RESEARCH ARTICLE

Pridopidine, a Clinic-Ready Compound, Reduces 3,4-Dihydroxyphenylalanine-Induced Dyskinesia in Parkinsonian Macaques

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ABSTRACT: **Background:** Pridopidine, in development for Huntington's disease, may modulate aberrant L-dopa-induced effects including L-dopa-induced dyskinesia (LID).
Objective: This study investigated whether pridopidine could reduce LID in the MPTP macaque model of Parkinson's disease and characterized the observed behavioral effects in terms of receptor occupancy.
Methods: The pharmacokinetic profile and effects of pridopidine (15-30 mg/kg) on parkinsonism, dyskinesia, and quality of on-time, in combination with L-dopa, were assessed in MPTP macaques with LID. Pridopidine receptor occupancy was estimated using known in vitro binding affinities to σ_1 and dopamine D_2 receptors, in vivo PET imaging, and pharmacokinetic profiling across different species.
Results: Pridopidine produced a dose-dependent reduction in dyskinesia (up to 71%, 30 mg/kg) and decreased the duration of on-time with disabling dyskinesia evoked by L-dopa by 37% (20 mg/kg) and 60% (30 mg/kg). Pridopidine did not compromise the anti-parkinsonian benefit of L-dopa. Plasma exposures following the ineffective dose (15 mg/kg) were associated with full σ_1 occupancy (>80%), suggesting that σ_1 engagement alone is unlikely to account for the antidyskinetic benefits of pridopidine. Exposures following effective doses (20-30 mg/kg), while providing full σ_1 occupancy, provide only modest dopamine D_2 occupancy (<40%). However, effective pridopidine doses clearly engage a range of receptors (including adrenergic-α_{2C}, dopamine-D_3, and serotoninergic-5-HT_{1A} sites) to a higher degree than D_2 and might contribute to the antidyskinetic actions.
Conclusions: In MPTP macaques, pridopidine produced a significant decrease in LID without compromising the antiparkinsonian benefit of L-dopa. Although the actions of pridopidine were associated with full σ_1 occupancy, effective exposures are more likely associated with occupancy of additional, non-sigma receptors. This complex pharmacology may underlie the effectiveness of pridopidine against LID. © 2018 International Parkinson and Movement Disorder Society

Key Words: dopidines; dyskinesia; Parkinson's disease; sigma-1 receptor

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The development of motor complications in Parkinson's disease (PD) is an unvarying consequence of therapeutic dopamine replacement with 3,4-dihydroxyphenylalanine (L-dopa).[1,2] Over time, the quality of benefit provided by L-dopa is compromised as a result of the development of involuntary movements, L-dopa-induced dyskinesia (LID), and a shortening of the duration for which therapy is efficacious, wearing-off[3] as well as abrupt changes in motor status (ON-OFF phenomena). LID can be as troublesome as the disease itself.[4] A 2014 Priority Setting Partnership identified LID third among a list of 96 unmet needs in PD.[5]

Pridopidine (TV-7820, ACR16) is a small molecule under development for the treatment of Huntington's disease (HD). Pridopidine's binding profile has been characterized using in vitro assays and binds with highest affinity to the sigma-1 receptor (σ_1R) with an inhibitor constant (K_i) of 82 nM (human) or 70 nM (rat),[6] considerably higher than its affinity for the dopamine D_2 receptor (D_2R) previously thought to represent the main target for pridopidine activity (binding K_i $\sim$10 μM).[7,8] Affinities in the moderate to low micromolar range of pridopidine were also found for other CNS receptors including the σ_2 receptor, adrenergic α_{2A}, α_{2C}, dopaminergic-D_3, serotoninergic (5-hydroxytryptamine) 5-HT_{1A}, 5-HT_{2A}, and 5-HT_7 receptors.

Pridopidine has been shown to affect motor activity in HD clinical trials and in animal models. Beneficial effects on motor function (measured as UHDRS-Total Motor Score) were previously demonstrated in two large, double-blind, placebo-controlled studies in HD patients MermaiHD (Pridopidine for the Treatment of Motor Function in Patients with Huntington's Disease) and HART (A Randomized, Double-Blind, Placebo-Controlled Trial of Pridopidine in Huntington's Disease)[9,10]. In the recently completed Efficacy, Safety, and Tolerability of Pridopidine in Huntington Disease (PRIDE-HD) study, the primary endpoint of change in UHDRS-Total Motor Score was not met. However, pridopidine provided a potentially beneficial effect on the UHDRS-Total Functional Capacity score.[11] Motor effects of pridopidine have been previously reported in preclinical models, including reduced hyperactivity (in models of elevated dopaminergic or decreased glutamatergic neurotransmission) or enhanced hypoactivity (in habituated animals with low baseline activity). The locomotor activity of normal animals is generally unaffected.[7,8] Pridopidine at a low dose (5 mg/kg) had no effect on dizocilpine-(MK-801) or amphetamine-induced hyperactivity in rats[7] and demonstrated partial occupancy of the σ_1R (57 ± 2%).[6] However, a higher dose of 15 mg/kg elicited motor effects in models of induced hyperactivity[7] together with full σ_1R occupancy but lacking D_2R occupancy.[6] Further increasing pridopidine dose produced even more robust motor effects along with D_2R occupancy. In addition, daily pridopidine treatment (30 mg/kg) improved motor coordination in the YAC128 HD mouse.[12] Thus, although these data might suggest that motor activity of pridopidine is mediated at least in part by σ_1R actions, the role of binding to its medium and lower affinity CNS-related targets may be crucial but has yet to be fully elucidated.

The potential of pridopidine to reduce motor complications of L-dopa in PD was initially demonstrated in the 6-hydroxydopamine-lesioned rat. Pridopidine significantly decreased the L-dopa-induced sensitization of contraversive rotation while showing no decrease in the anti-parkinsonian benefit of L-dopa.[13] The aim of this study was to evaluate the effect of pridopidine on LID in the MPTP-lesioned macaque (considered the gold standard for preclinical evaluation of compounds with antidyskinetic potential) [14-16] and to characterize any observed behavioural effects in terms of the receptor occupancy profile of pridopidine to sites including but not limited to the σ_1R and D_2R.

Material and Methods

Test Item

Pridopidine-hydrochloride (4-[3-(methylsulfonyl)phenyl]-1-propylpiperidine-hydrochloride), was provided by Teva Pharmaceutical Industries Ltd. and, for pharmacokinetic (PK) and behavioral studies, was formulated in sterile water.

Pharmacokinetic and Behavioral Assessment in the MPTP-Lesioned Macaque

Animals

A total of 8 cynomolgus macaques (8-14 years, 3.0-4.8 kg; Suzhou-Xishan-Zhongke Company, Suzhou, Jiangsu, People's Republic of China) were used in this study. Fresh fruit, primate pellets, and water were available ad libitum other than at times of overnight fasting (from 5 PM) prior to days of behavioral assessment. The animals were group housed with cage sizes exceeding Council of Europe, United Kingdom, European Union, National Institutes of Health, and Canadian Council on Animal Care minimum recommendations. The cages were enriched with environmental stimuli, including perch, foraging boards, and toys. Housing rooms were subject to a 12-hour light-dark cycle at 20°C to 25°C. All efforts were made to reduce to a minimum the number of animals necessary for statistically valid analyses and to minimize animal suffering. All studies were performed with local Institutional Animal Care and Use Committee (IACUC) approval and in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (Institute of Laboratory Animal Resources Committee on Care and Use of Laboratory Animals.[17]

MPTP Administration and Development of Motor Complications

Animals received once-daily subcutaneous injection of MPTP (0.2 mg/kg in sterile saline; Sigma-Aldrich, Oakville, Ontario, Canada) as previously described[18] and a parkinsonian syndrome allowed to develop for 90 days. LID that were of a predominantly dystonic phenotype (both during peak effect and periods of wearing on and off) and peak-dose chorea and were evoked by once-daily L-dopa (25 mg/kg; Madopar, Roche, Shanghai, China) for at least 4 months. During this period, the animals were acclimatized to the experimental setting.

Pharmacokinetic Profiling of Pridopidine in the MPTP-lesioned Macaque

A total of 3 doses of pridopidine (15, 20, and 30 mg/kg, N = 8 per dose) were administered via oral gavage and samples collected prior to (t = −10 minutes) and +10 and 30 minutes, 1, 2, 4, 6, 8, and 24 hours post-drug administration. All animals received each treatment separated by 1 week. Samples were placed into K_2-EDTA (ethylenediaminetetraacetic acid) tubes (Becton-Dickinson, Mississauga, Ontario, Canada) and centrifuged for 5 minutes at $1500g_{ave}$ and plasma analyzed for pridopidine via liquid chromatography—tandem mass spectrometry (LC-MS/MS) (see Supplementary Materials).

Efficacy Assessment in the MPTP-Lesioned Macaque

A high-dose of oral L-dopa (LDh) that produced optimal antiparkinsonian benefit but was compromised by disabling dyskinesia (range 30-35 mg/kg, mean 32.1 mg/kg), was assessed alone or in combination with 3 oral doses of pridopidine (15, 20, and 30 mg/kg). Based on the outcome of the PK profiling, it was determined that $vehicle_1$/pridopidine would be given 2 hours prior to $vehicle_2$/LDh and start of behavioral observations. The effects of treatments on parkinsonian disability, dyskinesia, and duration and quality of antiparkinsonian benefit (on-time) and activity were assessed and analyzed for a period of 6 hours.

Assessment of Parkinsonian Disability and Dyskinesia

The animals were transferred to individual observation cages (1.5 × 1.0 × 1.1 m) and their behavior recorded on HD video. Rating scales for parkinsonism and dyskinesia adapted from their clinical counterparts (UPDRS part III and Unified Dyskinesia Rating Scale (UDysRS), respectively) were used to assess recordings via post hoc analysis by a movement disorders neurologist blinded to treatment. A measure of total parkinsonian disability as described previously[18] was derived by adding scores for range of movement (0-4), bradykinesia (0-3), posture (0-2), and alertness (0-1). Dyskinesia, representative of the maximum of either chorea or dystonia, were scored as 0 = absent, 1 = mild, 2 = moderate, 3 = marked or 4 = severe. Parkinsonian disability and dyskinesia were assessed for 5 minutes every 10 minutes, with the score given being most representative of each 5-minute observation period. The scores were summed for each hour for time course analyses and across the entire observation period (0-6 hours). Thus, for measures of parkinsonian disability and dyskinesia, the maximum scores possible (equating to severe) during the period from 0 to 6 hours were 360 and 144, respectively.

The duration of antiparkinsonian action, on-time, was defined as the number of minutes for which the bradykinesia score was 0. In addition, the duration of on-time associated with dyskinesia of varying severity was calculated as follows: on-time with disabling dyskinesia was calculated as the number of minutes for which bradykinesia was 0 while the dyskinesia score was greater than 2. Meanwhile, on-time without disabling dyskinesia represents the number of minutes for which the bradykinesia score was 0 while the dyskinesia score was 2 or less.

Statistical Analyses

Data derived from assessment of duration and quality of on-time were plotted as mean ± standard error of the mean. Statistical analyses for these data were performed using parametric repeated-measures 2-way analysis of variance (ANOVA), followed by Holm-Sidak multiple comparison tests. Data for measures of parkinsonian disability and dyskinesia were graphed, where appropriate, as median scores alone (time course) or with individual values (cumulated totals). Time course data for parkinsonian disability and dyskinesia were first ranked within each animal across all treatments using Excel's (Microsoft, Redmond, Washington, USA) RANKAVG function. These transformed data were then analyzed in GraphPad Prism (version 7.03, La Jolla, California, USA) and subjected to non-repeated-measures 2-way ANOVA followed by Holm-Sidak multiple comparison tests. Cumulated disability and dyskinesia data were analyzed using a Friedman test followed by a Dunn's multiple comparison test.

Results

In Vitro Pridopidine Receptor Binding Profile

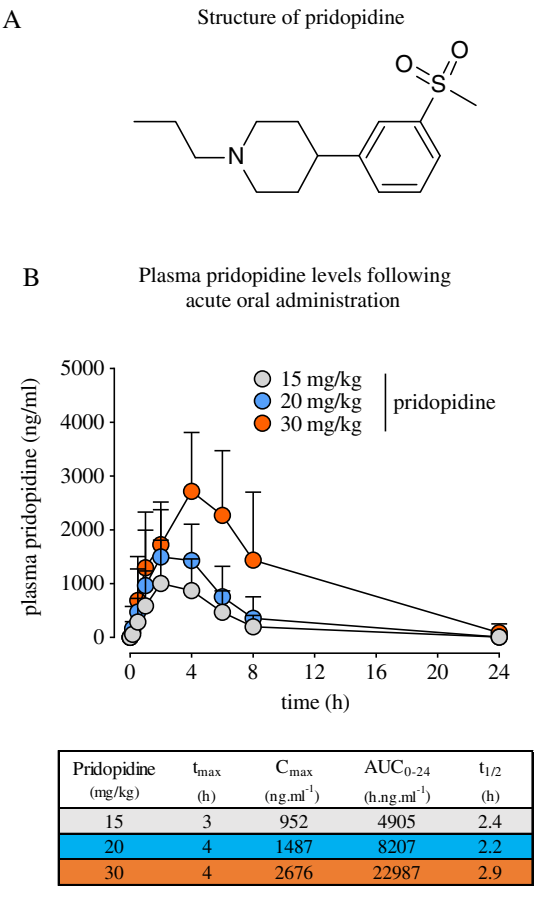
In vitro binding assays (See Supplementary Materials) were performed against a range of receptors and as validation of previously reported targets for pridopidine.[13] Pridopidine was found to have highest affinity for the σ_1R with a K_i of 57 nM with moderate-affinity binding in the micromolar range also evident to adrenergic α_{2C}, dopamine-D_3,

and serotoninergic 5-HT_{1A} and lower-affinity binding to sites including σ_2 receptor, 5-HT_{2A}; 5-HT_7, $\alpha_{2A,}$ histamine-H_3, and muscarinic-M_2 (Table 1). We detected only negligible binding of pridopidine to the D_2R (K_i 29.5 μM).

TABLE 1. Pridopidine binding profile ranked in descending order of affinity (K_i)

Target	IC_{50} (μM)	K_i (μM)	nH
Sigma-1 (σ_1R)	0.14	0.057	0.87
Adrenergic α_{2C}	3.56	1.58	0.76
Dopamine D_3	4.79	1.63	0.90
Serotonin 5-HT_{1A}	6.36	3.63	0.72
Sigma-2 (σ_2R)	7.16	5.45	0.80
Serotonin 5-HT_{2A}	24.5	7.00	0.81
Serotonin 5-HT_7	14.8	8.51	1.02
Adrenergic α_{2A}	22.0	11.0	0.98
Histamine H_3	37.6	18.3	0.85
Muscarinic M_2	58.1	24.4	0.62
Dopamine D_2	88.4	29.5	0.94

IC_{50}, half maximum inhibitory concentration; K_i, inhibition constant calculated using the equation of Cheng and Prusoff;[29] nH, Hill coefficient, defining the slope of the competitive binding curve, was calculated using MathIQTM; 5-HT, 5-hydroxytryptamine.



Pridopidine (mg/kg)	t_{max} (h)	C_{max} (ng.ml^{-1})	AUC_{0-24} (h.ng.ml^{-1})	$t_{1/2}$ (h)
15	3	952	4905	2.4
20	4	1487	8207	2.2
30	4	2676	22987	2.9

FIG. 1. Structure of pridopidine and plasma exposure profile following oral administration in the MPTP-lesioned macaque. Plasma samples prepared at times up to 24 hours following administration. Key pharmacokinetic data are also shown. Data are N = 8, mean ± standard deviation. [Color figure can be viewed at wileyonlinelibrary.com]

Additional targets were tested including the N-methyl-D-aspartate (NMDA), 5-HT_6 and neurokinin NK_1 receptors along with the dopamine, norepinephrine, and the serotonin transporters, all exhibiting no detectable levels of binding.

Pharmacokinetic Profile of Pridopidine in the MPTP-Lesioned Macaque

All doses of pridopidine assessed were well tolerated. Oral administration of pridopidine, 15, 20, and 30 mg/kg, was associated with geometric mean peak plasma concentration (C_{max}) values of 952, 1487, and 2676 ng/ml (corresponding to 3.4, 5.3, and 9.5 μM, respectively) occurring at median time of peak plasma concentration (T_{max}) of 3 hours (15 mg/kg) and 4 hours (20 and 30 mg/kg) and with corresponding AUC_{0-24} values of 4905, 8207, and 22,987 ng·h/ml (corresponding to 17.5, 29.2, and 81.8 h·μM). Structure and the mean plasma profile of pridopidine in the MPTP-lesioned macaque and key associated PK parameters are shown in Figure 1.

In Vivo Receptor Occupancy Profile of Pridopidine in the MPTP-Lesioned Macaque

We estimated σ_1R and D_2R occupancies using (1) known binding affinities of pridopidine to human and rodent receptors in vitro, (2) published in vivo PET imaging in rats[6] and PET imaging in monkeys, and (3) the extensive PK profiling of pridopidine in the different species. A summary of these data is shown in Table 2.

Effect of L-dopa in the MPTP-Lesioned Macaque With Established LID

A high dose of L-dopa evoked dyskinesia that were marked to severe during the first 2 hours following administration and a robust alleviation of parkinsonian disability such that median levels were absent mild during the first 4 hours following treatment (Figs. 2A,C). Duration of total on-time associated with LDh treatment was 254 ± 25 minutes (mean ± standard error of the mean) of which 168 ± 20 minutes (66% of total; mean ± standard error of the mean) was compromised by disabling dyskinesia (Fig. 3).

Effects of Pridopidine in MPTP-Lesioned Macaques With Established LID

Effects of Pridopidine on LID

Pridopidine produced a significant and dose-dependent reduction in dyskinesia evoked by LDh. Examining the entire 6-hour time course revealed a significant effect of combination treatment ($F_{3,28}$ = 4.981, P = .0068), but not time ($F_{5,140}$ = 0, P > .9999), or the interaction of treatment and time ($F_{15,140}$ = 0.9595, P = .5011) on levels of dyskinesia (2-way, repeated-measures ANOVA;

TABLE 2. Expected occupancy of NHP and human receptors at various pridopidine doses

Species	Dose	C_{max} (ng/ml)	C_{max} (μM)	AUC_{0-24} (h*ng/ml)	AUC_{0-24} (h*μM)	% σ_1R occupancy	% D_2R occupancy
NHP	15 mg/kg (PO)	952	3.4	4905	17.5	>80	15
	20 mg/kg (PO)	1487	5.3	8207	29.2	>80	25
	30 mg/kg (PO)	2676	9.5	22987	81.8	>80	40
Human	45 mg bid (90 mg/day, PO)	618	2.2	8600	31	>80	<15
	67.5 mg bid (135 mg/day, PO)	1008	3.6	12865	46	>80	15
	90 mg bid (180 mg/day, PO)	1480	5.3	17300	62	>80	25
	112.5 mg bid (225 mg/day, PO)	1717	6.1	21600	77	>80	30

C_{max} values for non-human primate (NHP) as a function of oral pridopidine dose are based on internally accumulated pharmacokinetic data (supplementary) in addition to data presented here. NHP dopamine D_2R occupancy data is based on in vivo PET imaging with pridopidine (SC) the specific D_2R ligand ^{11}C-raclopride (supplementary). Human D_2R data is estimated based on NHP data. NHP and human σ_1R occupancy data are extrapolated from in vitro binding investigations with ^{3}H-fluspidine, known and specific σ_1R tracer, against human σ_1R, (supplementary). AUC; area under the curve, bid; twice-daily dosing.

Fig. 2A). When compared with LDh-vehicle treatment, there was a significant decrease in dyskinesia during the first hour (20 mg/kg) and first and second hours (30 mg/kg) after the start of observation in response to LDh when combined with pridopidine, with median levels remaining between moderate and marked (20 mg/kg) or mild to moderate (30 mg/kg; all $P < .05$). Assessing levels of dyskinesia cumulated during the 2-hour period after start of observations (0-2 hour period) revealed a significant effect of pridopidine combination treatment (0-2 hours; Friedman statistic = 11.66, $P = .0087$; Fig. 2B) on levels of dyskinesia evoked by LDh. Median levels of dyskinesia in the LDh-treated animals combined with high-dose pridopidine (30 mg/kg) were reduced by 71% compared to those seen following LDh-vehicle such that median levels were below mild (non-disabling; $P < .01$).

Effects of Pridopidine on Parkinsonian Disability

Acute pridopidine treatment produced no change in the antiparkinsonian benefit afforded by a LDh at any point across the 6-hour observation when compared with vehicle treatment. Thus, examining the entire 6-hour time course period of observation revealed no effect of pridopidine treatment ($F_{3,28} = 0.4385$, $P = .7272$), time ($F_{5,140} = 0$, $P > .9999$), or the interaction of the 2 ($F_{15,140} = 0.6715$, $P = .8089$) on levels of disability (2-way, RM-ANOVA, Fig. 2C). At no point following LDh administration were levels of disability following pridopidine treatment (all doses) significantly different from those observed following vehicle treatment (all $P > .05$). Levels of parkinsonism cumulated during the 2-hour period after start of observations also revealed no effect of combination pridopidine treatment (Friedman statistic = 2.089, $P = .5542$; Fig. 2D), with no significant difference in levels of disability following treatment with combined LDh and pridopidine (all doses) compared to those seen following LDh alone (all $P > .05$).

Effects of Pridopidine on Duration and Quality of On-Time

Pridopidine produced no change in the total duration of on-time but did improve the quality of on-time associated with LDh (Fig. 3). Thus, pridopidine did not negatively impact on the duration of antiparkinsonian benefit of LDh but did alter the associated quality thereof in terms of the proportion associated with either non-disabling or disabling dyskinesia. Specifically, assessed during the 6-hour period of observation while there was no effect of treatment ($F_{3,21} = 1.659$, $P = .2062$), there was a significant effect of on-time subtype (total, without/with disabling; $F_{2,14} = 18.29$, $P = .0001$) and the interaction of treatment and subtype ($F_{6,42} = 2.887$, $P = .0190$) on duration and quality of on-time (2-way, RM-ANOVA; Fig. 3). Post hoc Holm-Sidak's analysis revealed no difference in either duration of total on-time or proportion of on-time that was devoid of disabling dyskinesia in response to LDh when combined with any dose of pridopidine compared to that observed following LDh-vehicle treatment. In contrast, pridopidine produced a significant reduction in on-time with disabling dyskinesia, a decrease of 60% evident following administration of the 30 mg/kg dose compared to vehicle treatment (66 minutes cf. 168 minutes, respectively; $P < .01$).

Discussion

The current study demonstrates, for the first time, that pridopidine reduces established LID without compromising the antiparkinsonian benefit of L-dopa in a MPTP-lesioned macaque model of PD. These findings offer significant promise with respect to the development of pridopidine as a treatment for LID in PD.

Methodological Considerations

As L-dopa plasma T_{max} is reached approximately 1.5 hours after administration,[19] and pridopidine T_{max}

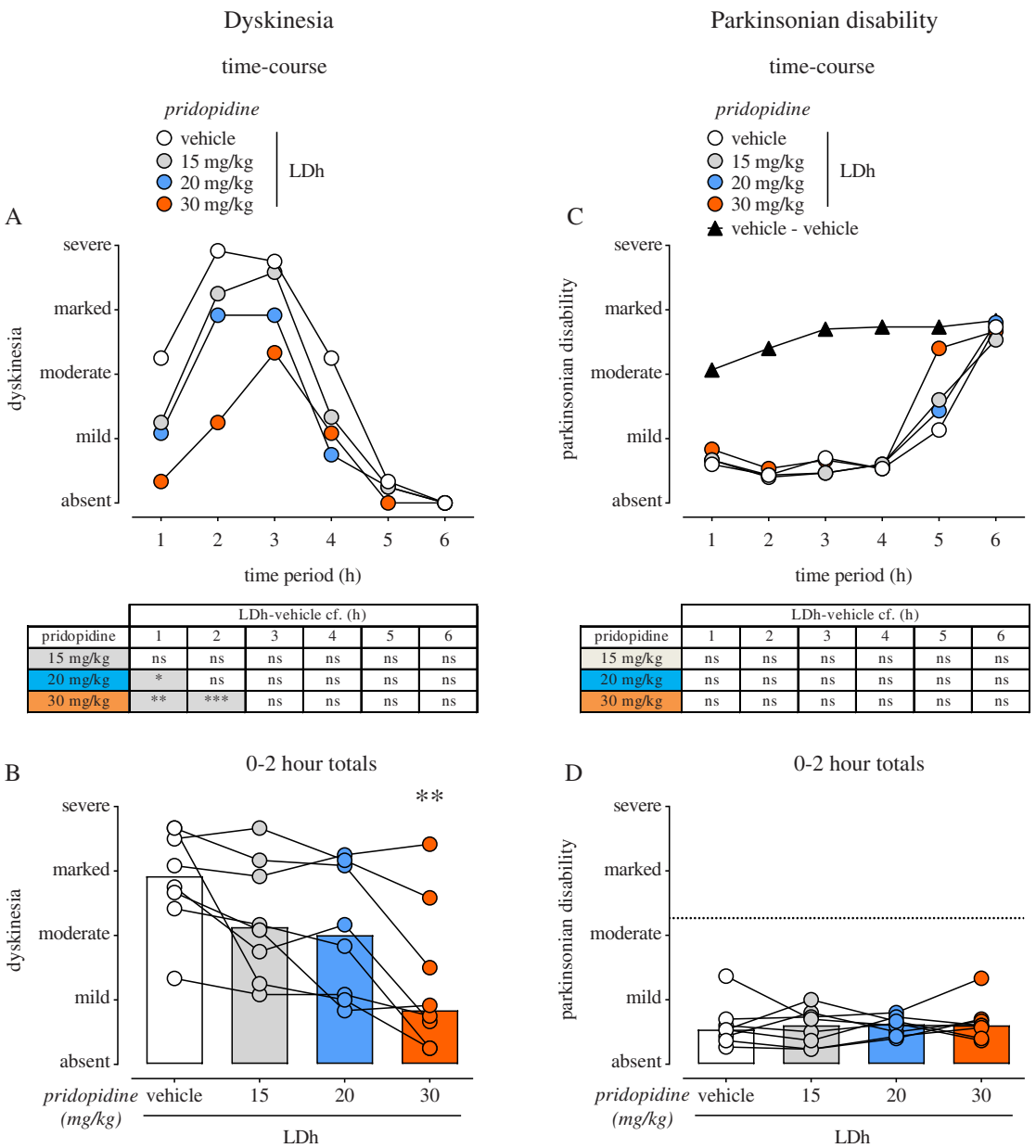


FIG. 2. Effect of acute pridopidine treatment in combination with high-dose L-dopa (LDh) on parkinsonian disability and dyskinesia in MPTP-lesioned primates with established motor complications. Levels of dyskinesia and parkinsonian disability were assessed during a 6-hour period and cumulated in either 1-hour epochs (A and C) or cumulated across the 0- to 2-hour period of peak effect (B and D). Data are median (A and C) with individual values (B and D). N = 8 for all treatment groups. Two-way repeated-measures ANOVA (A and C) with Holm-Sidak's test or Friedman test with Dunn's test (B and D). *P < .05, **P < .01, or ***P < .001 compared with vehicle treatment. [Color figure can be viewed at wileyonlinelibrary.com]

is 3 to 4 hours, for efficacy studies we administered pridopidine 2 hours prior to L-dopa to provide overlap of their respective peak plasma exposures. The macaques employed in this study were rendered parkinsonian using protocols and cumulative doses of MPTP consistent with those that we and others have previously employed.[18,20,21] The extent of lesion produced by this regimen[18] is comparable to that observed in advanced PD patients and typical of MPTP-lesioned animals with robust parkinsonism. The data generated by the current study are thus relevant to the treatment of moderate and advanced PD patients with established motor complications.

Pridopidine Does Not Negatively Impact on the Antiparkinsonian Benefit of L-dopa

Because the 6-hour period of observation encompassed the plasma T_{max} for pridopidine, we suggest that the timing of observation was ideal in which to see any potential for an effect of treatment (positive or negative) on parkinsonism. Indeed, in the current study

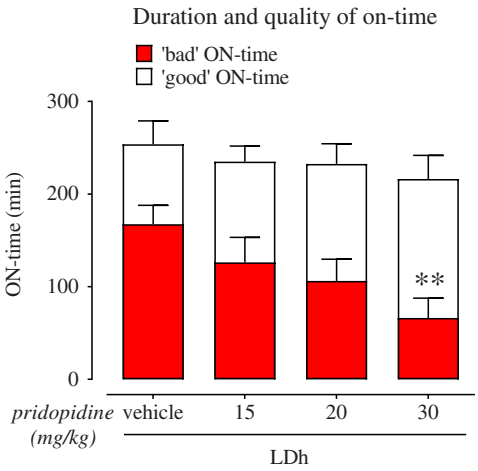


FIG. 3. Effect of acute pridopidine treatment in combination with high-dose L-dopa (LDh) on duration and quality of on-time in MPTP-lesioned primates with established motor complications. Total on-time was calculated as that time for which bradykinesia was absent while quality of on-time was assessed as the duration for which bradykinesia was absent but accompanied by either no or mild/moderate dyskinesia (non-disabling) or marked/severe dyskinesia (disabling). Data are mean ± standard error of the mean. N = 8 for all treatment groups. Two-way repeat-measures ANOVA with Holm-Sidak's multiple comparison test. $^{*}P < .05$ or $^{***}P < .001$ compared with vehicle treatment. [Color figure can be viewed at wileyonlinelibrary.com]

there were no negative effects of pridopidine observed on either peak antiparkinsonian actions or duration of on-time evoked by L-dopa. Regardless, the finite duration of the observation does not completely rule out the possibility that pridopidine could exacerbate parkinsonism outside of this period when L-dopa no longer exerts (and potentially masks) such an effect. Thus, in an associated study (data not shown) we have shown that pridopidine administered as monotherapy or in combination with low doses of L-dopa (in which parkinsonism is not fully alleviated and could therefore reveal an exacerbation as a result of pridopidine) causes no increase in levels of parkinsonian disability during the 6-hour period of observation and during which pridopidine plasma levels and subsequent target engagement were maximal.

Antidyskinetic Efficacy, Potential for Translation to the Clinic

In the current study, pridopidine demonstrated dose-dependent, acute antidyskinetic efficacy at oral doses of 20 and 30 mg/kg, but not 15 mg/kg. The PK profile for pridopidine in macaque plasma following 15, 20, and 30 mg/kg was associated with C_{max} of 952, 1487, and 2676 ng/ml and $AUC_{0\text{-}24}$ of 4905, 8207, and 22987 h·ng/ml, respectively. The PK profile in humans evaluated in past clinical trials[9,11] and data in Table 2 show that 90 mg/kg administered twice-daily is sufficient to provide plasma exposures equivalent to those associated with antidyskinetic actions in the macaque. Thus, in humans, a twice-daily 90 mg dose had a C_{max} of 1480 ng/ml, almost identical to that seen in the macaque at 20 mg/kg albeit with approximately twice the $AUC_{0\text{-}24h}$ (17,300 h·ng/ml), which might be expected when comparing single acute (macaque) with twice-daily (human) administration. From these data, we conclude that twice-daily doses of 90 mg or higher would provide plasma levels in excess of 1480 ng/ml in humans and would be appropriate for the evaluation of the antidyskinetic potential of pridopidine in a clinical trial. At doses of 90 mg administered twice-daily, we would expect high levels of σ_1R occupancy (>80%), with concurrent low levels of D_2R binding (~25%). In keeping with this, neither the 90 mg or 112.5 mg b.i.d. doses displayed any evidence of parkinsonian symptoms, suggesting no significant D_2R antagonist actions in any of the recent clinical trials with pridopidine.[9,11] Further to a lack of problematic D_2R antagonist actions, pridopidine, at least in HD patients, has a robust safety profile. It is well tolerated with incidence of adverse events no different to those observed in placebo-treated patients across multiple exploratory proof-of-principle, phase II, and phase III clinical trials,[9,10] including the phase II PRIDE-HD study, which investigated doses of pridopidine up to 112.5 mg b.i.d for 52 weeks.[11]

Among animal models of neurodegeneration, the MPTP-lesioned primate offers unparalleled predictive validity in determining the likelihood of a compound that displays efficacy at reducing established LID or enhancing the antiparkinsonian benefit of L-dopa in the primate being able to show similar efficacy in PD patients at phase II (reviewed extensively by Fox and colleagues)[16]. Thus, although we cannot predict the full potential of pridopidine to reduce dyskinesia in the clinic, there are many prior examples of compounds for which the primate was predictive of efficacy in man. For example, the cannabinoid, nabilone, not only effectively reduce LID in the primate[22] but also in man.[23] Similarly, the NMDA antagonist amantadine reduces dyskinesia in both the MPTP macaque[24,25] and in dyskinetic patients.[25]

Potential Mechanism of Action

At effective antidyskinetic doses (20 and 30 mg/kg), pridopidine had low D_2R occupancies of 25% to 40%, consistent with its low affinity binding to D_2R (Ki 29.5 μM). Thus, it is highly unlikely that the antidyskinetic effects of pridopidine are mediated by actions at the D_2R, a conclusion also supported by a lack of negative impact of pridopidine at these doses on antiparkinsonian benefit of L-dopa. Consistent with in vitro binding assays showing that pridopidine displays high affinity for the σ_1R (K_i of 57 nM), at all doses of pridopidine assessed, including those with (20 and 30 mg/kg) and without (15 mg/kg) antidyskinetic efficacy, pridopidine was also associated with very high (>80%) levels of σ_1R occupancy. It would therefore appear that σ_1R occupancy, in and of itself, is not sufficient to drive the

antidyskinetic effects observed in the current study. Pridopidine also binds with moderate affinity (K_i, in the micromolar range approximately 30-fold higher than to σ_1R, and approximately 10- to 30-fold lower than D_2R) to other targets, including adrenergic α_{2C} (K_i 1.58 μM), dopamine D_3 (K_i 1.63 μM), and serotonin 5-HT_{1A} (K_i 3.63 μM) receptors (Table 1). It is therefore possible that actions at these moderate affinity binding sites are involved in mediating the observed antidyskinetic benefits.

Our in vivo receptor occupancy data obtained at times of peak plasma pridopidine levels (T_{max}) are currently restricted to data for σ_1R and D_2R sites. Thus, the extent of occupancy by pridopidine to other binding sites must be inferred by their relative affinities, identified from in vitro data, to those for D_2R and σ_1R. Several types of receptors, including adrenergic α_{2C} and serotoninergic 5-HT_{1A}, will be engaged at doses of pridopidine above those providing full σ_1R occupancy and below those providing significant D_2R occupancy, for example, >40%. The doses found to have antidyskinetic efficacy here would fall within this window. Although we do not currently know the functional consequence of binding of pridopidine to these sites, we highlight adrenergic α_{2C} and serotoninergic 5-HT_{1A} as potential mediators of the antidyskinetic efficacy of pridopidine. Indeed, compounds more selectively targeting each of these sites have previously been shown to exhibit potential to reduce LID in both NHP models of PD as well as human PD patients. Thus, the selective α_2 receptor antagonist, fipamezole, alleviated LID in the MPTP-lesioned marmoset[26] and the MPTP-lesioned macaque [20] and PD patients.[27] Multiple studies in the 6-OHDA-lesioned rat, MPTP-lesioned NHP, and human PD patients have demonstrated the potential of multiple 5-HT_{1A} agonists to reduce LID (reviewed by Huot and colleagues)[28]. The efficacy of pridopidine in reducing LID may accrue from either of these actions individually or a synergy between the two or by an action of σ_1R occupancy to enhance either.

Conclusion

In MPTP-lesioned macaques, pridopidine produced a significant and meaningful decrease in LID without compromising the antiparkinsonian benefit of L-dopa. Although the antidyskinetic actions of pridopidine could be associated with high σ_1R occupancy, effective exposures may more likely be associated with occupancy of multiple non-sigma receptors, including adrenergic α_{2C} and 5-HT_{1A}. This complex pharmacology may underlie the effectiveness of pridopidine against LID.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.