

Pharmacokinetic/Pharmacodynamic Correlation Analysis of Amantadine for Levodopa-Induced Dyskinesia[§]

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ABSTRACT

Dyskinesia is a common motor complication associated with the use of levodopa to treat Parkinson's disease. Numerous animal studies in mice, rats, and nonhuman primates have demonstrated that the *N*-methyl-*D*-aspartate antagonist, amantadine, dose dependently reduces levodopa-induced dyskinesia (LID). However, none of these studies characterized the amantadine plasma concentrations required for a therapeutic effect. This study evaluates the pharmacokinetic (PK)/pharmacodynamic (PD) relationship between amantadine plasma concentrations and antidyskinetic efficacy across multiple species to define optimal therapeutic dosing. The PK profile of amantadine was determined in mice, rats, and macaques. Efficacy data from the 6-hydroxydopamine rat and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine macaque model of LID, along with previously published antidyskinetic efficacy data, were used to establish

species-specific PK/PD relationships using a direct-effect maximum possible effect model. Results from the PK/PD model were compared with amantadine plasma concentrations and antidyskinetic effect in a phase 2 study in patients with Parkinson's disease treated with ADS-5102, an extended-release amantadine capsule formulation. Outcomes from each of the species evaluated indicate that the EC₅₀ of amantadine for reducing dyskinesia range from 1025 to 1633 ng/ml (1367 ng/ml for an all-species model). These data are consistent with the mean amantadine plasma concentrations observed in patients with Parkinson's disease (~1500 ng/ml) treated with ADS-5102 at doses that demonstrated a statistically significant reduction in dyskinesia. These results demonstrate that the EC₅₀ of amantadine for reducing dyskinesia is consistent across multiple species and supports a plasma concentration target of ~1400 ng/ml to achieve therapeutic efficacy.

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Introduction

Dopamine replacement therapy with the dopamine precursor, L-3,4-dihydroxyphenylalanine (levodopa), remains the most effective symptomatic treatment of Parkinson's disease. However, long-term treatment with levodopa often leads to the development of motor complications including dyskinesia, which is characterized by involuntary movements that are nonrhythmic, purposeless, and may be unpredictable in onset and severity. In patients treated with levodopa, dyskinesia can develop early, affects nearly 90% of such patients within approximately 10 years of treatment (Ahlskog and Muenter, 2001), and has a substantial adverse effect on quality of life (Suh et al., 2012).

Although the progressive loss of dopaminergic neurons is the hallmark of Parkinson's disease, the dysregulation of glutamatergic signaling pathways is a major contributor to the development and expression of dyskinesia (Sgambato-Faure and Cenci, 2012). Adaptations to fluctuating levels of dopamine and loss of dopamine modulation have been associated with

ABBREVIATIONS: AE, adverse event; AIM, abnormal involuntary movement; ALO, axial, limb, and orolingual; AUC_{inf}, area under plasma concentration-time curve at infinity; CI, confidence interval; C_{max}, maximal plasma concentration; CNS, central nervous system; CRL, Charles River Laboratories; EC₅₀, 50% effective plasma concentration; E_{max}, maximum possible effect; IC₅₀, 50% inhibitory drug concentration; IR, immediate-release; LID, levodopa-induced dyskinesia; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NHP, nonhuman primate; NMDA, *N*-methyl-*D*-aspartate; 6-OHDA, 6-hydroxydopamine; PD, pharmacodynamics; PK, pharmacokinetics; UDysRS, Unified Dyskinesia Rating Scale.

increased concentrations of extracellular glutamate in animal models (Jonkers et al., 2002; Robelet et al., 2004; Dupre et al., 2011), and increased expression and/or increased activity of the *N*-methyl-D-aspartate (NMDA)-type glutamate receptor in animal models and humans (Calon et al., 2002). The role of glutamate dysregulation in the development and expression of dyskinesia is supported by the antidyskinetic activity of diverse NMDA receptor antagonists in animal models of Parkinson's disease and in humans. For example, a competitive NMDA receptor antagonist [(3*S*,4*a**R*,6*S*,8*a**R*)-6-(phosphonomethyl)-1,2,3,4,4*a*,5,6,7,8,8*a*-decahydroisoquinoline-3-carboxylic acid (LY235959)] significantly decreased dyskinesia in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned parkinsonian nonhuman primates (NHPs) treated with levodopa (Papa and Chase, 1996). Treatment with the high-affinity, uncompetitive NMDA receptor antagonist MK-801 (dizocilpine) in rodent and NHP models of levodopa-induced dyskinesia (LID) resulted in reduced dyskinesia (Bibbiani et al., 2005), and treatment with dextromethorphan, which is also an uncompetitive NMDA receptor antagonist, reduced dyskinesia in humans with Parkinson's disease (Verhagen Metman et al., 1998a).

Amantadine is a low-affinity, uncompetitive NMDA receptor antagonist (Parsons et al., 1995). Numerous studies in mice, rats, and NHPs have demonstrated that amantadine reduces dyskinesia in models of LID in a dose-dependent manner. In mouse and rat, effective doses of amantadine ranged from 10 to 60 mg/kg (subcutaneous or intraperitoneal administration) in the 6-hydroxydopamine (6-OHDA) model. Amantadine had a consistent benefit at a doses ≥ 40 mg/kg, whereas lower doses had variable effects (Dekundy et al., 2007; Bido et al., 2011; Kobylecki et al., 2011; Paquette et al., 2012; Papathanou et al., 2014; Bortolanza et al., 2016; Sebastianutto et al., 2016). In NHPs (cynomolgus macaques and marmosets) that were administered MPTP to induce parkinsonian disability, chronic treatment with levodopa produces dyskinesia similar to that in humans. Acute treatment (oral or subcutaneous) with amantadine at 0.3–30 mg/kg resulted in a significant reduction of dyskinesia (Blanchet et al., 1998; Hill et al., 2004; Bibbiani et al., 2005; Kobylecki et al., 2011; Bezdard et al., 2013; Grégoire et al., 2013; Ko et al., 2014). Results from multiple small clinical studies indicate an immediate-release (IR) form of amantadine provides antidyskinetic benefits in patients with Parkinson's disease, with efficacy increasing at higher plasma concentrations (Verhagen Metman et al., 1998b).

Despite a half-life of approximately 17 hours, the total daily dose of amantadine IR is typically split to two or three times daily administration due to dose-limiting central nervous system (CNS) adverse events (AEs) associated with once-daily dosing (Parkes et al., 1970; Hayden et al., 1983). Higher doses that may produce a greater antidyskinetic effect are associated with an increased frequency of CNS AEs, such as dizziness, hallucinations, and sleep disturbances (Parkes et al., 1970). The ADS-5102 (amantadine) extended-release capsule (GOCOVRI; Adamas Pharmaceuticals, Inc., Emeryville, CA) is the first and only US Food and Drug Administration–approved medicine for the treatment of dyskinesia in patients with Parkinson's disease receiving levodopa-based therapy, with or without concomitant dopaminergic medications. The recommended dose of ADS-5102 for the treatment of dyskinesia is 274 mg once daily at bedtime

(equivalent to a daily 340-mg dose of amantadine HCl). Bedtime administration of ADS-5102 provides high amantadine plasma concentrations in the morning, which are sustained throughout the day when dyskinesias occur (Hauser RA, Pahwa R, Wargin W, et al., manuscript in preparation). Multiple randomized, placebo-controlled trials demonstrated that ADS-5102 significantly reduced dyskinesia in patients with Parkinson's disease who were treated with levodopa, with a secondary benefit of reduced OFF-time associated with motor complications (Pahwa et al., 2015, 2017; Oertel et al., 2017).

To date, none of the reported animal studies included a determination of amantadine plasma concentrations required to obtain a therapeutic effect. The objective of the current analysis was to determine the amantadine plasma concentrations required to reduce dyskinesia in multiple species and any correlations across species. The pharmacokinetic (PK) profile of amantadine was determined in mice, rats, and cynomolgus macaques, and the efficacy of amantadine in reducing LID was assessed in Parkinson's disease models (6-OHDA-rat and MPTP-macaque). The PK data were used to build species-specific PK models that were applied to new efficacy data reported herein and previously published efficacy data in mice, rats, and macaques. A PK/pharmacodynamics (PD) relationship was established to determine the EC_{50} values of amantadine required for the reduction of dyskinesia in these LID animal models. In a recent clinical study, amantadine plasma concentrations associated with the antidyskinetic efficacy of ADS-5102 were reported in patients with Parkinson's disease (Pahwa et al., 2015); these concentrations were compared with the results of the model. These results provide the rationale for target therapeutic plasma amantadine concentrations, with the goal of maximizing antidyskinetic efficacy, in patients with Parkinson's disease treated with levodopa as well as supporting the validity of these animal models for testing novel antidyskinetic drugs.

Materials and Methods

The PK time course of amantadine plasma levels was determined in mice (eight per group), rats (six per group), and macaques (eight per group), and efficacy was evaluated in rat and macaque models of Parkinson's disease with established LID ($n = 6$ /group for rats and $n = 8$ /group for macaques). All studies were performed with local Institutional Animal Care and Use Committee approval as well as in accordance with the *Guide for the Care and Use of Laboratory Animals* (1996) as adopted by the National Institutes of Health Committee on Care and Use of Laboratory Animals. The mouse PK study was conducted at Charles River Laboratories (CRL; Wilmington, MA) in normal C57BL/6J mice (The Jackson Laboratory, Sacramento, CA), the rat PK study was conducted at CRL in normal Sprague-Dawley rats (CRL, Raleigh, NC), and the NHP PK and LID efficacy study was conducted at Atuka (Suzhou, People's Republic of China) in levodopa-treated MPTP macaques (*Macaca fascicularis*; Suzhou Xishan Zhongke Laboratory Animal Company, Suzhou City, People's Republic of China).

Blood samples (0.1–0.15 ml) were collected at the time points specified for each species below, and the collected plasma was frozen (-70° to -80° C) and stored until processed and analyzed by liquid chromatography-tandem mass spectrometry, as described in the bioanalysis section of the Supplemental Material. A rat LID efficacy study was conducted at Atuka (Toronto, ON, Canada) in levodopa-treated 6-OHDA-lesioned Sprague-Dawley rats (CRL, Senneville, QC, Canada). Plasma concentrations were reported in this experiment. Additional PD data were included from a selection of publications that

reported antidyskinetic efficacy in mouse, rat, or cynomolgus macaque to support the PK/PD model. In addition, amantadine plasma concentrations and antidyskinetic efficacy data from a phase 2 study in Parkinson's disease patients treated with ADS-5102, an extended-release amantadine capsule formulation (Pahwa et al., 2015), were compared with the model. Greater detail for all rodent, macaque, and human studies is provided in the Supplemental Material.

PK Studies and PK Modeling. Normal male and female C57BL/6J mice were administered a single dose of 10, 30, or 60 mg/kg, i.p., amantadine HCl (MOEHS Catalana SL, Barcelona, Spain), and sparse blood samples were collected from the submandibular vein at 0.25, 0.5, 1, 2, 4, 6, 8, and 12 hours after dosing. Each mouse contributed two samples to the analysis, and each time point represented samples from four animals per sex.

Normal male and female Sprague-Dawley rats were administered a single dose of 15, 45, or 90 mg/kg, i.p., amantadine HCl (Sigma-Aldrich, St. Louis, MO), and blood samples were obtained via jugular vein cannula at predose, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after dosing. Plasma also was collected in levodopa-treated 6-OHDA-lesioned rats infused subcutaneously continuously for 12 days with amantadine via ALZET (Cupertino, CA) pump (see below).

Four oral doses of amantadine (1, 3, 10, and 30 mg/kg) were given to each of eight MPTP-lesioned macaques in an ascending, nonrandomized treatment design after an overnight fast. Blood samples were collected from the saphenous vein predose, and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, and 48 hours postdose. A 1-week washout occurred between each treatment.

Time-course PK data for each species were fit to an appropriate model and curve using Phoenix WinNonlin version 6.3 (Build 6.3.0.395; Certara, Princeton, NJ). For each species, the critical constants for the exposure-time curve for each dose level were averaged to produce a dose-independent exposure-time curve that could be used to simulate exposure-time curves for dose levels used in the literature for which no experimentally determined PK data were available.

Behavioral Assessment of Effects of Amantadine on Dyskinesia and Abnormal Involuntary Movements: Overview. Results from a 6-OHDA rat study and an MPTP macaque study described below along with the previously published studies identified in Supplemental Table 1 were used as the data source for the effect of amantadine on NHP dyskinesia or the rodent correlate [abnormal involuntary movements (AIMs)]. Published mouse and rat studies in which amantadine was administered intraperitoneally and reported a time course of reduction of AIMs after levodopa treatment, and published NHP studies in which macaques received oral amantadine were included in the analyses. Variations in the key study design components are also presented in Supplemental Table 1.

Continuous Amantadine Administration in 6-OHDA-Lesioned Rats. Female Sprague-Dawley rats (six per group) received unilateral nigrostriatal dopaminergic lesions of the median forebrain bundle using standard stereotaxic techniques (Paxinos and Watson, 1986). Only animals demonstrating a $\geq 85\%$ asymmetry score in the forelimb asymmetry cylinder test were included in the study. These animals were treated with 10 mg/kg levodopa methyl ester HCl in combination with 15 mg/kg, i.p., benserazide HCl once daily in a dose volume of 1 ml/kg for 58 days to induce stable AIMs.

Three subtypes of AIMs [axial, limb, and orolingual (ALO)] were assessed (Supplemental Material). Animals were observed for 1 minute before levodopa treatment and 1 minute every 20 minutes for 3 hours after levodopa treatment. Animals obtaining a score of 3 (marked) or 4 (severe) for AIMs during at least one assessment point were included in the study. Animals were assigned to treatment groups so that the baseline AIM scores (via blinded assessment) were not different between each group. ALZET osmotic pumps (model 2ML2; 5 μ l/h, 14-day pump) containing either vehicle (25 mM sodium acetate buffer, pH 5.0) or amantadine HCl delivering 22.5, 45, or 83 mg/kg per day, s.c., were used. During the amantadine treatment period, all animals received once-daily levodopa (10 mg/kg

levodopa/15 mg/kg benserazide, i.p.), and the effects of treatments on AIMs were assessed on treatment day 12, as described in the Supplemental Material. Cumulative AIMs (20–120 minutes) resulting from the levodopa challenge on amantadine treatment day 12 were compared with cumulative AIMs scored at baseline, before pump implantation.

Referenced AIMs Data from Published Rodent Studies. In addition to the de novo efficacy data generated and reported herein, efficacy data from mouse and rats studies were collected from literature reports (Bido et al., 2011; Papanthanou et al., 2014; Bortolanza et al., 2016; Sebastianutto et al., 2016), in which amantadine was administered as a single intraperitoneal dose; ALO AIMs in these reported studies were assessed as described in the Supplemental Material and Supplemental Table 1. Where not explicitly provided, data on AIMs were extracted using WebPlotDigitizer (version 3.6).

Oral Administration of Amantadine in MPTP Macaques. For the NHP, both the de novo efficacy data reported here as well as data obtained from literature reports were used to build the PK/PD model (Bezard et al., 2013; Grégoire et al., 2013; Ko et al., 2014). For our study, the development of the MPTP-macaque LID model is described in the Supplemental Material.

For efficacy assessments, in an acute challenge randomized design, each of eight macaques was administered (via oral gavage) vehicle (water) or four doses of amantadine (1, 3, 10, or 30 mg/kg) after an overnight fast. Amantadine or vehicle was administered 1 hour before levodopa, and dyskinesia was assessed over a 6-hour period of observation (Supplemental Material). A 72-hour washout period occurred between treatments. Data derived from the assessment of NHP measures of dyskinesia were graphed as median scores (time course).

For the efficacy data from the literature (Bezard et al., 2013; Grégoire et al., 2013; Ko et al., 2014) included in the NHP PK/PD analysis, the severity of dyskinesia was rated using dyskinesia scales described in the original publication and summarized in Supplemental Table 1. Where not explicitly provided, dyskinesia data were extracted using WebPlotDigitizer (version 3.6).

PK/PD Analysis. A similar methodology was used for all species. Simulated PK exposure-time curves were used to provide plasma levels of amantadine at the time points after amantadine treatment that correlated with the AIM or dyskinesia observation time points described below. Peak dyskinesia or AIMs generally occur beginning approximately 20 minutes after the administration of levodopa and are sustained through 120 minutes after levodopa administration. Amantadine plasma concentrations were used to assess the exposure-response relationship for the reduction of dyskinesia at time points in which moderate dyskinesia was measured in the vehicle group (Supplemental Fig. 1). Values less than zero were assigned a value of zero. For the PK/PD relationship, a sigmoidal maximum possible effect (E_{\max}) model using Prism version 3.6 (GraphPad Software, La Jolla, CA) was fit to the data assuming an E_{\max} of 100%. Details of doses and time points from previously published rodent and macaque studies are provided in Supplemental Material and Supplemental Table 1.

Results

Amantadine PK/PD Analysis in Mice. The mean amantadine plasma concentration-time profiles after the administration of a single dose of amantadine (10, 30, or 60 mg/kg, i.p.) in mice are displayed in Fig. 1A. Amantadine exposures increased proportionally with dose over this range, with mean C_{\max} values ranging from 1268 to 8826 ng/ml and mean area under plasma concentration-time curve at infinity (AUC_{∞}) ranging from 1781 to 13,877 ng/h per milliliter (Table 1). The composite profile of each dose was modeled using a one-compartment model with an intravenous bolus to optimize the

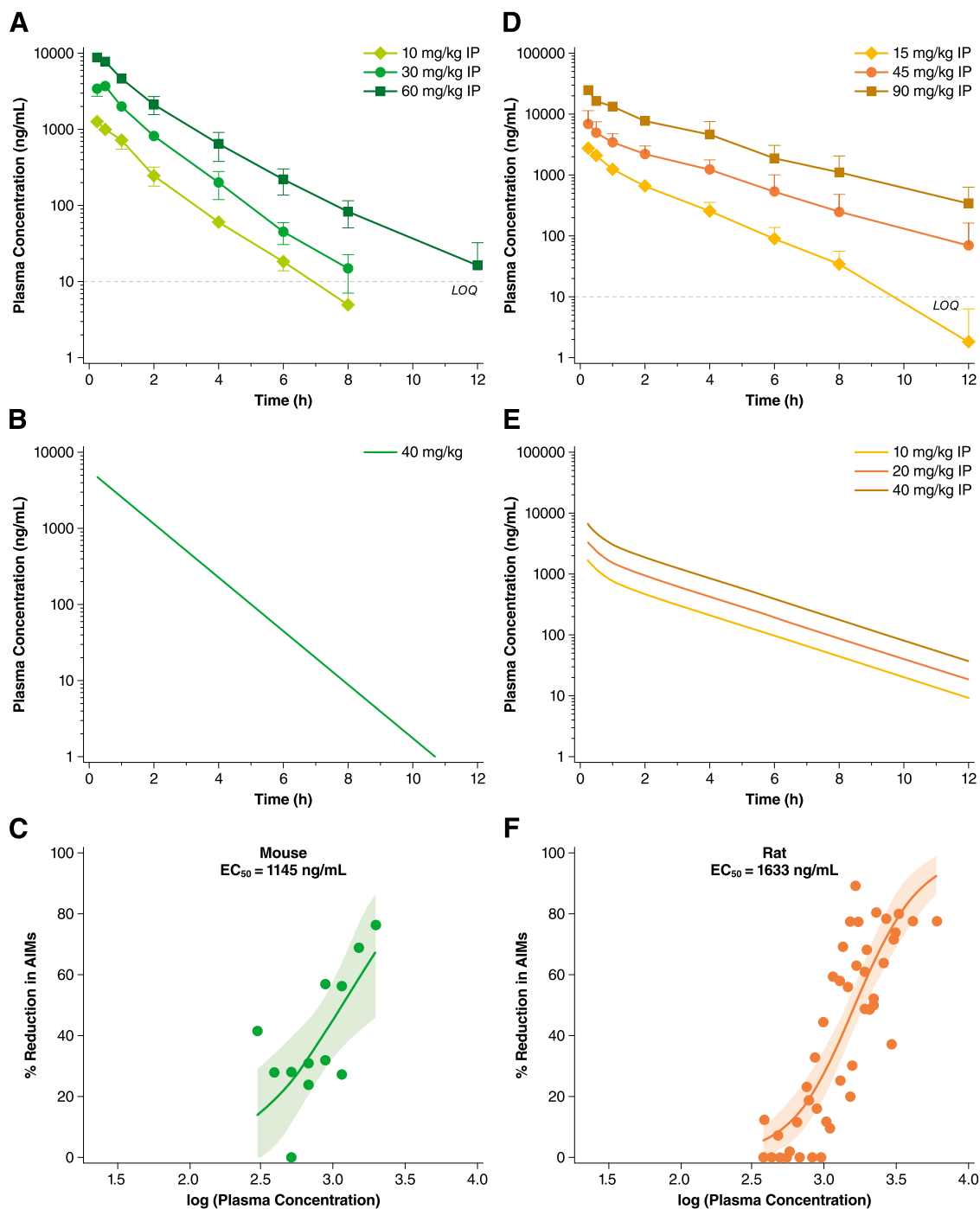


Fig. 1. Amantadine plasma concentration-time profiles after a single intraperitoneal injection dose in mice for observed plasma concentrations (mean \pm S.D.) (A) and simulated plasma concentrations (the PK model) (B). (C) Determination of the PK/PD relationship and the EC₅₀ in mouse. The percentage reduction from vehicle in AIMs is plotted as a function of the log(amantadine plasma concentration) in mouse. EC₅₀ = 1145 ng/ml. The shaded area represents 95% CI. Amantadine plasma concentration-time profiles after a single intraperitoneal injection dose in rat for observed plasma concentrations (mean \pm S.D.) (D) and simulated plasma concentrations (the PK model) (E). The simulated model was fit optimized to maximum plasma concentration, two-compartment intravenous bolus model. (F) Determination of the PK/PD relationship and the EC₅₀ value in rat. The percentage reduction from vehicle in AIMs is plotted as a function of the log(amantadine plasma concentration) in rat. EC₅₀ = 1633 ng/ml. The shaded area represents the 95% CI.

fit to C_{max} , and the average parameters (Table 1) were used to simulate a 40 mg/kg dose (Fig. 1B). Bido et al. (2011) and Sebastianutto et al. (2016) demonstrated that 40 mg/kg, i.p., amantadine decreased AIMs as a function of time after a levodopa challenge, achieving maximum reductions of 76% and 42%, respectively, compared with vehicle.

The percentage of reduction in AIMs was plotted as a function of simulated amantadine plasma concentrations (Fig. 1C). A sigmoidal E_{max} model provided a good fit for the data with an amantadine EC₅₀ of 1145 ng/ml (95% confidence interval [CI], 756–1735 ng/ml) for the reduction of AIMs (Supplemental Table 2).

TABLE 1
Amantadine PK parameters by species

	Mouse, Sexes Combined 10 mg/kg	30 mg/kg	60 mg/kg	Mean ^a	
NCA parameter					
C_{max} (ng/ml)	1268	3733	8826		
$C_{max}/dose$ (ng/ml)/(mg/kg)	127	124	147		
T_{max} (h)	0.25	0.5	0.25		
AUC_{inf} (ng/h per milliliter)	1781	5522	13,877		
$AUC_{inf}/dose$ (ng/h per milliliter)/(mg/kg)	178	184	231		
$t_{1/2}$ (h)	1.1	1.0	1.5		
Modeled parameters (using C(T) = $D/V \times \exp(-K10 \times T)$)					
V (ml/kg)	6378	6459	5436	6091	
K10 (1/h)	0.8424	0.7811	0.8075	0.810	
	Rat, Sexes Combined 15 mg/kg	45 mg/kg	90 mg/kg	Mean ^b	
NCA parameter					
C_{max} (ng/ml)	2769	6982	24,899		
$C_{max}/dose$ (ng/ml)/(mg/kg)	185	155	277		
T_{max} (h)	0.25	0.25	0.25		
AUC_{inf} (ng/h per milliliter)	4199	14,114	52,563		
$AUC_{inf}/dose$ (ng/h per milliliter)/(mg/kg)	280	314	578		
$t_{1/2}$ (h)	1.18	1.95	1.91		
Modeled parameters (using C(T) = $A \times \exp(-\alpha \times T) + B \times \exp(-\beta \times T)$)					
V1 (ml/kg)	3816	3862	1518	3839	
K10 (1/h)	0.87	0.77	1.09	0.82	
K12 (1/h)	0.61	1.63	3.49	1.12	
K21 (1/h)	1.14	1.68	2.31	1.41	
	Cynomolgus Macaque, Sexes Combined 1 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	Mean ^c
NCA parameter					
C_{max} (ng/ml)	161	539	1599	4633	
$C_{max}/dose$ (ng/ml)/(mg/kg)	161	180	160	154	
T_{max} (h)	4.0	3.5	3.75	5.75	
AUC_{inf} (ng/h per milliliter)	2287	5710	21,746	65,372	
$AUC_{inf}/dose$ (ng/h per milliliter)/(mg/kg)	2287	1903	2175	2179	
$t_{1/2}$ (h)	11.5	6.49	6.95	6.27	
Modeled parameters (using C(T) = $K \times (D/V) \times T \times \exp(-K \times T)$)					
V_F (ml/kg)	2466	2171	2324	2663	2406
K (1/h)	0.3	0.32	0.26	0.22	0.274
T_{lag} (h) ^d	0.39	0.45	0.41	0.52	0.440

A and B, coefficients; alpha, alpha phase elimination rate constant; beta, beta phase elimination rate constant; D, dose administered; K and K01 and K10 and K21, rate constant; NCA, noncompartmental analysis; T_{lag} , time lag; T_{max} , time to C_{max} ; $t_{1/2}$, half-life; V, volume; V_F, apparent volume; V1, volume of central compartment.

^aMean of 10, 30, and 60 mg/kg doses used to simulate the 40 mg/kg PK profile.

^bMean of 15 and 45 mg/kg doses used to simulate 10, 20, and 40 mg/kg PK profile.

^cMean of 1, 3, 10, and 30 mg/kg doses used to simulate PK profiles.

^d T_{lag} max = 1.73 h.

Amantadine PK/PD Analysis in Rats. The mean amantadine concentration-time profile for single intraperitoneal dose administration in rats is displayed in Fig. 1D. Plasma concentrations were dose proportional between 15 and 45 mg/kg, which was most appropriate for the range used in the LID efficacy studies. The mean C_{max} for 15 and 45 mg/kg doses were 2769 and 6982 ng/ml, respectively, and the mean AUC_{inf} estimates were 4199 and 14,114 ng/h per milliliter, respectively (Table 1). A two-compartment intravenous bolus model using the full time course of available data (12 hours) was fit to the 15 and 45 mg/kg dose data (Table 1), and the average parameters were used to simulate PK profiles for 10, 20, and 40 mg/kg doses (Fig. 1E).

Two previous publications, Papathanou et al. (2014) and Bortolanza et al. (2016), demonstrated a dose-dependent reduction of AIMs after a single dose of 10, 20, or 40 mg/kg, i.p., amantadine in the 6-OHDA rat model with a maximum reduction of AIMs >75%. In another publication, Bido et al. (2011) demonstrated that 40 mg/kg, i.p., amantadine

decreased AIMs, achieving a maximum reduction of 63% compared with vehicle.

The percentage reduction in AIMs was plotted as a function of the simulated amantadine plasma concentrations (Fig. 1F). A sigmoidal E_{max} model provided a good fit for the data with an amantadine EC_{50} of 1633 ng/ml (95% CI, 1419–1879 ng/ml) for the reduction of AIMs (Supplemental Table 2).

Amantadine PK/PD Analysis in Cynomolgus Macaques. Dose proportionality was observed over the dose range of 1–30 mg/kg amantadine (Fig. 2A), with the mean C_{max} ranging from 161 to 4633 ng/ml and the mean AUC_{inf} ranging from 2287 to 65,372 ng/h per milliliter (Table 1). A one-compartment model with a constrained input rate equivalent to output was fit to the data, and the average parameters (Table 1) were used to simulate PK profiles for doses of 0.3, 1, 5, 10, 20, and 30 mg/kg (Fig. 2B) that were used in the three referenced studies (Bezard et al., 2013; Grégoire et al., 2013; Ko et al., 2014). In our efficacy study, single oral doses of amantadine resulted in a dose-dependent decrease in

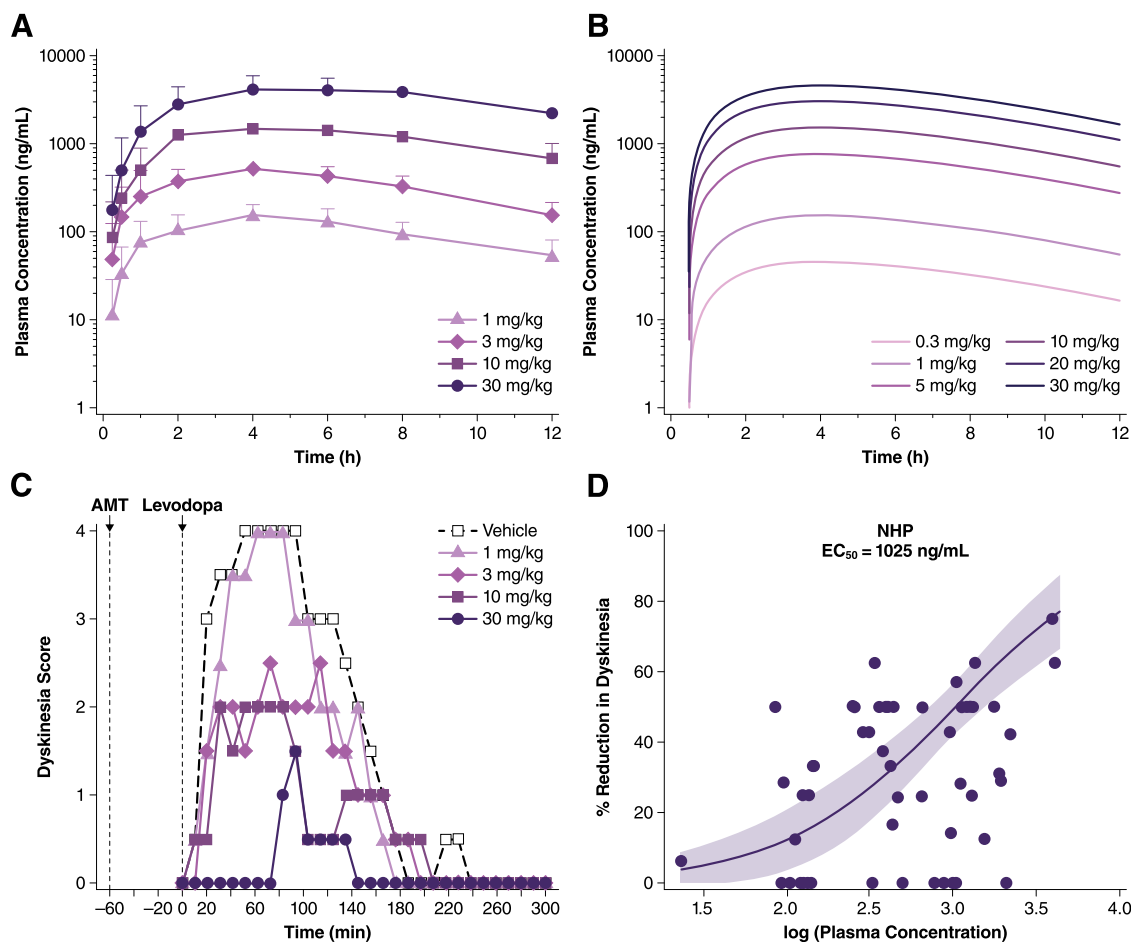


Fig. 2. Amantadine (AMT) plasma concentration-time profiles and dyskinesia score after a single oral dose in dyskinetic cynomolgus macaque for observed plasma concentrations (mean \pm S.D.) (A), simulated plasma concentrations (the PK model) (B), and dyskinesia score by treatment (median) (C). The PK model was fit optimized to maximum plasma concentration, one-compartment model with constrained input rate equivalent to output. (D) Determination of PK/PD relationship and the EC_{50} in macaque. The percentage reduction from vehicle in dyskinesia is plotted as a function of the log(amantadine plasma concentration) in macaque. $EC_{50} = 1025$ ng/ml. The shaded area represents the 95% CI.

dyskinesia in MPTP macaques, with up to a 78% reduction in median levels of LID occurring at 30 mg/kg amantadine (Fig. 2C).

The percentage reduction in dyskinesia was plotted as a function of the simulated amantadine plasma concentrations (Fig. 2D). A sigmoidal E_{max} model provided a good fit for the data with an amantadine EC_{50} of 1025 ng/ml (95% CI, 716–1467 ng/ml) for the reduction of dyskinesia (Supplemental Table 2).

PK/PD Relationships Across Species. The exposure-response relationships for mouse, rat, and macaque data each fit the sigmoidal E_{max} model fairly well, with R^2 values ranging from 0.407 to 0.682. The relationships for each species also showed close agreement with regard to the EC_{50} , which ranged from 1025 to 1633 ng/ml. Combining data from all species for model fitting resulted in estimates similar to those obtained individually, with an R^2 value of 0.429 and an EC_{50} value of 1367 ng/ml (95% CI, 1139–1639 ng/ml) (Fig. 3; Supplemental Table 2).

A study was conducted in dyskinetic 6-OHDA-lesioned rats using ALZET pumps to ensure constant amantadine plasma levels that bracketed the EC_{50} (1367 ng/ml). Subcutaneous infusion for 12 days of 83 mg/kg per day amantadine resulted in plasma concentrations of 2922 ng/ml (Fig. 4A), and a

significant reduction from baseline in cumulative AIMs was observed at this dose compared with vehicle (37.2% vs. 0.1% reduction, respectively; $P = 0.008$) (Fig. 4B). Conversely, sustained plasma concentrations below 900 ng/ml, achieved with infusion for 12 days of 22.5 or 45 mg/kg per day, s.c., amantadine, did not significantly impact AIMs. These data are included in the rat, and all-species E_{max} models are presented in Figs. 1F and 3, respectively.

In a previously reported clinical study (Pahwa et al., 2015), steady-state amantadine plasma concentrations were measured in patients with Parkinson's disease administered 210, 274, or 338 mg ADS-5102 (amantadine) extended-release capsules once daily at bedtime (equivalent to 260, 340, or 420 mg amantadine HCl, respectively). Mean (S.D.) steady-state amantadine plasma concentrations were 1383 (354), 1431 (707), and 1677 (512) ng/ml for the respective doses (Fig. 4C). The least-squares mean change from baseline at week 8 in the Unified Dyskinesia Rating Scale (UDysRS) total score was -6.7 , -12.3 , -17.9 , and -16.7 for the placebo, 210-, 274-, and 338-mg groups, respectively (Fig. 4D). Reductions in the UDysRS total score for the 274- and 338-mg groups (corresponding to 43% and 41% reduction, respectively) were significant compared with placebo, and are consistent with the nonclinical PK/PD relationship described above.

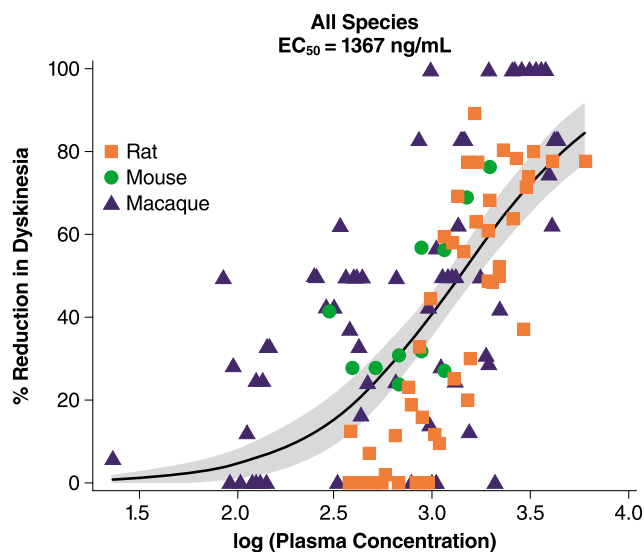


Fig. 3. Determination of the PK/PD relationship and the EC_{50} values across species. The percentage reduction from vehicle in AIMs (mouse and rat) or dyskinesia (macaque) is plotted as a function of the log(amantadine plasma concentration) in mouse, rat, and macaque. $EC_{50} = 1367$ ng/ml. The shaded area represents the 95% CI.

Discussion

Amantadine has been shown to be effective in reducing dyskinesia in animal models of LID as well as in patients with Parkinson's disease treated with levodopa, but the plasma concentrations associated with efficacy have not been well characterized. The analyses described herein are the first to correlate amantadine plasma concentrations with efficacy endpoints for reducing dyskinesia in different species and models of LID. Using an approach that correlates simulated amantadine plasma concentrations from mouse, rat, and macaque PK studies with the previously published and new efficacy data for reducing dyskinesia in LID models, these results define exposure-response relationships that fit sigmoidal E_{max} models and predict EC_{50} values between 1025 and 1633 ng/ml when assessed individually or combining data from all species. Differences in plasma protein binding, blood-brain partitioning, NMDA receptor affinity, or models of Parkinson's disease might have been anticipated to affect the efficacy of amantadine in reducing LID across the species. However, these results show that without correcting for any differences, an EC_{50} of approximately 1400 ng/ml appears to be a commonality across the species examined and provides a target therapeutic plasma amantadine concentration for reducing dyskinesia in patients with Parkinson's disease.

Further support for the efficacy of this range of concentrations is provided by the study in the rat 6-OHDA model dosed with a constant subcutaneous infusion of amantadine, which showed significant percentage reductions from baseline in cumulative AIM assessments compared with vehicle for average plasma concentrations of 2922 ng/ml, whereas no significant effect on AIMs was observed in the dose groups resulting in average amantadine plasma concentrations below 900 ng/ml. Additionally, clinical data from patients with Parkinson's disease treated with the recommended dose of 274 mg ADS-5102 showed a statistically significant reduction in dyskinesia and a mean steady-state amantadine plasma concentration of ~ 1500 ng/ml, which approximates the EC_{50}

value described for the nonclinical species (Pahwa et al., 2015). In the study by Pahwa et al. (2015), the median amantadine plasma concentration in patients who discontinued due to CNS-related AEs was approximately 2100 ng/ml. Verhagen Metman et al. (1998b) reported that amantadine IR reduced dyskinesia in patients with Parkinson's disease treated with levodopa, with plasma concentrations that were consistent with the PK/PD model presented herein (average plasma concentration, ~ 1600 ng/ml), despite differences in study design elements and endpoint measures. Although limited, the PK/PD data in patients with Parkinson's disease provide support that the animal models are predictive of a relationship between amantadine plasma concentration and reduction in dyskinesia in humans.

Improvements in continuous delivery of amantadine would provide a smooth PK profile achieving high concentrations and better coverage throughout the day compared with the peaks and troughs associated with bolus dosing. As reported in the study by Hauser et al. (2018), ADS-5102 can be dosed once daily at bedtime to achieve high morning and sustained daytime amantadine plasma concentrations when symptoms of dyskinesia occur. PK modeling suggested the recommended daily ADS-5102 dosage (274 mg once daily at bedtime) provided maximum steady-state concentrations of ~ 1500 ng/ml in patients with Parkinson's disease, which is approximately 2-fold higher than that achieved with amantadine IR dosing (81 mg, equivalent to amantadine HCl 100 mg, administered at 8:00 AM and 4:00 PM, based on prescription data, which indicated that $\sim 85\%$ of patients with Parkinson's disease treated with amantadine IR were prescribed a dose of ≤ 161 mg/day) (Navarro et al., 2017). Therefore, target amantadine plasma concentrations required to reduce dyskinesia based on the current model can be achieved throughout the day with the recommended once-daily dose of ADS-5102 but not with the commonly used dosing paradigm for amantadine IR.

Collectively, these data show good correlation across species between amantadine plasma concentrations and reduction in LID, highlighting an EC_{50} value of approximately 1400 ng/ml ($\sim 9 \mu\text{M}$) as an efficacious target plasma concentration. Although the mechanism of amantadine for the reduction in dyskinesia and OFF remains to be fully elucidated, these results also correlate well with the range of 50% inhibitory drug concentration values (IC_{50}) reported for amantadine for the NMDA receptor (10–90 μM) (Kornhuber et al., 1991; Bresink et al., 1995; Parsons et al., 1995, 1996). In particular, the IC_{50} of amantadine for the inhibition of striatal NMDA receptors was reported to be 12 μM (Parsons et al., 1996), or 1800 ng/ml, and support the involvement of the NMDA receptor and the glutamatergic pathway in mediating the expression of dyskinesia and motor complications in Parkinson's disease.

In summary, the correlation between the effective concentrations in animals (rodents and NHPs, ~ 1400 ng/ml) and efficacious plasma concentrations in humans (1500 ng/ml) demonstrates that animal models of LID are predictive of efficacy in humans. These results provide a benchmark amantadine plasma concentration of ~ 1400 ng/ml for the reduction of dyskinesia, and provide evidence that the recommended dose of ADS-5102 (274 mg) can provide plasma concentrations in patients with Parkinson's disease that achieve that benchmark, further supporting the use of ADS-5102 for the treatment of dyskinesia in patients with Parkinson's disease.

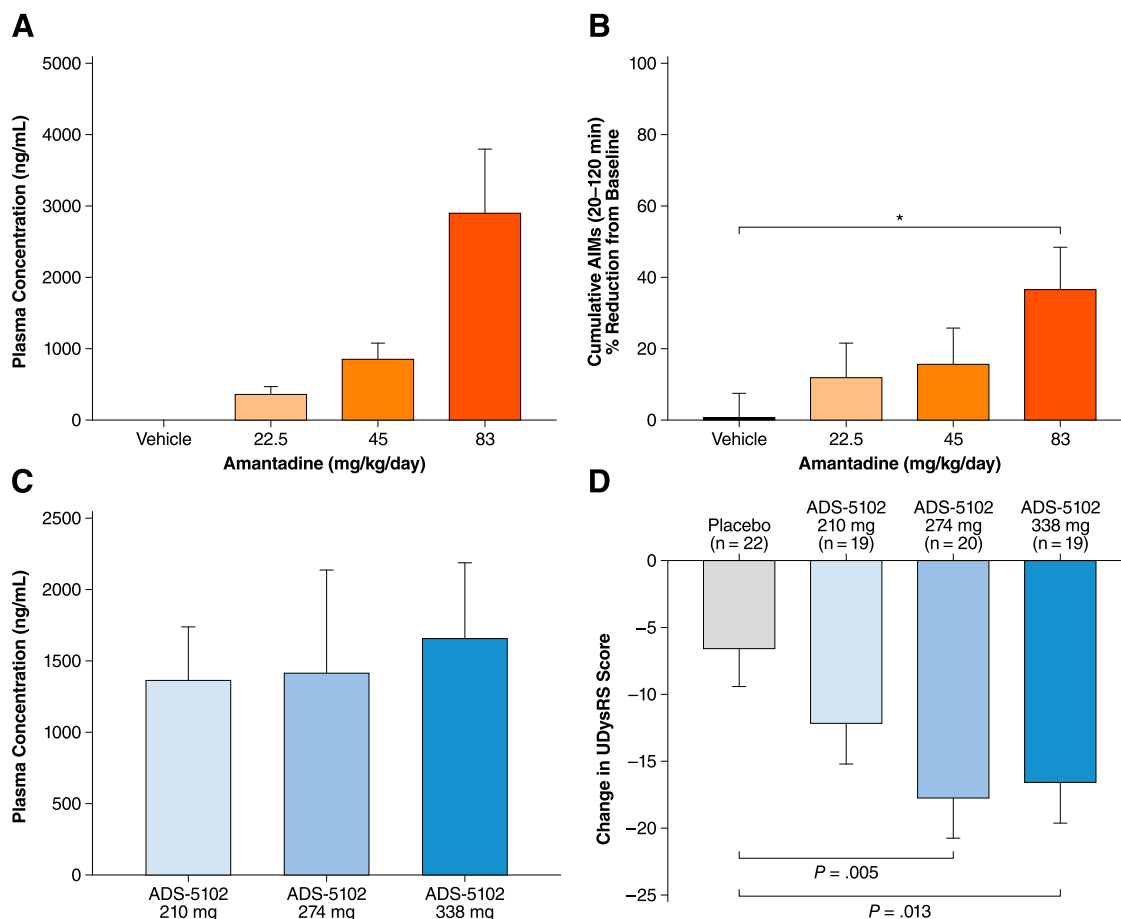


Fig. 4. Comparison of amantadine plasma concentrations (mean \pm S.D.) (A) and associated cumulative AIMs (percentage reduction from baseline, mean \pm S.E.M.) (B) in a rat 6-OHDA levodopa-induced dyskinesia model dosed with a constant subcutaneous infusion of amantadine for 12 days. The percentage reduction of AIMs was statistically significant ($P = 0.008$) for the 83 mg/kg per day amantadine dose, designated by the asterisk. Mean (\pm S.D.) steady-state amantadine plasma concentrations (C) and observed change in UDysRS total score (least-squares mean \pm S.E.) (D) from baseline to week 8 (a decrease indicates improvement). Doses are represented as freebase. These plasma concentrations are predicted to represent high, sustained amantadine exposure throughout the waking hours based on steady-state PK in healthy subjects (Hauser et al., 2018). Source: Pahwa et al. (2015): reprinted with permission.

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Performed data analysis: Brigham, Johnston, and Brown.

Wrote or contributed to the writing of the manuscript: Brigham, Johnston, Brown, Holt, Fox, Hill, Howson, Brotchie, and Nguyen.

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