1521-0103/367/2/373–381$35.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

https://doi.org/10.1124/jpet.118.247650
J Pharmacol Exp Ther 367:373–381, November 2018

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

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Received January 3, 2018; accepted June 13, 2018

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 EC_{50} 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 EC_{50} of amantadine for reducing dyskinesia is consistent across multiple species and supports a plasma concentration target of ~1400 ng/ml to achieve therapeutic efficacy.

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

This study was supported by funding from Adamas Pharmaceuticals, Inc. E.F.B., C.B., J.D.S.H., and J.T.N. are employees of and have received compensation and stock options from Adamas Pharmaceuticals, Inc. T.H.J. has received consultancy payments from and holds an equity stake in Atuka Inc. S.H.F. has received consultancy and research funding from Avanir, Biotie, Britannia, C2N, Cynapsus, Kyowa, Orion, and Zambon; honoraria from the International Parkinson and Movement Disorder Society, CHDI, and American Academy of Neurology; research funding from the Michael J. Fox Foundation for Parkinson's Research, NIH, Parkinson Society Canada, and Toronto Western Foundation; and a salary from the University Health Network Department of Medicine Practice Plan. M.P.H. has received consultancy payments from and holds an equity stake in Atuka Inc. P.A.H. has received consultancy from Atuka Inc. J.M.B. has received consultancy payments from Atuka Inc. and Adamas Pharmaceuticals, Inc.; and holds an equity stake in Atuka Inc. No other potential conflicts of interest relevant to this article are reported.

Part of this work was presented as follows: Brigham EF, Johnston TH, Brown C, Holt JDS, Fox SH, Hill MP, Howson PA, Brotchie JM, and Nguyen JT. PK-PD analysis identifies similar high amantadine plasma concentrations needed to reduce L-DOPA induced dyskinesia across multiple species. *Neuroscience 2017: The Society for Neuroscience Annual Meeting*; 11–15 November 2017; Washington, DC. The Society for Neuroscience, Washington, DC.

https://doi.org/10.1124/jpet.118.247650.

[S] This article has supplemental material available at jpet.aspetjournals.org.

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; EC_{50}, 50% effective plasma concentration; E_{max}, maximum possible effect; IC_{50}, 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 [(3S,4aR,6S,8aR)-6-(phosphonomethyl)-1,2,3,4,4a,5,6,7,8,8a-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; Bezard 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 μl/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; Papathanou 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_{inf}) 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

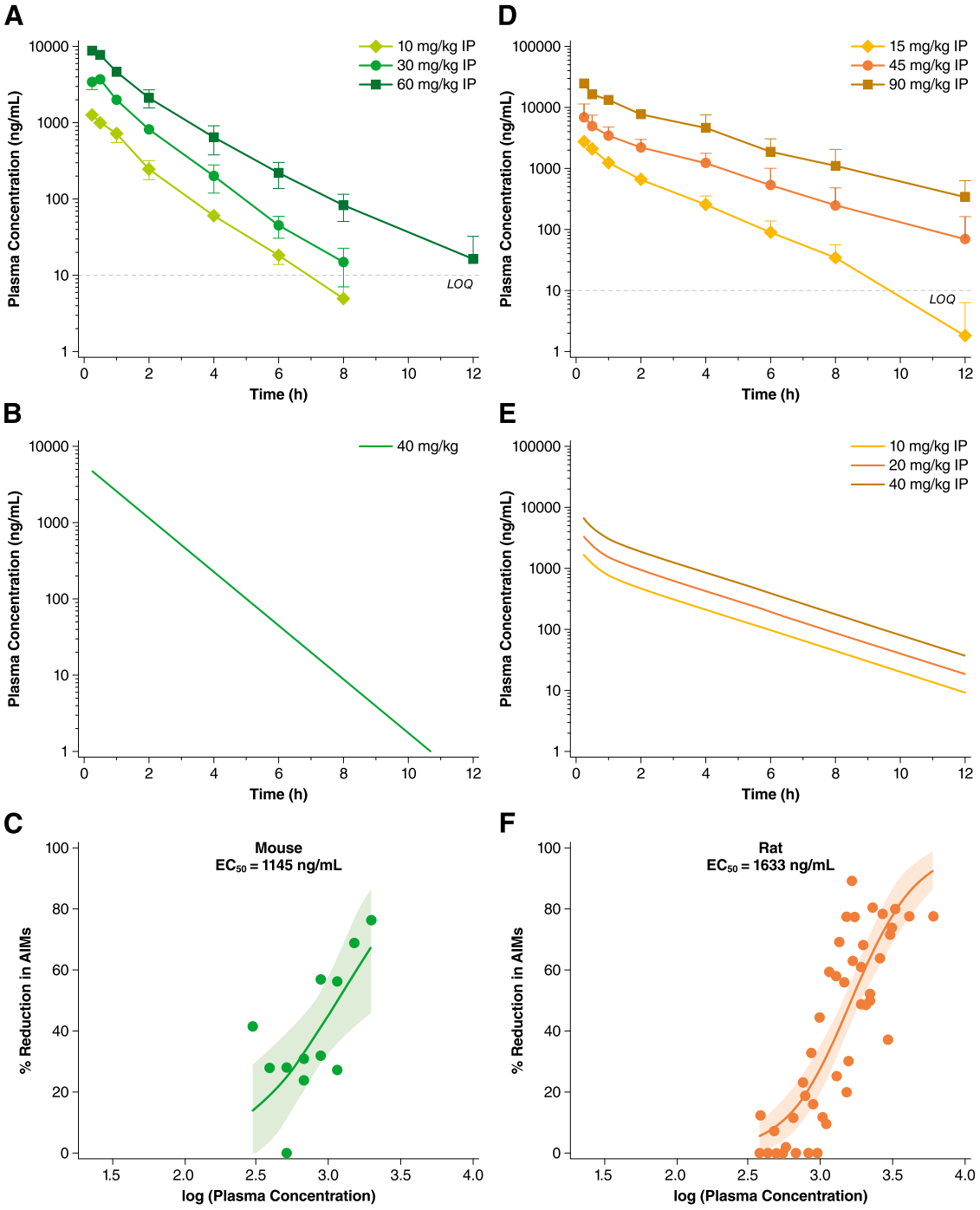

Fig. 1. Amantadine plasma concentration-time profiles after a single intraperitoneal injection dose in mice for observed plasma concentrations (mean ± S.D.) (A) and simulated plasma concentrations (the PK model) (B). (C) Determination of the PK/PD relationship and the EC_{50} in mouse. The percentage reduction from vehicle in AIMs is plotted as a function of the log(amantadine plasma concentration) in mouse. EC_{50} = 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 ± 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 EC_{50} value in rat. The percentage reduction from vehicle in AIMs is plotted as a function of the log(amantadine plasma concentration) in rat. EC_{50} = 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 EC_{50} 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 × exp(–K10 × 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 × exp(–alpha × T) + B × exp(–beta × 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 × (D/V) × T × exp(–K × 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.
[a]Mean of 10, 30, and 60 mg/kg doses used to simulate the 40 mg/kg PK profile.
[b]Mean of 15 and 45 mg/kg doses used to simulate 10, 20, and 40 mg/kg PK profile.
[c]Mean 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

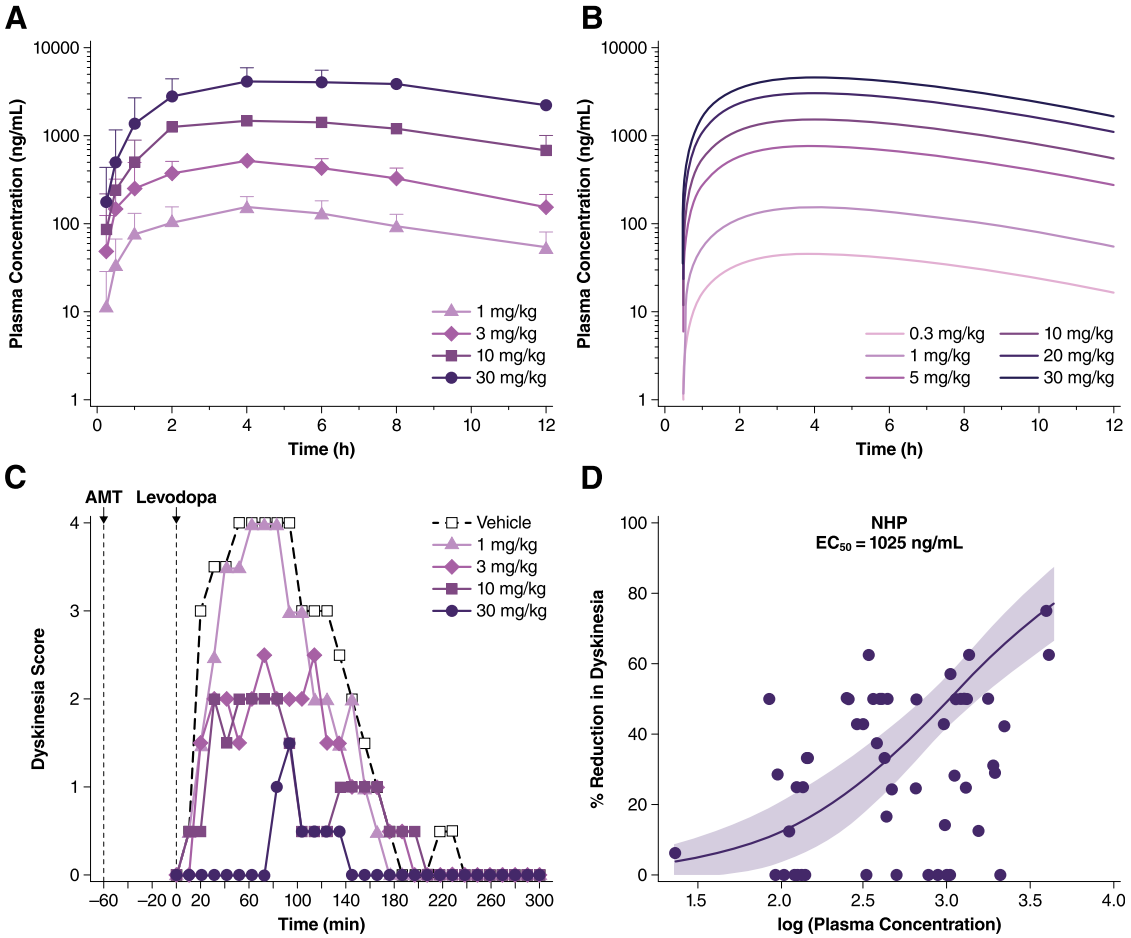

Fig. 2. Amantadine (AMT) plasma concentration-time profiles and dyskinesia score after a single oral dose in dyskinetic cynomolgus macaque for observed plasma concentrations (mean ± S.D.) (A), simulated plasma concentrations (the PK model) (B), and dyskinesia score by treatment (median) (C). The PK model 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.

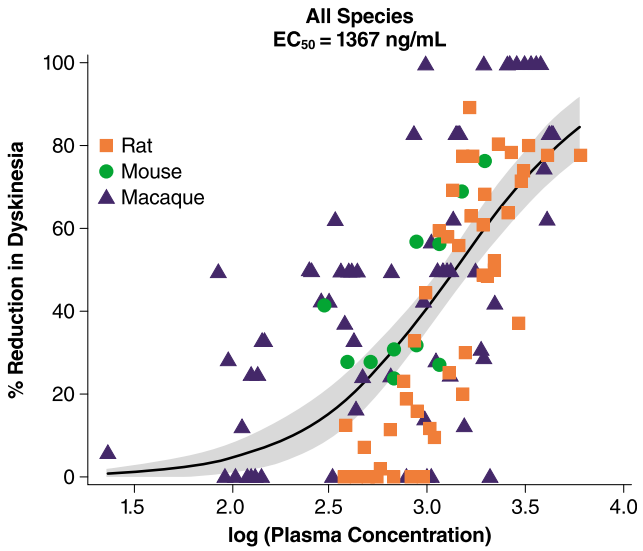

Fig. 3. Determination of the PK/PD relationship and the EC_{50} values across species. The percentage reduction from vehicle in AIMs (mouse and rat) or dyskinesia (macaque) is plotted as a function of the log(amantadine plasma concentration) in mouse, rat, and macaque. EC_{50} = 1367 ng/ml. The shaded 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 ~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 (~9 μM) 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. Theseresults 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.

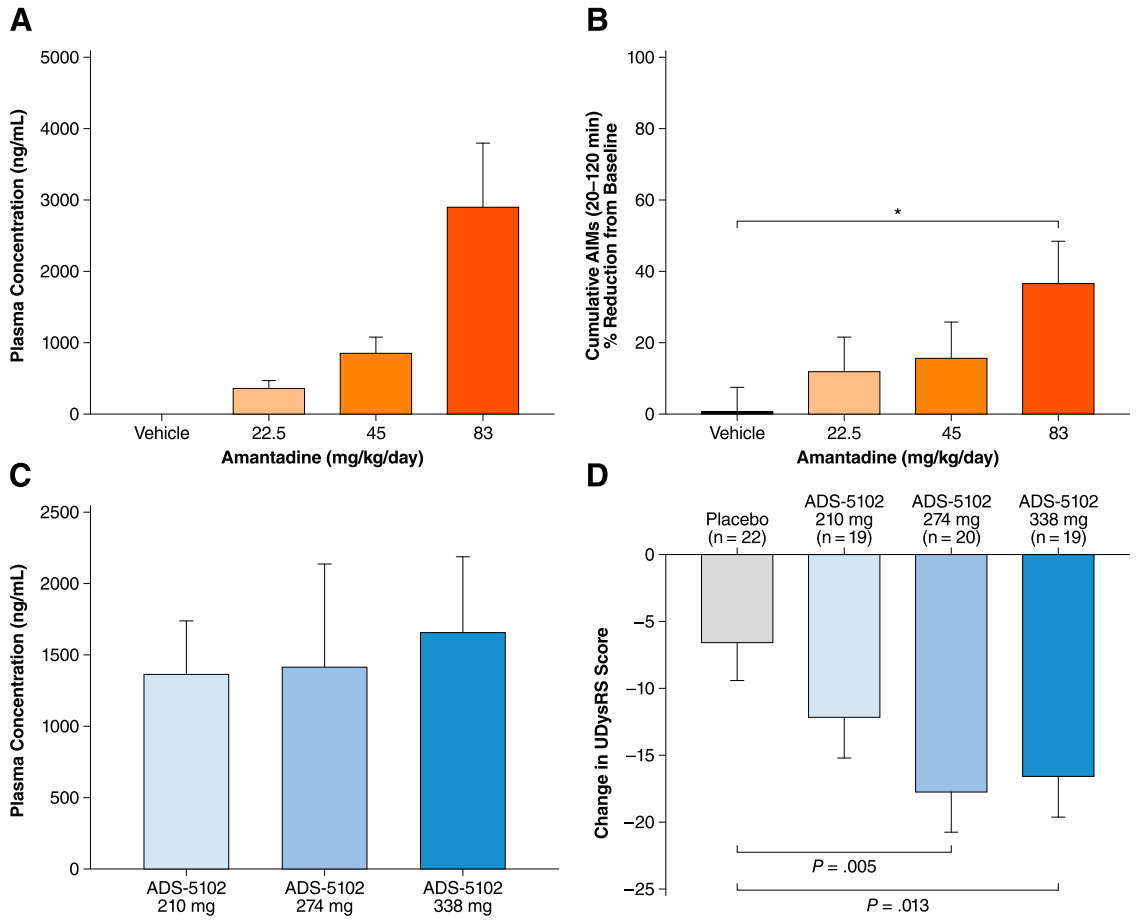

Fig. 4. Comparison of amantadine plasma concentrations (mean ± S.D.) (A) and associated cumulative AIMs (percentage reduction from baseline, mean ± S.E.M.) (B) in a rat 6-OHDA levodopa-induced dyskinesia model dosed with a constant subcutaneous infusion of amantadine for 12 days. The percentage reduction of AIMs was statistically significant ($P = 0.008$) for the 83 mg/kg per day amantadine dose, designated by the asterisk. Mean (±S.D.) steady-state amantadine plasma concentrations (C) and observed change in UDysRS total score (least-squares mean ± 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.

Acknowledgments

We thank The Curry Rockefeller Group, LLC, and Nuventra Pharma Sciences for editorial support, which was funded by Adamas Pharmaceuticals, Inc.

Authorship Contributions

Participated in research design: Brigham, Johnston, Holt, and Nguyen.

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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.

References

Ahlskog JE and Muenter MD (2001) Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov Disord* **16**: 448–458.

Bezard E, Tronci E, Pioli EY, Li Q, Porras G, Björklund A, and Carta M (2013) Study of the antidyskinetic effect of eltoprazine in animal models of levodopa-induced dyskinesia. *Mov Disord* **28**:1088–1096.

Bibbiani F, Oh JD, Kielaite A, Collins MA, Smith C, and Chase TN (2005) Combined blockade of AMPA and NMDA glutamate receptors reduces levodopa-induced motor complications in animal models of PD. *Exp Neurol* **196**:422–429.

Bido S, Marti M, and Morari M (2011) Amantadine attenuates levodopa-induced dyskinesia in mice and rats preventing the accompanying rise in nigral GABA levels. *J Neurochem* **118**:1043–1055.

Blanchet PJ, Konitsiotis S, and Chase TN (1998) Amantadine reduces levodopa-induced dyskinesias in parkinsonian monkeys. *Mov Disord* **13**:798–802.

Bortolanza M, Bariotto-Dos-Santos KD, Dos-Santos-Pereira M, da-Silva CA, and Del-Bel E (2016) Antidyskinetic effect of 7-nitroindazole and sodium nitroprusside associated with amantadine in a rat model of Parkinson's disease. *Neurotox Res* **30**:88–100.

Bresink I, Danysz W, Parsons CG, and Mutschler E (1995) Different binding affinities of NMDA receptor channel blockers in various brain regions—indication of NMDA receptor heterogeneity. *Neuropharmacology* **34**:533–540.

Calon F, Morissette M, Ghribi O, Goulet M, Grondin R, Blanchet PJ, Bédard PJ, and Di Paolo T (2002) Alteration of glutamate receptors in the striatum of dyskinetic 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated monkeys following dopamine agonist treatment. *Prog Neuropsychopharmacol Biol Psychiatry* **26**:127–138.

Dekundy A, Lundblad M, Danysz W, and Cenci MA (2007) Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. *Behav Brain Res* **179**:76–89.

Dupre KB, Ostock CY, Eskow Jaunarajs KL, Button T, Savage LM, Wolf W, and Bishop C (2011) Local modulation of striatal glutamate efflux by serotonin 1A receptor stimulation in dyskinetic, hemiparkinsonian rats. *Exp Neurol* **229**:288–299.

Grégoire L, Jourdain VA, Townsend M, Roach A, and Di Paolo T (2013) Safinamide reduces dyskinesias and prolongs L-DOPA antiparkinsonian effect in parkinsonian monkeys. *Parkinsonism Relat Disord* **19**:508–514.

Hayden FG, Hoffman HE, and Spyker DA (1983) Differences in side effects of amantadine hydrochloride and rimantadine hydrochloride relate to differences in pharmacokinetics. *Antimicrob Agents Chemother* **23**:458–464.

Hauser RA, Pahwa R, Wargin WA, Souza-Prien CJ, McClure N, Johnson R, Nguyen JT, Patni R, and Went GT (2018) Pharmacokinetics of ADS-5102 (amantadine) extended release capsules administered once daily at bedtime for the treatment of dyskinesia. *Clin Pharmacokinet* DOI:10.1007/s40262-018-0663-4.

Hill MP, Ravenscroft P, Bezard E, Crossman AR, Brotchie JM, Michel A, Grimée R, and Klitgaard H (2004) Levetiracetam potentiates the antidyskinetic action of amantadine in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned primate model of Parkinson's disease. *J Pharmacol Exp Ther* **310**:386–394.

Jonkers N, Sarre S, Ebinger G, and Michotte Y (2002) MK801 suppresses the L-DOPA-induced increase of glutamate in striatum of hemi-Parkinson rats. *Brain Res* **926**:149–155.

Ko WK, Pioli E, Li Q, McGuire S, Dufour A, Sherer TB, Bezard E, and Facheris MF (2014) Combined fenobam and amantadine treatment promotes robust anti-dyskinetic effects in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned primate model of Parkinson's disease. *Mov Disord* **29**:772–779.

Kobylecki C, Hill MP, Crossman AR, and Ravenscroft P (2011) Synergistic anti-dyskinetic effects of topiramate and amantadine in animal models of Parkinson's disease. *Mov Disord* **26**:2354–2363.

Kornhuber J, Bormann J, Hübers M, Rusche K, and Riederer P (1991) Effects of the 1-amino-adamantanes at the MK-801-binding site of the NMDA-receptor-gated ion channel: a human postmortem brain study. *Eur J Pharmacol* **206**:297–300.

Navarro R, Whangbo A, Pahwa R, Isaacson SH, Schmidt P, and Went GT (2017) An assessment of the persistence and medication possession ratio of adjunctive treatments to levodopa in patients with Parkinson's disease (PD), in *ISPOR 2017: The International Society for Pharmacoeconomics and Outcomes Research 22nd Annual International Meeting;* 2017 May 20–24; Boston, MA.

Oertel W, Eggert K, Pahwa R, Tanner CM, Hauser RA, Trenkwalder C, Ehret R, Azulay JP, Isaacson S, Felt L, et al. (2017) Randomized, placebo-controlled trial of ADS-5102 (amantadine) extended-release capsules for levodopa-induced dyskinesia in Parkinson's disease (EASE LID 3). *Mov Disord* **32**:1701–1709.

Pahwa R, Tanner CM, Hauser RA, Isaacson SH, Nausieda PA, Truong DD, Agarwal P, Hull KL, Lyons KE, Johnson R, et al. (2017) ADS-5102 (amantadine) extended-release capsules for levodopa-induced dyskinesia in Parkinson disease (EASE LID study): a randomized clinical trial. *JAMA Neurol* **74**:941–949.

Pahwa R, Tanner CM, Hauser RA, Sethi K, Isaacson S, Truong D, Struck L, Ruby AE, McClure NL, Went GT, et al. (2015) Amantadine extended release for levodopa-induced dyskinesia in Parkinson's disease (EASED study). *Mov Disord* **30**:788–795.

Papa SM and Chase TN (1996) Levodopa-induced dyskinesias improved by a glutamate antagonist in Parkinsonian monkeys. *Ann Neurol* **39**:574–578.

Papathanou M, Jenner P, Iravani M, Jackson M, Stockwell K, Strang I, Zeng BY, McCreary AC, and Rose S (2014) The H3 receptor agonist immepip does not affect l-dopa-induced abnormal involuntary movements in 6-OHDA-lesioned rats. *Eur J Pharmacol* **741**:304–310.

Paquette MA, Martinez AA, Macheda T, Meshul CK, Johnson SW, Berger SP, and Giuffrida A (2012) Anti-dyskinetic mechanisms of amantadine and dextromethorphan in the 6-OHDA rat model of Parkinson's disease: role of NMDA vs. 5-HT1A receptors. *Eur J Neurosci* **36**:3224–3234.

Parkes JD, Zilkha KJ, Marsden P, Baxter RC, and Knill-Jones RP (1970) Amantadine dosage in treatment of Parkinson's disease. *Lancet* **1**:1130–1133.

Parsons CG, Panchenko VA, Pinchenko VO, Tsyndrenko AY, and Krishtal OA (1996) Comparative patch-clamp studies with freshly dissociated rat hippocampal and striatal neurons on the NMDA receptor antagonistic effects of amantadine and memantine. *Eur J Neurosci* **8**:446–454.

Parsons CG, Quack G, Bresink I, Baran L, Przegalinski E, Kostowski W, Krzascik P, Hartmann S, and Danysz W (1995) Comparison of the potency, kinetics and voltage-dependency of a series of uncompetitive NMDA receptor antagonists in vitro with anticonvulsive and motor impairment activity in vivo. *Neuropharmacology* **34**:1239–1258.

Paxinos G and Watson C (1986) *The Rat Brain in Stereotaxic Coordinates*, 2nd ed, Academic Press, Orlando, FL.

Robelet S, Melon C, Guillet B, Salin P, and Kerkerian-Le Goff L (2004) Chronic L-DOPA treatment increases extracellular glutamate levels and GLT1 expression in the basal ganglia in a rat model of Parkinson's disease. *Eur J Neurosci* **20**: 1255–1266.

Sebastianutto I, Maslava N, Hopkins CR, and Cenci MA (2016) Validation of an improved scale for rating l-DOPA-induced dyskinesia in the mouse and effects of specific dopamine receptor antagonists. *Neurobiol Dis* **96**:156–170.

Sgambato-Faure V and Cenci MA (2012) Glutamatergic mechanisms in the dyskinesias induced by pharmacological dopamine replacement and deep brain stimulation for the treatment of Parkinson's disease. *Prog Neurobiol* **96**:69–86.

Suh DC, Pahwa R, and Mallya U (2012) Treatment patterns and associated costs with Parkinson's disease levodopa induced dyskinesia. *J Neurol Sci* **319**:24–31.

Verhagen Metman L, Blanchet PJ, van den Munckhof P, Del Dotto P, Natté R, and Chase TN (1998a) A trial of dextromethorphan in parkinsonian patients with motor response complications. *Mov Disord* **13**:414–417.

Verhagen Metman L, Del Dotto P, van den Munckhof P, Fang J, Mouradian MM, and Chase TN (1998b) Amantadine as treatment for dyskinesias and motor fluctuations in Parkinson's disease. *Neurology* **50**:1323–1326.

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SUPPLEMENTAL MATERIALS

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

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Adamas Pharmaceuticals, Inc., Emeryville, California, USA (E.F.B., C.B., J.D.S.H., J.T.N.); Atuka Inc, Toronto, Ontario, Canada (T.H.J., M.P.H., P.A.H., J.M.B.); Krembil Research Institute, University Health Network, Toronto, Ontario, Canada (T.H.J., S.H.F., J.M.B.); Morton and Gloria Shulman Movement Disorders Centre and the Edmond J. Safra Program in Parkinson's Disease, Toronto Western Hospital, University Health Network, Division of Neurology, University of Toronto, Toronto, Ontario, Canada (S.H.F.)

Methods

Bioanalysis

Rodent

A validated LC-MS/MS method was used to determine the concentration of amantadine in mouse and rat plasma samples. For the calibration standards, a 10-µL aliquot of the appropriate concentration of amantadine (10.0-4000 ng/mL) was added to 50-µL blank rat K_2EDTA plasma. All other samples (blanks and mouse or rat plasma samples) received 10 µL 50% methanol instead of the standard. Samples received a 25-µL aliquot of internal standard (1-aminoadamantane-d6, 5000 ng/mL in 50% methanol) except the blank, which received 50% methanol in place of the internal standard. Samples were vortexed and 200 µL acetonitrile:methanol (4:1, v:v) was then added to each sample. Samples were vortexed and centrifuged and 25 µL of the supernatant was added to a 96-well plate containing 1000 µL 0.1% formic acid. Samples were vortexed and centrifuged and a 10-µL aliquot was injected onto an LC-MS/MS (API 5000™, Applied Biosystems, Foster City, California) for analysis.

Amantadine was eluted from the high performance liquid chromatography column (Phenomenex Luna 5 µm PFP (2), 50 × 2.0 mm, Phenomenex, Torrance, California) for mass quantification with the mass spectrometer set at mass-to-charge ratios of 152.40>135.00 and 158.55>141.06 (m/z) to quantify amantadine and internal standard, respectively. An automated data acquisition system (Analyst 1.4.2, Applied Biosystems, Foster City, California) processed and calculated the data.

Cynomolgus macaque

For cynomolgus macaque samples, proteins were precipitated by combining 30 µL plasma and 5 µL water containing 0.1% formic acid, the internal standard (desloratadine, 20 µg/mL), and 60 µL trichloroacetic acid (10%, w/v). The mixture was vortexed for 3 min and centrifuged at 3700 rpm for 15 min and 15 µL of the supernatant was combined with 150 µL water (with 0.1% formic acid) and vortexed for 5 min. An aliquot of 10 µL of the mixture was injected into the LC-MS/MS system. LC-MS/MS analyses were performed on a Shimadzu LC-10AD pump equipped with a CTC-HTS auto-sampler (Zwingen, Switzerland) and a column oven. The MS/MS system was an MDS Sciex API-4000 mass spectrometer with an electrospray ionization probe (Toronto, Canada). Chromatographic separation of the analytes was achieved on an Agilent ZORBAX SB-C18 column. The linearity was from 10 to 2000 ng/mL.

Rodent ALO AIMs Scale

Three subtypes of AIMs were assessed by raters blind to treatment: axial—dystonic postures or choreiform twisting of the neck and upper body towards the contralateral side; limb—

random uncontrollable movements of forelimb contralateral to the lesion; and orolingual–excess chewing and jaw movements with protrusion of the tongue. For each subtype, the duration of AIMs was scored between 0 and 4 as follows: 0 = absent; 1 = present for less than 30 seconds; 2 = present for more than 30 seconds; 3 = present throughout the minute but suppressed by external stimuli; and 4 = present throughout the minute but not suppressible by external stimuli. A maximum score denotes the highest score obtained at any point of observation. For the published studies, AIMs data were expressed for each observational time point as the maximal score of ALO AIMs, the sum of ALO AIMs, or as the product of the ALO AIMs multiplied by an amplitude score.

Development of MPTP-Macaque LID Model

Eight female macaques received a once-daily SC injection of MPTP (0.2 mg/kg) for up to 30 days. Over the next 90 days, MPTP was administered as needed until a moderate level of parkinsonian syndrome was observed. Animals with MPTP lesions producing stable symptoms for at least 60 days were administered 25 mg/kg levodopa (levodopa:benserazide ratio: 4:1; Madopar, Roche, Auckland, New Zealand) orally for a minimum of 4 months to induce choreiform and dystonic dyskinesia. Before the start of the amantadine study, dose-finding observations were conducted (data not shown) to identify a dose of levodopa intended to produce optimal antiparkinsonian actions but which was compromised by disabling dyskinesia (range, 30-35 mg/kg; mean ± standard deviation, 31.9 ± 2.6 mg/kg). Responses to this dose of levodopa were assessed to ensure stability and reproducibility within each animal on successive levodopa administrations. The behavior of the animals was recorded by video after being placed in an observation cage. The rating scale for dyskinesia,

adapted from its clinical counterpart (Unified Dyskinesia Rating Scale), was used to assess the video recordings via post-hoc analysis by a movement disorders neurologist blinded to treatment. Dyskinesia data representative of the maximum of either chorea or dystonia were scored between 0 and 4 as follows: 0 = absent; 1 = mild; 2 = moderate; 3 = marked; and 4 = severe. Dyskinesia was assessed for 5 minutes every 10 minutes, the score given being most representative of each 5-minute observation period.

Mouse PK/PD Analysis

Data on AIMS from Bido et al (Bido et al., 2011) and Sebastianutto et al (Sebastianutto et al., 2016) were included in the analysis. In the Bido et al (Bido et al., 2011) study, 40 mg/kg amantadine or vehicle was administered IP 60 minutes before levodopa, while in the Sebastianutto et al (Sebastianutto et al., 2016) study, 40 mg/kg amantadine or vehicle was administered IP 100 minutes before levodopa. For both studies, the percent reduction in the AIMs score was calculated for the mice administered amantadine relative to mice treated with vehicle at 20, 40, 60, 80, 100, and 120 minutes after levodopa administration.

Rat PK/PD Analysis

In addition to the de novo PK and efficacy data generated herein, efficacy data from rat studies reported by Bido et al (Bido et al., 2011), Papathanou et al (Papathanou et al., 2014), and Bortolanza et al (Bortolanza et al., 2016) were included in the analysis. In the Bido et al (Bido et al., 2011) study, 40 mg/kg amantadine or vehicle was administered IP 60 minutes before levodopa, and the percent reduction in the AIMs score was calculated for the rats administered amantadine relative to rats treated with vehicle at 40, 60, 80, 100, and 120

minutes after levodopa administration. In the Papathanou et al (Papathanou et al., 2014) study, levodopa was administered IP concurrently with vehicle or with 10, 20, or 40 mg/kg amantadine, and the percent reduction in the AIMs score was calculated for the rats administered amantadine relative to rats treated with vehicle at 20, 40, 60, 80, 100, and 120 minutes after levodopa administration. In the Bortolanza et al (Bortolanza et al., 2016) study, 10, 20, or 40 mg/kg amantadine or vehicle was administered IP 35 minutes before levodopa, and the percent reduction in the AIMs score was calculated for the rats administered amantadine relative to rats treated with vehicle at 20, 40, 60, 80, 100, and 120 minutes after levodopa administration.

Cynomolgus Macaque PK/PD Analysis

In addition to the de novo PK and efficacy data generated herein, efficacy data from macaque studies reported by Bezard et al (Bezard et al., 2013), Gregoire et al (Gregoire et al., 2013) and Ko et al (Ko et al., 2014) were included in the analysis. In the Bezard et al (Bezard et al., 2013) study, 10 and 20 mg/kg amantadine or vehicle was administered orally 15 minutes before levodopa, and the percent reduction in the dyskinesia score was calculated for the animals administered amantadine relative to animals treated with vehicle at 20, 50, 80, 110, and 140 minutes after levodopa administration. In the Gregoire et al (Gregoire et al., 2013) study, 0.3, 1, 5, or 20 mg/kg amantadine or vehicle was administered orally 60 minutes before levodopa, and the percent reduction in the dyskinesia score was calculated for the animals administered amantadine relative to animals treated with vehicle at 60 minutes after levodopa administration. In the Ko et al (Ko et al., 2014) study, 10, 20, or 30 mg/kg amantadine or vehicle was administered concurrently with levodopa, and the percent reduction in the dyskinesia score using the NHP dyskinesia rating scale was calculated for

the animals administered levodopa and amantadine relative to animals treated with vehicle at 50, 80, 110, and 140 minutes after levodopa administration.

Human Studies PK/PD Analysis

As previously reported by Pahwa et al (Pahwa et al., 2015) patients with Parkinson's disease experiencing dyskinesia were administered 210, 274, or 338 mg ADS-5102 (equivalent to 260, 340, or 420 mg amantadine HCl, respectively), an extended release capsule formulation of amantadine. This study was designed as a multicenter, randomized, double-blind, placebo-controlled, 4-arm, parallel-group study in which 83 patients were randomly assigned in a 1:1:1:1 ratio to 1 of 4 treatments including placebo. Each patient received an oral dose once daily at bedtime (no earlier than 9 PM if possible) for 8 weeks. Blood samples were collected on day 1 (predose) and weeks 1, 2, 4, 6, and 8 (if the week 6 sample was missed) between 9 am and 4 PM, time points which are predicted to occur during high, sustained amantadine exposure based on steady-state PK in healthy subjects (Hauser RA, Pahwa R, Wargin W, et al. Pharmacokinetics of ADS-5102 [amantadine] extended release capsules administered once-daily at bedtime for the treatment of dyskinesia. [In Review]). Amantadine plasma concentrations were determined by LC-MS/MS using a validated method as described in Hauser et al (Hauser RA, Pahwa R, Wargin W, et al. Pharmacokinetics of ADS-5102 [amantadine] extended release capsules administered once-daily at bedtime for the treatment of dyskinesia. [In Review]). Dyskinesia was assessed using the Unified Dyskinesia Rating Scale (UDysRS). For comparison across the species, the dyskinesia data at week 8 were recalculated as a percent reduction in dyskinesia from the mean baseline UDysRS score for each group.

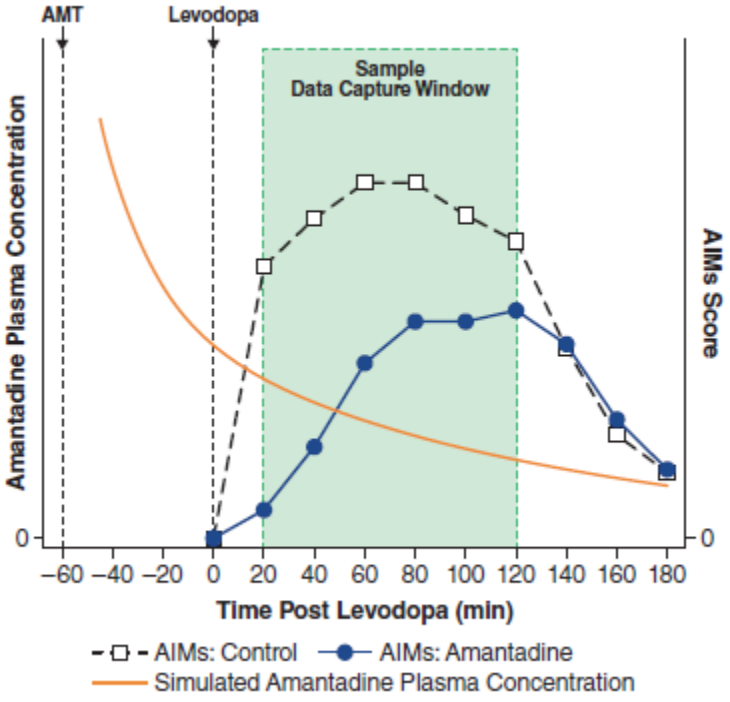
SUPPLEMENTARY INFORMATION, TABLES AND FIGURES

SUPPLEMENTAL TABLE 1. Overview of nonclinical studies conducted in present publication and referenced studies

SUPPLEMENTAL TABLE 2. Comparison of EC_{50}, HillSlope, and R^2 for individual species to pooled data

SUPPLEMENTAL FIG. 1. Example time course for pharmacokinetic/pharmacodynamic assessments. Amantadine (AMT) and levodopa timing of administrations are indicated by arrows. Simulated amantadine plasma concentrations are indicated by the solid line, control abnormal involuntary movements (AIMs) represented by lines with open circles, and amantadine AIMs represented by lines with closed circles. In this example, the data capture window (dashed vertical lines) brackets between 20 and 120 minutes post levodopa administration.

Supplemental Fig. 1.



SUPPLEMENTAL TABLE 2. Overview of nonclinical studies conducted in present publication and referenced studies

Species and strain	Amantadine Dosing regimen	Sampling schedule	Bioanalysis method[a]	Reference
C57BL/6J mouse	IP 10, 30, and 60 mg/kg	0.25, 0.5, 1, 2, 4, 6, 8, and 12 h postdose	LC-MS/MS	Current publication
Sprague Dawley rat	IP 15, 45, and 90 mg/kg	Predose, 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h postdose	LC-MS/MS	Current publication
Sprague Dawley rat	SC ALZET Osmotic Minipump 22.5, 45, and 83 mg/kg per day Constant infusion for 12 days	Amantadine treatment day 12	LC-MS/MS	Current publication
Cynomolgus macaque	Oral 1, 3, 10, and 30 mg/kg	Predose, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, and 48 h postdose	LC-MS/MS	Current publication
Human	Oral capsule 210, 274, or 338 mg ADS-5102 (equivalent to 260, 340, or 420 mg amantadine HCl)	Blood samples were collected on day 1 (predose), and weeks 1, 2, 4, 6, and 8 (if the week 6 sample was missed) between 9 am and 4 pm	LC-MS/MS	Pahwa et al., 2015

Species and strain	Amantadine Dosing regimen	6-OHDA or MPTP dose paradigm	Lesion acceptance criteria	Daily levodopa/benserazide dose and route	Endpoint: AIMs or dyskinesia Total duration of observations	Reference
Swiss mouse	IP 40 mg/kg 60 min before levodopa	6 µg/2 µL 6-OHDA Striatum	Contralateral forelimb placements <40% total in cylinder test	IP 15 mg/kg levodopa/12mg/kg benserazide	Global AIMs[b] (ALO) 1 min every 20 min for 180 min post levodopa	Bido et al., 2011
C57Bl/6J mouse	IP 40 mg/kg 100 min before levodopa	3.2 µg/1 µL 6-OHDA Median forebrain bundle	Contralateral forelimb placements <25% total in cylinder test	SC Escalating doses of 3 and 6 mg/kg levodopa/12 mg/kg benserazide	Global AIMs[b] (ALO) 1 min every 20 min for 180 min post levodopa	Sebastianutto et al., 2016
Sprague Dawley rat	SC ALZET Osmotic Minipump 22.5, 45, or 83 mg/kg per day Constant infusion for 12 days	12 µg/2.5 µL 6-OHDA Median forebrain bundle	Contralateral forelimb placements <15% total in cylinder test	IP 10 mg/kg levodopa/15 mg/kg benserazide	AIMs (ALO) 1 min every 20 min for 180 min post levodopa	Current publication
Sprague Dawley rat	IP 40 mg/kg 60 min before levodopa	8 µg/4 µL 6-OHDA Median forebrain bundle	Amphetamine (5 mg/kg IP) induced rotations >7/min	IP 6 mg/kg levodopa/12 mg/kg benserazide	Global AIMs (ALO) 1 min every 20 min for 180 min post levodopa	Bido et al., 2011
Wistar rat	IP 10, 20, and 40 mg/kg Immediately after levodopa	8 µg/4 µL 6-OHDA Median forebrain bundle	Amphetamine (2.5 mg/kg IP) or apomorphine (0.5 mg/kg SC)	IP 6.25 mg/kg levodopa/15 mg/kg benserazide	AIMs (ALO) 1 min every 20 min for 120 min post levodopa	Papathanou et al., 2014

Species and strain	Amantadine Dosing regimen	6-OHDA or MPTP dose paradigm	Lesion acceptance criteria	Daily levodopa/benserazide dose and route	Endpoint: AIMs or dyskinesia Total duration of observations	Reference
			induced rotations >6/min			
Wistar rat	IP 10, 20, and 40 mg/kg 35 min before levodopa	4 µg/3 µL 6-OHDA Median forebrain bundle	Apomorphine (0.5 mg/kg SC) induced rotations >2/min	Oral 20 mg/kg levodopa/5 mg/kg benserazide	Global AIMs (ALO) 1 min every 20 min for 180 min post levodopa	Bortolanza et al., 2016
Cynomolgus macaque	Oral 1, 3, 10, and 30 mg/kg 60 min before levodopa	0.2 mg/kg MPTP Daily injections for 90 days as needed	Lesion stable for minimum 60 days	Oral 25 mg/kg levodopa/6.25 mg/kg benserazide for at least 4 months Challenge levodopa dose adjusted for each macaque (30-35 mg/kg)	Dyskinesia (NHPDysR: choreic and dystonic components, adapted from UDysRS) 5 min every 10 min up to 6 h	Current publication
Cynomolgus macaque	Oral 10 and 20 mg/kg 15 min before levodopa	MPTP Dose and route not specified	N/A	Dose and route not specified (The authors cited another paper that indicated QID 6-8 months with 4:1	Dyskinesia Dyskinesia Disability Scale (choreic and dystonic components)	Bezard et al., 2013

Species and strain	Amantadine Dosing regimen	6-OHDA or MPTP dose paradigm	Lesion acceptance criteria	Daily levodopa/benserazide dose and route	**Endpoint:** **AIMs or dyskinesia** Total duration of observations	Reference
				levodopa/carbidopa ratio)	10 min every 30 min for 250 min post levodopa	
Cynomolgus macaque	Oral 0.3, 1, 5, and 20 mg/kg 60 min before levodopa	SC ALZET Osmotic Minipump 0.5 mg/24 h MPTP Continuous infusion using SC osmotic minipump until stable parkinsonian syndrome developed	N/A	QD with levodopa/benserazide 100/25 mg capsule orally until reproducible dyskinesias developed Levodopa/benserazide 100/25 or 50/12.5 mg/kg was administered orally 3 times per week, 3 weeks before study Challenge dose (SC): levodopa doses were adjusted for each macaque and varied from 15/50 to 35/50 mg/kg	Dyskinesia Dyskinesias were rated for the face, neck, trunk, arms, and legs and summed (maximal score: 21) Severity of chorea and dystonia scored For amantadine experiment only, peak effect is reported 60 min post levodopa	Gregoire et al., 2013

Species and strain	Amantadine Dosing regimen	6-OHDA or MPTP dose paradigm	Lesion acceptance criteria	Daily levodopa/benserazide dose and route	Endpoint: AIMs or dyskinesia Total duration of observations	Reference
Cynomolgus macaque	Oral 10, 20, and 30 mg/kg Concurrent with levodopa	0.2 mg/kg MPTP Daily injections until parkinsonian signs appeared	N/A	Twice daily with an individually titrated dose of levodopa that provided maximum reversal of PD motor behaviors 4:1 levodopa/carbidopa ratio (range, 9-17 mg/kg); chronic levodopa treatment lasted for 4-5 months; maintenance dose is 2×/week	Dyskinesia NHPDysR 10 min every 30 min for 240 min post levodopa	Ko et al., 2014
Human	Oral capsule 210, 274, or 338 mg ADS-5102 (equivalent to 260, 340, or 420 mg amantadine HCl)	N/A	N/A	N/A	UdysRS evaluated at the baseline, week 2, week 4, and week 8 visits	Pahwa et al., 2015

[a]LC-MS/MS was validated in rat and macaque and used for studies reported in this publication.

[b]Global AIMs: A composite AIM score produced by multiplying the basic score and an amplitude score for each AIMs subtype.

IP, intraperitoneal; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LID, levodopa-induced dyskinesia; 6-OHDA, 6-hydroxydopamine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; AIMs, abnormal involuntary movements; ALO, axial, limb,

and orolingual; SC, subcutaneous; NHPDysR, Nonhuman Primate Dyskinesia Rating Scale; UDysRS, Unified Dyskinesia Rating Scale; N/A, not applicable; QID, 4 times daily; QD, once daily.

SUPPLEMENTAL TABLE 2. Comparison of EC_{50}, HillSlope, and R^2 for individual species to pooled data

Variable	Mouse	Rat	Macaque	Mouse, rat, macaque (pooled)
$LogEC_{50}$	3.059	3.213	3.011	3.136
HillSlope	1.341	1.924	0.840	1.148
R^2	0.490	0.682	0.407	0.429
EC_{50} (95% CI), ng/mL	1145 (756-1735)	1633 (1419-1879)	1025 (716-1467)	1367 (1139-1639)
DF	42	10	68	124

CI, confidence interval; DF, degrees of freedom; EC_{50}, 50% effective plasma concentration.