L-745,870 Reduces L-DOPA-Induced Dyskinesia in the 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine-Lesioned Macaque Model of Parkinson’s Disease

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ABSTRACT

L-DOPA-induced dyskinesia remains an unmet challenge in the treatment of Parkinson’s disease (PD). Here, we investigate the potential antidyshkinetic efficacy of 3-[[4-(4-chlorophenyl)piperazin-1-yl]methyl]-1H-pyrido[2,3-b]pyridine (L-745,870), a potent and selective dopamine D4 receptor antagonist with a good toxicology profile and an excellent safety and tolerability record in phase I/II clinical studies, for non-PD indications. Six macaques were rendered parkinsonian by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (L-DOPA) significantly reduced the severity of dyskinesia, by up to 59%, in comparison with L-DOPA alone (P < 0.01). L-745,870 had no effect on the duration of antiparkinsonian benefit (ON-time) (P > 0.05). However, L-745,870 (1 mg/kg) significantly increased the duration of ON-time without disabling dyskinesia (+204%; P < 0.001) and decreased duration of ON-time with disabling dyskinesia compared with L-DOPA alone (−56%; P < 0.01). Brain levels of L-745,870 (−600 ng/g) were within the range at which L-745,870 provides selective D4 receptor antagonism. Plasma levels were comparable with those demonstrated to be well tolerated in human studies. These data suggest that selective D4 receptor antagonists represent a potential therapeutic approach for L-DOPA-induced dyskinesia. It is noteworthy that L-745,870 has already undergone significant clinical development, has an excellent profile for a therapeutic candidate, and could be advanced rapidly to phase IIa clinical studies for dyskinesia in PD.

Introduction

The classic manifestations of Parkinson’s disease (PD) are caused by the degeneration of dopaminergic neurons of the substantia nigra, which leads to a deficit in dopamine within the striatum (Hassler, 1938; Ehringer and Hornykiewicz, 1960). As such, dopamine replacement therapy with the dopamine precursor L-DOPA is the most effective treatment for PD (Fahn, 2008). However, chronic treatment with L-DOPA is marred by the development of abnormal involuntary movements, dyskinesia (Hely et al., 2005; Fabbrini et al., 2007), which negatively affects the quality of life (Péchevis et al., 2005).

Although the involvement of D1, D2, and D3 receptors in dyskinesia has been extensively studied (Gold et al., 2007; Guigoni et al., 2007; Dupre et al., 2008; Jenner, 2008; Visanji et al., 2009a), D4 receptors have been relatively neglected,
even though they are present within the basal ganglia, which are key structures involved in both parkinsonism and dyskinesia (DeLong, 1990; DeLong and Wichmann, 2007). Thus D₄ receptors are encountered within the striatum (Rivera et al., 2002), pallidium (Mauger et al., 1998), and subthalamic nucleus (Flores et al., 1999) and therefore could play an important role in basal ganglia signaling.

In addition to D₄ receptors localization within the basal ganglia, pharmacological studies hint that D₄ receptors might represent a promising therapeutic target for l-DOPA-induced dyskinesia. Thus, sarizotan is a molecule that effectively alleviated l-DOPA-induced dyskinesia in two experimental models of parkinsonism, the 6-hydroxydopamine-lesioned rat (Marin et al., 2009) and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned nonhuman primate (NHP) (Grégoire et al., 2009) and in phase II studies (Olanow et al., 2004; Bara-Jiménez et al., 2005). Unfortunately, sarizotan was not superior to placebo in phase III studies (Goetz et al., 2008).

Antagonizing D₄ receptors thus might represent a way to alleviate l-DOPA-induced dyskinesia, but the antidyskinetic efficacy of D₄ antagonists has yet to be demonstrated. In the present study, we have assessed the antidyskinetic efficacy of L-745,870 in the MPTP-lesioned macaque.

Materials and Methods

Animals

Six cynomolgus macaques (Macaca fascicularis; three females and three males; 5.88 ± 1.02 kg; 8.5 ± 0.3 years) were obtained from Shared Animal Health (Beijing, China). Animals were housed individually in conditions of controlled temperature (23 ± 2°C), humidity (50 ± 2%), and light (12-h light/dark cycle; 7:00 AM lights on). Animals were group-housed, and housing conditions exceeded National Institutes of Health, Canadian Council on Animal Care, and United Kingdom Home Office guidelines (2.4 × 3.0 × 1.0 m). Animals had unrestricted access to primate diet and water, and fresh fruits were supplemented daily. Housing cages were enriched with auditory and tactile stimuli. Efforts were made to keep to a minimum the number of animals required for statistically valid analyses and minimize their suffering. Experiments were carried out in Suzhou, Jiangsu, China with local institutional animal care and use committee approval and in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Institute of Laboratory Animal Resources, 1996).

Induction of Parkinsonism and Dyskinesia in the Cynomolgus Macaque

Animals were rendered parkinsonian by once-daily injection of MPTP hydrochloride (Sigma-Aldrich, Oakville, Canada) dissolved into 0.9% sterile NaCl (0.2 mg/kg), administered intravenously as described previously (Johnston et al., 2010a,b), until the first appearance of parkinsonism (mean cumulative dose 14.4 ± 5.4 mg). After stabilization of the parkinsonian phenotype, dyskinesia, both choreiform and dystonic, was induced by chronic l-DOPA/benserazide treatment (25/6.25 mg/kg p.o. twice daily, 4:1 ratio; administered as Madopar, Hoffmann-La Roche Limited, Shanghai, China).

Administration of L-745,870 in Combination with l-DOPA to MPTP-Lesioned Macaques

On days of behavioral assessment, at 9:00 AM, macaques were administered l-DOPA/benserazide orally (dose was individually tailored for each animal; 34.2 ± 1.5 mg/kg; 4:1 l-DOPA/benserazide ratio as Madopar) in combination with either vehicle (0.00037% HCl in water) or L-745,870 trihydrochloride, (equivalent to 0.01, 0.1, 0.3, and 1.0 mg/kg of drug-free base orally; Tocris Bioscience, Ellisville, MO). The drug administration schedule was randomized according to a Latin square design. After administration of treatment, each macaque was placed individually into an observation cage (1.5 × 1.0 × 1.1 m) containing food, water, and primate toys and left undisturbed for the 6-h duration of the experiment.

Motor activity was detected by automated computer-based passive infrared activity monitoring, and behavior was recorded on disc for post hoc analysis by a neurologist specialized in movement disorders who was blinded to the treatment given. At least 72 h were left between each treatment in any animal.

Behavioral Assessment of L-745,870 in the MPTP-Lesioned Macaque

The scales used for assessment of behavior were described in detail previously (Visani et al., 2009b; Johnston et al., 2010a). Parkinsonian disability was rated for 5 min every 10 min by using a parkinsonian disability scale combining measures of range of movement, bradykinesia, posture, and attention/alertness. Range of
movement was rated on a 0 to 4 scale: 0, walking on the floor and/or climbing on the walls or roof of the cage to 4, no movement. Bradykinesia was rated on a 0 to 3 scale: 0, normal speed and initiation of movement to 3, marked slowing, or unable to move, with prolonged freezing episodes. Posture was rated on a 0 to 2 scale: 0, normal, upright to 2, hunched body and neck, face down, may lose balance. Attention/alertness was rated on a 0 to 1 scale: 0, present, looking around, observing and 1, absent. The score attributed to each of the behaviors assessed was the most representative of the 5-min observation period. A global parkinsonian disability score was derived, summing the scores of the aforementioned behaviors. The maximal parkinsonian disability score per 5-min observation period was 10.

Dyskinesia was scored, for 5 min every 10 min, on a 0 to 4 scale: 0, absent; 1, mild, fleeting, not interfering with normal activity, present less than 30% of the observation period; 2, moderate, not interfering with normal activity, present more than 30% of the observation period; 3, marked, at times disabling, i.e., interfering with normal activity, present less than 70% of the observation period; and 4, severe, continuous, disabling, replacing normal activity, present more than 70% of the observation period. Choreiform and dystonic dyskinesia were assessed separately, and the dyskinesia score attributed reflected the most disabling dyskinesia observed, whether chorea or dystonia. Parkinsonian disability and dyskinesia scores were cumulated for each hour according to the entire 6 h of observation. The duration of antiparkinsonian benefit, i.e., ON-time, was defined as the number of minutes for which the bradykinesia score was 0, based on widely used criteria for clinical diagnosis of idiopathic PD (Hughes et al., 1992). ON-time was further divided as “good” or “bad” quality, depending on the severity of dyskinesia present. Thus, good-quality ON-time was defined as the number of minutes when the bradykinesia score was 0 and dyskinesia was either absent or nondisabling, i.e., mild, or moderate in intensity (scores of 0, 1, and 2), whereas bad-quality ON-time was defined as the number of minutes during which bradykinesia was 0 and dyskinesia was disabling, i.e., either marked or severe (scores of 3 and 4). ON-time without disabling dyskinesia was defined as the sum of ON-time without dyskinesia (score of 0) and ON-time with nondisabling dyskinesia (scores of 1 and 2). These scales were developed to provide a NHP analog of the measures such as ON-time and ON-time with troublesome dyskinesia that are widely used in clinical studies (Rascò et al., 2005).

Determination of L-745,870 Levels in the Plasma, Cerebrospinal Fluid, and Brain of the MPTP-Lesioned Macaque

**Plasma Pharmacokinetic Study: Administration of L-745,870 and Blood Sampling.** In a series of studies, conducted independently from behavioral observations, in three MPTP-lesioned cynomolgus macaques, plasma levels of drug were assessed after a single administration of L-745,870 (0.1, 0.3, and 1 mg/kg p.o. and 0.3 mg/kg i.v.) given in combination with L-DOPA/benserazide (30 mg/kg p.o.). Plasma levels of L-745,870 (0.3 mg/kg p.o.) were also assessed in the absence of L-DOPA to determine whether L-DOPA alters L-745,870 PK profile. All animals received all treatments once, in a balanced, randomized, and nonrepetitive manner across drug concentrations. Blood samples were collected for determination of plasma L-745,870 levels to correlate plasma, CSF, and brain levels. Two milliliters of CSF were collected from the cisterna magna and immediately stored at −80°C. The brain was rapidly removed, and the primary motor cortex and putamen were dissected out, flash-frozen in isopentane, and stored at −80°C until processing.

**Assessment of L-745,870 Levels via Liquid Chromatography-Tandem Mass Spectrometry.** Brain tissue was homogenized into a ddH2O solution on ice by using an ultrasonic tissue homogenizer (Mandel Scientific Company Inc., Guelph, Canada). Aliquots of 45 μl of macaque biological samples (plasma, CSF, or brain homogenate) were supplemented with 5 μl of methanol and 5 μl of mirtazapine solution (the internal standard, 100 ng/ml), transferred into a 1.5-ml microcentrifuge Eppendorf tube, and vortexed. After alkalization with an additional 10 μl of alkalizer (ammonium chloride dissolved into ammonia water, pH 10), 1 ml of methyl tert-butyl ether (MTBE) was added to the microcentrifuge tube. Samples were then vortexed for 3 min and centrifuged at 14,000 rpm for 3 min. Supernatant was then transferred to a 96-well plate. Extraction solutions were evaporated to dryness under a stream of nitrogen at room temperature. Residues were reconstituted with 100 μl of methanol in ddH2O (1:1 v/v) and transferred into injection vials. Ten microliters of each solution were injected for liquid chromatography-tandem mass spectrometry (MS/MS) analysis.

Chromographic separation was performed on a Shimadzu (Kyoto, Japan) LC-10A VP system equipped with two binary pumps, a CTC-HTS autosampler (CTC Analytics, Zwingen, Switzerland), and a Kinetex PFP (2.1 × 50 mm; 2.6 μm; 100 Å) column (Phenomenex, Torrance, CA). Chromatography was performed at 40°C with an isocratic gradient at 0.1% formic acid and 5 mM ammonium acetate in water and 0.1% formic acid in acetonitrile (10:90, v/v). Flow rate was set at 0.6 ml/min. The MS/MS system was an MDS Sciex (Concord, ON, Canada) API-4000 mass spectrometer with an electrospray ionization probe. Analytes were detected by multiple reaction monitoring in positive mode with a dwell time of 150 ms. MS/MS conditions were optimized by T-tube infusion of 100 ng/ml for the analyte in methanol/ddH2O (1:1 v/v) at a flow rate of 20 μl/min. The optimal transitions from the protonated molecular ion to a diagnostic fragment ion were mass-to-charge ratio (m/z) of 327.2 → 131.1 for L-745,870 and m/z 266.1 → 195.1 for mirtazapine. Collision gas was nitrogen, collision energy was set at 21 V, the source temperature was 450°C, and the ion spray voltage was set at 5000 V.

Calibration curves (0.1–100 ng/ml) were generated, and their linearity was fitted with the liquid chromatography-MS/MS software Analyst version 1.4.1 (Applied Biosystems, Foster City, CA). Concentrations of biological samples were calculated with the calibration curves and expressed as nanomole/millilitre.

**Statistical Analysis**

Continuous motor activity scores were analyzed by one-way repeated-measures (RM) analysis of variance (ANOVA) followed by Tukey’s multiple-comparison post hoc tests. Categorical, discontinuous scores for parkinsonian disability and dyskinesia severity were analyzed by using nonparametric Friedman’s followed by Dunn’s multiple-comparison post hoc tests. Continuous ON-time data were analyzed by one-way RM ANOVA followed by Tukey’s multiple-comparison post hoc tests. Time-course data for motor activity counts were analyzed by two-way RM ANOVA followed by Bonferroni’s multiple-comparison post hoc tests. Levels of L-745,870 in the primary motor cortex and putamen were compared by using the parametric one-tailed Student’s t test. PK parameters of L-745,870 administered with and without L-DOPA were compared by using the parametric one-tailed Student’s t test. Sta-
tistical significance was assigned at $P < 0.05$. Statistical analyses were computed by using Prism 5.03 (GraphPad Software Inc., San Diego, CA).

Determination of L-745,870 PK parameters, area under the curve (AUC), maximal plasma concentration ($C_{\text{max}}$), time of maximal plasma concentration ($t_{\text{max}}$), elimination rate constant ($K_{\text{e}}$), $t_{1/2}$, clearance (CL), and bioavailability was done by using Microsoft (Redmond, WA) Excel and Prism 5.03.

Results

L-745,870 Reduces the Severity of L-DOPA-Induced Dyskinesia and Extends the Duration of Good-Quality ON-Time

Administration of 1 mg/kg L-745,870 in combination with L-DOPA provided a significant reduction of L-DOPA-induced dyskinesia severity compared with L-DOPA/vehicle treatment. Administration of L-745,870 also significantly extended the duration of good-quality ON-time, while reducing the duration of bad-quality ON-time.

Over the 360-min observation period, there was a significant effect of treatment and an interaction between treatment and time, but no effect of time alone on the severity of L-DOPA-induced dyskinesia ($F_{\text{time,5,210}} = 0.0, P > 0.05; F_{\text{treatment,6,210}} = 35.63, P < 0.001; F_{\text{interaction,30,210}} = 5.539, P < 0.001; $two-way ANOVA after ranking of data) (Fig. 1A). Thus, L-DOPA, in combination with vehicle or L-745,870, elicited significantly more severe dyskinesia than vehicle/vehicle or vehicle/L-745,870 treatments from 0 to 180 min after drug administration (all $P < 0.01$; Bonferroni’s post hoc test). However, 1 mg/kg L-745,870 significantly alleviated the severity of dyskinesia compared with L-DOPA alone (47% from 0–60 min, $P < 0.01$; 30% from 60–120 min, $P < 0.01$; 59% from 120–180 min, $P < 0.01$; Bonferroni’s post hoc test). L-745,870 at 1 mg/kg also reduced the severity of dyskinesia compared with lower doses of L-745,870 ($P < 0.01$ from 60–180 min compared with L-DOPA/L-745,870 and $P < 0.05$ from 120–180 min compared with L-DOPA/L-745,870; Bonferroni’s post hoc test). Lower doses of L-745,870 did not alleviate L-DOPA-induced dyskinesia ($P > 0.05$; Bonferroni’s post hoc test).

Duration of ON-time with dyskinesia was unchanged by the addition of L-745,870 ($F_{5,30} = 48.99, P < 0.001$; one-way RM ANOVA; $P > 0.05$ when L-DOPA/L-745,870, regardless of the dose of L-745,870, was compared with L-DOPA alone; Tukey's post hoc test) (Fig. 1B).

However, duration of ON-time with disabling dyskinesia was significantly reduced by combining 1 mg/kg L-745,870 and L-DOPA ($F_{5,30} = 33.08; P < 0.001$; one-way RM ANOVA) (Fig. 1C). Thus, the duration of ON-time with disabling dyskinesia was 55 ± 15 min in the L-DOPA/1 mg/kg L-745,870 treatment compared with 127 ± 18 min in the L-DOPA/vehicle treatment (56% decrease; $P < 0.001$; Tukey’s post hoc test), 135 ± 13 min in the L-DOPA/0.1 mg/kg L-745,870 treatment (59% decrease; $P < 0.001$; Tukey’s post hoc test), 110 ± 10 min in the L-DOPA/0.1 mg/kg L-745,870 treatment (50% decrease; $P < 0.05$; Tukey’s post hoc test), and 100 ± 8 min in the L-DOPA/0.3 mg/kg L-745,870 treatment (45% decrease; $P < 0.05$; Tukey’s post hoc test).

Accordingly, the duration of ON-time without disabling dyskinesia was significantly increased when 1 mg/kg L-745,870 was added to L-DOPA ($F_{5,30} = 12.43; P < 0.001$; one-way RM ANOVA) (Fig. 1D). Thus, the duration of ON-time without disabling dyskinesia was 132 ± 30 min in the L-DOPA/1 mg/kg L-745,870 treatment compared with 43 ± 10 min in the L-DOPA/vehicle treatment (204% increase; $P < 0.001$; Tukey’s post hoc test), 33 ± 13 min in the L-DOPA/0.01 mg/kg L-745,870 treatment (295% increase; $P < 0.001$; Tukey’s post hoc test), 52 ± 13 min in the L-DOPA/0.1 mg/kg L-745,870 treatment (155% increase; $P < 0.01$; Tukey’s post hoc test), and 53 ± 13 min in the L-DOPA/0.3 mg/kg L-745,870 treatment (147% increase; $P < 0.01$; Tukey’s post hoc test).

The duration of ON-time without dyskinesia was also significantly extended by adding 1 mg/kg L-745,870 to L-DOPA ($F_{5,30} = 6.596; P < 0.001$; one-way RM ANOVA) (Fig. 1E). Thus, the duration of ON-time without dyskinesia was 55 ± 16 min in the L-DOPA/1 mg/kg L-745,870 treatment compared with 15 ± 7 min in the L-DOPA/vehicle treatment (267% increase; $P < 0.01$; Tukey’s post hoc test), 15 ± 9 min in the L-DOPA/0.01 mg/kg L-745,870 treatment (267% increase; $P < 0.01$; Tukey’s post hoc test), and 27 ± 7 min in the L-DOPA/0.3 mg/kg L-745,870 treatment (106% increase; $P > 0.05$; Tukey’s post hoc test).

L-745,870 Does Not Influence L-DOPA Antiparkinsonian Efficacy

Motor activity counts, parkinsonian scores, and duration of ON-time were not significantly altered by the addition of L-745,870 to L-DOPA, compared with L-DOPA/vehicle treatment, indicating that L-745,870 did not impair the antiparkinsonian action of L-DOPA.

Motor Activity. Over the 360-min observation period, there was a significant effect of time, treatment, and interaction between the two variables on the motor activity of the macaques ($F_{5,180} = 10.30, P < 0.001; F_{6,180} = 29.03, P < 0.001; F_{30,180} = 5.179, P < 0.001$; two-way RM ANOVA) (Fig. 2A). There was a significant increase in motor activity counts after L-DOPA administration (in combination with vehicle or L-745,870), compared with vehicle/vehicle and vehicle/L-745,870 treatments ($P < 0.05$ from 0–60 min and $P < 0.001$ from 120–180 min; Bonferroni’s post hoc test). At no time during the observation period did L-745,870 have any effect on L-DOPA- or vehicle-induced motor activity ($P > 0.05$; Bonferroni’s post hoc test). Accordingly, L-DOPA (in combination with vehicle or L-745,870) significantly increased total motor activity over the 360-min observation period, compared with vehicle/vehicle or vehicle/L-745,870 treatments ($F_{5,30} = 22.05; P < 0.001$, one-way RM ANOVA; $P < 0.001$, Tukey’s post hoc test) (Fig. 2B). whereas L-745,870 had no effect on L-DOPA- or vehicle-induced motor activity during the whole 360-min experiment ($P > 0.05$; Tukey’s post hoc test).

Parkinsonian Disability. Over the 360-min observation period, there was a significant effect of treatment and an interaction between time and treatment, but no effect of time alone on the degree of parkinsonism of the macaques ($F_{5,210} = 0.0, P > 0.05; F_{6,210} = 11.61, P < 0.001; F_{30,210} = 2.867, P < 0.001$; two-way ANOVA after ranking of data) (Fig. 3A). L-DOPA, whether combined with vehicle or L-745,870, significantly alleviated the severity of parkinsonism from 0 to 180 min after drug administra-
tion \((P < 0.05\) for all; Bonferroni’s post hoc test). The addition of L-745,870 to vehicle or l-DOPA did not result in a worsening of parkinsonism \((P > 0.05);\) Bonferroni’s post hoc test).

**ON-Time Duration.** l-DOPA in combination with vehicle or L-745,870 significantly extended the duration of ON-time compared with vehicle or l-DOPA/vehicle/L-745,870 treatments \((F_{5,30} = 60.09; P < 0.001,\) one-way RM ANOVA; \(P < 0.001,\) Tukey’s post hoc test) (Fig. 3B). The addition of L-745,870 to l-DOPA did not result in a reduction of ON-time duration \((P > 0.05;\) Tukey’s post hoc test); for instance, ON-time duration was 170 ± 15 min in the l-DOPA/vehicle treatment compared with 186 ± 22 min after the l-DOPA/1 mg/kg L-745,870 treatment \((P > 0.05;\) Tukey’s post hoc test).

**Pharmacokinetic Profile of L-745,870 in the MPTP-Lesioned Macaque**

In the MPTP-lesioned macaque, L-745,870 was detectable in the plasma as early as 15 min after oral administration of
the 1 mg/kg dose and was still above the detection threshold 24 h after administration. No L-745,870 was detectable in the pretreatment sample.

PK parameters are illustrated in Fig. 4 and detailed in Table 1. C\textsubscript{max} reached after oral administration of 1 mg/kg L-745,870 was 181.3 ± 5.3 nM, and t\textsubscript{max} was 3.0 ± 0.0 h.
Following intravenous administration of L-745,870, $t_{1/2}$ was $4.2 \pm 1.0$ h, and CL rate was $0.072 \pm 0.008$ l/min. Bioavailability of L-745,870 was $42.0 \pm 11.5\%$. The shape of the curve after a single intravenous administration was indicative of first-order kinetic metabolism at therapeutically relevant plasma concentrations (Fig. 4B). L-DOPA had no effect on the L-745,870 plasma PK time course (Fig. 4C).

**L-745,870 Levels in the Plasma, Cerebrospinal Fluid, and Brain of the MPTP-Lesioned Macaque at Peak Behavioral Effect**

As presented in Table 2, 90 min after oral administration plasma L-745,870 levels were $193.9 \pm 55.0$ nmol/ml, whereas CSF levels were $12.7 \pm 5.6$ nmol/ml, which represents a 6% CSF/plasma ratio. L-745,870 levels were $650 \pm 263$ ng/g wet tissue in the primary motor cortex and $560 \pm 253$ ng/g wet tissue in the putamen ($t = 0.2476$; df = 4; $P > 0.05$; one-tailed Student’s $t$ test).

**Discussion**

This study has demonstrated that oral administration of acute challenges of L-745,870 (1 mg/kg) in combination with L-DOPA significantly alleviates dyskinesia. Whether the antidyskinetic effect of L-745,870 would be maintained in the context of chronic administration of the drug remains unknown, and further studies are needed to investigate the effects of long-term administration of L-745,870 on dyskinesia severity. The magnitude of the antidyskinetic effect achieved here with L-745,870 compares advantageously with the magnitude of the antidyskinetic effect we previously achieved with famotidine (Johnston et al., 2010c) and fipamezole (Johnston et al., 2010b). This reduction in dyskinesia severity was accompanied by a reduction in bad-quality ON-time and an extension in good-quality ON-time duration. It is noteworthy that the antidyskinetic efficacy of L-745,870 was achieved without compromising L-DOPA antiparkinsonian benefit and at brain levels at which L-745,870 is selective for D$_4$ receptors, thereby identifying D$_4$ receptors as a promising target for dyskinesia. Moreover, effective plasma levels of L-745,870 obtained in our study are in the range of those that were demonstrated to be well tolerated in human trials.

**The Antidyskinetic Effect of L-745,870 Is Observed at Doses at Which It Is Known to Act as a Selective Antagonist of Dopamine D$_4$ Receptors.** L-745,870 is a potent and selective D$_4$ receptor antagonist with an inhibition constant ($K_i$) of 0.43 nM (Kulagowski et al., 1996; Patel et al., 1997; Stewart et al., 2004; Nakane et al., 2005). L-745,870 exhibits nearly 100-fold selectivity over its next target, $\alpha$-2 adrenoceptors ($K_i$ of 33–49 nM) (Patel et al., 1997; Nakane et al., 2005).

The pharmacokinetics of L-745,870 had not been characterized previously in the cynomolgus macaque and had never been assessed in any animal model of Parkinsonism to our knowledge. In comparison with the rhesus macaque (Patel et al., 1997), in cynomolgus macaques L-745,870 has higher bioavailability but comparable $t_{1/2}$. However, the drug $t_{1/2}$ is longer in humans (15 h at steady state) (Bristow et al., 1997b). To our knowledge, most of the PK parameters reported in Fig. 4 and Tables 1 and 2 have never been evaluated in the NHP, and our study is the first to determine brain and CSF levels of L-745,870 in the cynomolgus macaque.
L-745,870 Reduces L-DOPA-Induced Dyskinesia

TABLE 1
L-745,870 pharmacokinetic parameters in the MPTP-lesioned macaque

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0.1 mg/kg p.o.</th>
<th>0.3 mg/kg p.o.</th>
<th>1.0 mg/kg p.o.</th>
<th>0.3 mg/kg i.v.</th>
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<tr>
<td>C_{max}, nmol/ml, 0–1 h</td>
<td>3.4 ± 2.6</td>
<td>11.1 ± 9.4</td>
<td>97.8 ± 22.8</td>
<td>183.8 ± 13.8</td>
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<tr>
<td>C_{max}, nmol/ml, 0–2 h</td>
<td>9.8 ± 3.4</td>
<td>27.5 ± 8.6</td>
<td>142.7 ± 12.3</td>
<td>183.8 ± 13.8</td>
</tr>
<tr>
<td>C_{max}, nmol/ml, 0–3 h</td>
<td>10.1 ± 3.2</td>
<td>28.4 ± 7.7</td>
<td>181.3 ± 5.3</td>
<td>183.8 ± 13.8</td>
</tr>
<tr>
<td>C_{max}, nmol/ml, 0–6 h</td>
<td>10.1 ± 3.2</td>
<td>28.6 ± 7.6</td>
<td>181.3 ± 5.3</td>
<td>183.8 ± 13.8</td>
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<tr>
<td>C_{max}, nmol/ml, 0–8 h</td>
<td>10.1 ± 3.2</td>
<td>28.6 ± 7.6</td>
<td>181.3 ± 5.3</td>
<td>183.8 ± 13.8</td>
</tr>
<tr>
<td>C_{max}, nmol/ml, 0–24 h</td>
<td>10.1 ± 3.2</td>
<td>28.6 ± 7.6</td>
<td>181.3 ± 5.3</td>
<td>183.8 ± 13.8</td>
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<tr>
<td>AUC_{0–1 h}, h•nmol/ml</td>
<td>7.5 ± 0.6</td>
<td>22.4 ± 9.7</td>
<td>176.1 ± 36.2</td>
<td>235.1 ± 21.6</td>
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<tr>
<td>AUC_{0–2 h}, h•nmol/ml</td>
<td>16.1 ± 5.9</td>
<td>48.8 ± 15.4</td>
<td>338.1 ± 42.3</td>
<td>307.1 ± 40.4</td>
</tr>
<tr>
<td>AUC_{0–3 h}, h•nmol/ml</td>
<td>33.3 ± 9.9</td>
<td>112.4 ± 28.9</td>
<td>700.9 ± 15.4</td>
<td>431.6 ± 72.7</td>
</tr>
<tr>
<td>AUC_{0–6 h}, h•nmol/ml</td>
<td>38.5 ± 11.0</td>
<td>142.0 ± 35.6</td>
<td>767.1 ± 11.9</td>
<td>477.4 ± 79.9</td>
</tr>
<tr>
<td>AUC_{0–8 h}, h•nmol/ml</td>
<td>50.7 ± 6.1</td>
<td>266.9 ± 77.6</td>
<td>815.5 ± 51.9</td>
<td>648.3 ± 84.2</td>
</tr>
<tr>
<td>t_{max}, h</td>
<td>2.3 ± 0.3</td>
<td>3.3 ± 1.3</td>
<td>3.0 ± 0.0</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>t_{1/2}, h</td>
<td>1.3 2.6 3.2 1.02</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CL, l/h</td>
<td>0.072 ± 0.008</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioavailability, %</td>
<td>42.0 ± 11.5</td>
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</table>

Plasma levels reached in our study are in accordance with those obtained in a human phase IIa study (Kramer et al., 1997), in which plasma levels of L-745,870 were 12 ± 7 ng/ml, which corresponds to 36.7 ± 21.4 nmol/ml. It is noteworthy that in that study plasma was collected before medication intake, which corresponds to 1.6 drug half-lives. In our study, administration of 1 mg/kg L-745,870 led to plasma levels slightly under 50 nmol/ml after 1.6 half-lives (approximately 7 h; Fig. 4A). Thus, the effective dose in our study led to plasma concentrations similar to those that were demonstrated to be safe and well tolerated by human subjects. Moreover, at such plasma concentrations in humans L-745,870 remains highly selective for dopamine D₄ receptors and antagonizes approximately 90% of D₄ receptors (Bristow et al., 1997b; Kramer et al., 1997).

Accordingly, we describe that the antidysonetic actions of L-745,870 were seen at doses where brain levels were 650 ng/g of tissue in the primary motor cortex and 560 ng/g of tissue in the putamen, corresponding to concentrations of 397.8 nM in the motor cortex and 342.5 nM in the putamen. It has previously been demonstrated that only 2 to 2.5% of L-745,870 present within the brain is available for biological activity (Patel et al., 1997). Thus, with doses of L-745,870 providing antidysonetic benefit active brain levels would be 6 to 10 nM. Given the affinity of L-745,870 for D₄ receptors and selectivity described above, this would represent a concentration at which D₄ receptors were antagonized by >95% while remaining highly selective for D₄ receptors, because 6 to 10 nM is well below the affinity of L-745,870 for its next target (Patel et al., 1997; Nakane et al., 2005). As such, it seems most likely that the blockade of D₄ receptors is a promising target for alleviating L-DOPA-induced dyskinesia.

However, further studies, with other dopamine D₄ receptor antagonists such as 2-(4-(3,4-dimethylphenyl)piperazin-1-ylmethyl)-1H-benzimidazole (A-381,393) (see below), are required to validate D4 receptor blockade as an effective means of alleviating dyskinesia.

L-745,870 Does Not Impair L-DOPA Antiparkinsonian Efficacy. The antidysonetic effect of L-745,870 was achieved without compromising L-DOPA antiparkinsonian action. Thus, at no time during the experiment did parkinsonism scores differ between L-DOPA/L-745,870 and L-DOPA/vehicle treatments. In addition, the antidysonetic effects of L-745,870 were not accompanied by a reduction in motor activity. Finally, L-745,870 did not reduce ON-time duration, which was defined primarily by the absence of bradykinesia, based on the United Kingdom PD Society Brain Bank criteria for the diagnosis of idiopathic PD (Hughes et al., 1992).

However, because L-745,870 was not tested at doses above 1 mg/kg, it cannot be ruled out that higher doses of the compound might impair L-DOPA antiparkinsonian action, although an interaction with targets other than D₄ receptors might then mediate such action. Accordingly, considering the argument put forth in the previous section, selective and virtually complete blockade of D₄ receptors does not seem to interfere with L-DOPA antiparkinsonian efficacy.

Dopamine D₄ Receptors and Basal Ganglia Neurotransmission. Although the current study validates the D₄ receptor as a therapeutic target for L-DOPA-induced dyskinesia, the mechanism whereby antagonizing D₄ receptors alleviates dyskinesia has yet to be determined. Because they exert similar effects on intracellular signaling, D2, D₃, and D₄ receptors are members of the D2-like receptor family (Missale et al., 1998). D₄ receptors are present within the striatum (Mauger et al., 1998; Rivera et al., 2002), in which they are located on medium spiny neurons (Rivera et al., 2002). However, it remains unknown whether D₄ receptors are preferentially expressed on striatofugal neurons of the direct or indirect pathway. D₄ receptors are also found on large neurons of the globus pallidus (GP) (Ariano et al., 1997; Mauger et al., 1998). D₄ receptors are thus well localized to modulate dopaminergic nigrostriatal and nigropallidal transmissions. Moreover, D₄ receptor binding levels increase in the rat stria-
tum after 6-hydroxydopamine lesion (Zhang et al., 2001), thereby providing an obvious site for the potential enhancement of D₄ signaling as a mechanism underlying dyskinesia expression. According to the classic model of basal ganglia function (DeLong and Wichmann, 2007), antagonizing striatal D₄ receptors along the indirect pathway would result, like antagonizing D₂ receptors, in a reduction of dyskinesia, although at the expense of worsening the parkinsonian phenotype (Klawans and Weiner, 1974).

D₄ receptors are also encountered in the subthalamic nucleus (STN) (Flores et al., 1999). Stimulating D₄ receptors within the STN leads to a reduction of GABA levels, a phenomenon reversed by L-745,870 (Floran et al., 2004). According to the classic model of basal ganglia organization (DeLong and Wichmann, 2007), reversing a reduction in GABA transmission within the STN would reduce dyskinesia, but also compromise L-DOPA antiparkinsonian action. On the other hand, antagonizing striatal D₄ receptors along the direct pathway would exert an antiparkinsonian effect (DeLong and Wichmann, 2007). D₄ receptors might thus be localized in such a way that it is possible to achieve balance between an antidyskinetic effect mediated by the indirect pathway and an antiparkinsonian effect mediated by the direct pathway.

The preservation of the antiparkinsonian action of L-DOPA demonstrated in the current study might also be explained by a direct effect of L-745,870 on the output structures of the basal ganglia. Thus, there is an important dopaminergic innervation of the GP pars interna (GPI) in primates (Parent et al., 1995), and these dopaminergic afferents are relatively spared after MPTP administration (Parent et al., 1990). Although less profuse, the substantia nigra pars reticulata (SNr) also receives dopaminergic input from the substantia nigra pars compacta (Isaacs and Jacobowitz, 1994). The activation of D₄ receptors within the SNr results in a reduction of GP pars externa (GPe)-SNr GABA release, a phenomenon reversed by L-745,870 (Acosta-Garcia et al., 2009). Although such a phenomenon has not been demonstrated for the GPe-GPI projections, because some GPe-SNr fibers emit collaterals to the GPi (Sato et al., 2000), it is possible that L-745,870 exerts a similar effect on GPe-GPI GABAergic fibers, which would support an antiparkinsonian benefit.

Another possible site mediating the antidyskinetic action of D₄ antagonists might be within the cortex. Thus, D₄ receptors modulate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate receptor-mediated currents in the frontal cortex (Rondou et al., 2010; Yuen et al., 2010). As such, antagonizing D₄ receptors might decrease cortical excitability, which, according to the classic model of basal ganglia organization, would alleviate dyskinesia (DeLong and Wichmann, 2007).

**L-745,870 as a Development Candidate in Parkinson’s Disease.** L-745,870 seems to have several advantages over other drugs known to modulate D₄ receptors. For instance, clozapine binds to D₄ receptors with high affinity, but is not selective for this type of receptor (Huot et al., 2011) and, importantly, a 1 to 2% risk of agranulocytosis limits its use (Alvir et al., 1993). 2-[(2-phenyl-1H-imidazol-5-yl) methyl]-1-piperazinyl]-pyrimidine (NGD 94-1) is a selective D₄ antagonist that exhibits 50-fold selectivity over 5-HT₁₆ receptors (Tallman et al., 1997), but its development does not seem to have been pursued. A-381,393 (Cowart et al., 2004; Nakane et al., 2005) is a selective D₄ receptor antagonist that exhibits more than 2000-fold selectivity over its next target. Although A-381,393 is more selective for D₄ receptors than L-745,870, the molecule is not available commercially and, to our knowledge, has not been tested in primates or humans. As such, its in vivo profile, PK, safety, and tolerability have not been characterized, and the drug’s developmental path to clinic is considerably less advanced than that of L-745,870.

L-745,870 was initially developed as an antipsychotic compound and assessed through to a phase IIa study in acutely psychotic schizophrenic patients (Kramer et al., 1997). Although L-745,870 was ineffective in psychosis, the compound was demonstrated to be safe and well tolerated in human subjects (Kramer et al., 1997), and its pharmacokinetics and pharmacodynamics have been well characterized (Bristow et al., 1997b). The fact that the medication can be administered once daily also makes it attractive, especially in the context of PD, where patients experience dyskinesia as a complication of treatment throughout the waking day.

We propose that the safety and pharmacokinetic characteristics of L-745,870 make it an ideal candidate for rapid transition to clinical development. As such, the antidyskinetic efficacy of L-745,870 might be considered in the context of phase II clinical trials in PD. Furthermore, because L-745,870 has already undergone significant clinical development and has an excellent PK and safety profile for a therapeutic candidate, it could be advanced rapidly to such phase IIa clinical studies for dyskinesia in PD. The translation of these findings would be facilitated by the therapeutically relevant target levels for plasma and CSF exposure levels that we provide herein.

**Authorship Contributions**

**Participated in research design:** Huot, Johnston, Koprich, Aman, Fox, and Brotridge.

**Conducted experiments:** Huot, Johnston, Koprich, and Aman.

**Performed data analysis:** Huot, Aman, and Brotridge.

**Wrote or contributed to the writing of the manuscript:** Huot, Johnston, Koprich, Aman, Fox, and Brotridge.

**References**


