Observed isradipine concentrations (natural log transformed) versus population predicted concentrations. (B) Observed isradipine concentrations versus individual predicted concentrations. (C) Conditional weighted residuals versus population predicted concentrations. (D) Conditional weighted residuals versus time since last dose in hours. Dashed red lines represent the local Loess regression line. (E) Prediction-corrected visual predictive check plot of natural log transformed isradipine concentration ("Dependent variable") versus time since last dose ("Independent variable").
Table A1. Hazard ratios for time to need antiparkinson therapy according to CL/F or AUC_{24} tertile groups treated as nominal variable with gender or age as covariates.
Table A2. Occurrence of dizziness, peripheral edema by isradipine exposure tertile.
Table A3. Correlations between blood pressure changes and isradipine plasma exposures.

