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DPI-289, a novel mixed delta opioid agonist / mu opioid antagonist (DAMA), has L-DOPA-sparing potential in Parkinson's disease.

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ABSTRACT

L-DOPA-induced dyskinesia (LID) remains a significant problem in the management of Parkinson's disease (PD). In rodent and macaque models of PD, delta opioid receptor agonists have anti-parkinsonian actions while mu opioid antagonists can reduce the expression of LID. DPI-289 is a novel molecule with a unique combination of opioid receptor DAMA actions: delta agonist (K_i: 0.73 nM); mu antagonist (K_i: 12 nM). We demonstrated that DPI-289 has oral bioavailability and established its pharmacokinetic profile in both rat and primate. We hypothesised that these combined DAMA actions would provide an enhancement of L-DOPA effect without an associated increase in dyskinesia. In parkinsonian 6-OHDA lesioned rats and MPTP-lesioned macaques, DPI-289 provided anti-parkinsonian actions as monotherapy and an enhancement of L-DOPA benefit. Thus, acute administration of DPI-289 (3 mg/kg, *p.o.*) to 6-OHDA-lesioned rats produced a significant reduction in forelimb asymmetry (by 48%) that was maintained throughout the fifteen-day repeat-treatment period. Importantly, and in contrast to L-DOPA administration (6 mg/kg, *i.p.*), these benefits were not compromised by the development of abnormal involuntary movements. In the macaque, as monotherapy, DPI-289 (10 and 20 mg/kg) had significant, though incomplete, anti-parkinsonian actions lasting approximately 4 h. These benefits were not associated with dyskinesia. In fact, over the 6 h period of observation, DPI-289 (20 mg/kg) decreased parkinsonism by 19% and increased activity by 67% compared to vehicle treatment. By contrast, while high-dose L-DOPA (LDh) alone alleviated parkinsonism (for 3 h) this benefit was accompanied by significant dyskinesia that was disabling in nature. LDh provided a 50% reduction in parkinsonism over 6 h and 151% increase in activity. The combination of DPI-289 (20 mg/kg) and a low-dose of L-DOPA (LDl) provided anti-parkinsonian benefits greater than LDl alone without eliciting any significant dyskinesia. Treatment with LDl alone provided only transient statistically significant anti-parkinsonian benefit. However, the combination of LDl and DPI-289 reduced parkinsonism for 6 h (duration of monitoring), with parkinsonism being reduced by 35% and activity increased by 90% but with no increase in dyskinesia over that observed with LDl alone. Thus, DPI-289 has potential to improve the benefits of dopaminergic therapy in Parkinson's disease.

1. Introduction

Treatment of Parkinson's disease (PD) with dopaminergic agents such as 3,4-dihydroxyphenylalanine (L-DOPA) is invariably compromised by motor-complications (Kumar et al., 2005; Manson et al., 2012). Thus, with time, the quality of benefit afforded by L-

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DOPA is reduced because of the appearance of involuntary movements, dyskinesia, and a shortening of the duration (on-time) for which therapy is efficacious (Poewe and Mahlknecht, 2009).

Opioid peptides play an important role in modulating neurotransmission within the basal ganglia. Opioidergic transmission is regulated by dopamine (Gerfen et al., 1991) and becomes dysfunctional in both parkinsonism and motor complications. For instance, in the parkinsonian brain, elevation of pre-proenkephalin-A/met-enkephalin and consequent enhanced delta opioid signalling in the "indirect" striatopallidal pathway, may represent a mechanism attempting to compensate for loss of dopamine as PD progresses (Brotchie and Fitzer-Attas, 2009). A corollary of this concept is that enhancement of delta transmission might alleviate parkinsonian symptoms. Indeed, delta opioid receptor agonists have been validated as an approach to reduce parkinsonian disability in animal models. The anti-parkinsonian role of delta receptor activation has been demonstrated with the delta receptor-selective agonist, SNC-80, in rodent and non-human primate (NHP) studies (Hille et al., 2001). SNC-80 restored all behavioural deficits observed in rats treated with reserpine or dopamine receptor antagonists (haloperidol or SCH23390) including ambulatory behaviour, grooming, rearing, social interaction and exploration (Hille et al., 2001). When administered to MPTP-treated NHPs, SNC-80 enhanced motor activity and reduced bradykinesia and parkinsonian disability to levels seen in normal animals (Hille et al., 2001). These animals had not received prior dopaminergic therapy and had not been "primed" to elicit dyskinesia, thus representing a model of patients in the early stages of the disease. These data, from both rodent and non-human primate models of PD indicate that, at least in early disease, delta opioid receptor agonists have potential to provide a powerful anti-parkinsonian effect that could be useful clinically. Delta agonists are an attractive drug class as, while designated as a member of the opioid receptor family based on genetic sequence homology and shared endogenous ligands, studies in rodents and primates indicate that delta receptor activation does not produce the addiction and abuse liabilities associated with classical mu-receptor stimulating opiates (Negus et al., 1998).

While delta agonists might have value in patients without established L-DOPA-induced complications, their use in more advanced patients where dyskinesia has emerged may, based on animal models, be limited or even contraindicated. In MPTP-lesioned NHPs with already established LID, preliminary reports suggested that delta agonists might elicit dyskinesia even as monotherapy and exacerbate dyskinesia if given in combination with L-DOPA (Johnston et al., 2004). Indeed, delta receptor stimulation likely contributes to the expression of established LID as the delta receptor antagonist naltrindole reduced established LID in MPTP NHPs (Henry et al., 2001). Therefore, to maximize the benefits of delta agonists across the lifespan of the disease it might be necessary to control their propensity to elicit dyskinesia. Here we propose that combining delta opioid agonist with mu antagonist action within a single molecule will achieve such a goal. The rationale for this is that mu antagonists can reduce LID (Koprich et al., 2011) and there is significant overlap between the pharmacophores of delta agonists and mu antagonists to suggests than a multi-functional molecule, with high selectivity and affinity at both sites could have utility. In fact, the selective mu opioid receptor antagonists cyprodime and ADL-5510 can abolish L-DOPA-induced dyskinesia in the MPTP-lesioned NHP model of PD without attenuation of the anti-parkinsonian actions of L-DOPA (Henry et al., 2001; Koprich et al., 2011). We have previously proposed that stimulation of mu opioid receptors, resulting from over-expression of peptides, including alpha-neo-endorphin, produced from the precursor pre-proenkephalin-B (PPE-B) in the "direct" striatal output pathway, contributes to the expression of LID (Henry et al., 2001; Koprich et al., 2011).

DPI-289 is a novel, small molecule drug that combines delta opioid agonist and mu opioid antagonist actions and may therefore combine potential anti-parkinsonian and anti-dyskinetic mechanisms within the same molecule. The aim of the current study was to assess the behavioural and pharmacokinetic profiles of DPI-289 in rodent and primate models of PD.

2. Material and methods

2.1. Chemistry

DPI-289 was synthesised by Atuka Inc. DPI-289 was a white powder with a melting point of 195–196 °C, an optical rotation of −4.85° (c = 1%, $CHCl_3$), a purity of 98.8% and a diastereomeric purity of 99.4%. No single impurity was greater than 0.6%.

2.2. Pharmacological characterisation

2.2.1. Membrane preparation for radioligand binding

The brains from male albino Sprague-Dawley rats were obtained from Pel Freez Biologicals (Rogers, AR) and cerebellum from male albino guinea pigs from Accurate Chemical and Scientific Corporation (Westbury, NY). The tissue was rinsed with ice-cold 50 mM Tris-HCl buffer (pH 7.4, 25 °C) containing the following protease inhibitors: 50 μg/ml soybean trypsin inhibitor, 0.1 mM phenylmethylsulfonyl floride (PMSF) and 1 mM ethylenediaminetetraacetic acid (EDTA), 10 μg/ml Leupeptin, 200 μg/ml Bacitracin, and 0.5 μg/mL Aprotinin. Brains were homogenized in 5–10 vol/g wet weight in ice-cold 50 mM Tris buffer containing protease inhibitors using a motor-driven glass-Teflon homogenizer (nominal clearance, 0.13–0.18 mm). The homogenate was centrifuged at 6000 × g for 15 min at 4 °C, and the resulting supernatant centrifuged at 41,000 × g for 30 min. This membrane pellet was resuspended in 10 vol/g wet wt of 10 mM Tris-sucrose (0.32 M) buffer and sonicated with a Polytron tissue grinder (10 s, low speed). The homogenate was centrifuged at 41,000 × g for 30 min at 4 °C. The resulting membrane pellet was resuspended in 50 mM Tris buffer with protease inhibitors at a final protein concentration that ranged from 40 μg/ml to 50 μg/ml. This membrane fraction was frozen under liquid N2 and then stored at −80 °C prior to use in radioligand binding studies. Protein concentration was determined by the method of Bradford.

2.2.2. Radioligand binding on animal opiate receptors

Membrane fractions were incubated with 0.1 nM [^{3}H]-deltorphin II – delta receptor (specific activity 38.5–40.6 Ci/mmol), 0.1 nM [^{3}H]-DAMGO – mu receptor (specific activity 50.0 Ci/mmol) or 0.1 nM [^{3}H]-U69593 – kappa receptor (specific activity 41.4 Ci/mmol) in 2 ml of 10 mM Tris-HCl buffer containing 5 mM $MgCl_2$ and protease inhibitors (all radioligands purchased from Dupont-New England Nuclear, Boston, MA). Incubation was carried out for 90 min at 25 °C. These conditions permitted the complete equilibration of the radioligand with its receptor. The reaction was terminated by rapid filtration through Whatman GF/C glass fiber filters using a cell harvester (model M-48R, Brandel Instruments, Gaithersberg, MD) followed by two 5-ml rinses with ice-cold 50 mM Tris buffer. Specific binding was defined as that radioligand displaced by 1×10^{-6} M naloxone. Filters were counted by liquid scintillation spectrometry at an efficiency, determined by external standards, of 40–45%.

2.2.3. Intrinsic activity at delta receptor: mouse vas deferens

Following cervical dislocation, vasa deferentia were isolated

from male CD-1 mice (Charles River, Raleigh, NC) weighing 20–25 g. Muscles were suspended in individual organ baths containing Mg-free Krebs-Henseleit solution (37 °C, aerated with O_2-CO_2, 95:5) of the following composition (millimolar): NaCl, 117.5; KCl, 4.75; $CaCl_2$, 2.6; KH_2PO_4, 1.2; $NaHCO_3$, 24.5; and glucose, 11. The vas deferens segments were positioned between platinum electrodes and connected to a Grass FTO3 isometric force transducer. Muscles were stimulated to contract by administering 400-msec pulse trains (1msec duration, supramaximal voltage, 10 Hz) with a Grass S88 stimulator; resting tension was 0.5 g. To establish cumulative concentration-response relationships, compounds were added to organ baths and allowed to produce maximal response before addition of the next higher concentration.

2.2.4. Intrinsic activity at the mu receptor: guinea pig ileum

Tension developed in response to electrical stimulation of guinea pig isolated ileum was recorded as follows. Male albino guinea pigs (Charles River, Raleigh, NC) weighing 300–500 g were euthanized by decapitation and an 8 cm section of ileum removed and divided into 2–3 cm segments. Individual segments were suspended in standard organ baths (Radnoti) and continuously bathed in Krebs-Henseleit solution (37 °C, aerated with O_2/CO_2 95:5) of the following composition: 117.5 mM NaCl, 4.75 mM KCl, 2.4 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.5 mM $NaHCO_3$, and 11 mM glucose. Tissues were suspended from Grass FTO3 isometric force transducers under a resting tension of 1 g and contractions were elicited by a field stimulation of 0.1 Hz pulses of 0.5 ms duration at supramaximal voltage using platinum electrodes and a Grass S88 stimulator. Compound effects on electrically-induced contractions of the ileum were examined through the addition of cumulative concentrations (1×10^{-7} to 1×10^{-6} M; N = 3) to the bathing solution. The maximum concentration tested was limited by the solubility of the compounds in these assay conditions. A fentanyl dose response curve was used as a positive control in all tissues. Concentration-response curves were analyzed using Prism (GraphPad Software Inc., San Diego, CA, USA) to determine EC_{50}, IC_{50}, or K_i values.

2.2.5. In-vivo antagonism of fentanyl-induced analgesia

Male C57BL/6 mice (N = 15 per group, 8 weeks old at time of testing, Harlan, Madison, WI, USA), housed 4 per cage at standard temperature (21 ± 2 °C) and with access to food (Teklad 7912, Harlan, Madison, WI) and water *ad libitum*, were acclimatised for at least one week prior to the start of any procedures. Mice were administered a subcutaneous bolus of fentanyl (100 μg/kg, Taylor Pharmaceuticals, Decatur, IL, USA) 30 min after receiving an oral dose of either vehicle (5% dextrose) or DPI-289 (10 mg/kg). Tail pinch reaction time was determined at 10-min intervals from the time of fentanyl dosing for 60 min. Analgesic effects of fentanyl were expressed as the Mean Percent Effect; (response time − basal time)/(maximum time − basal time).

2.3. Test item

DPI-289 for use in efficacy components was formulated in a vehicle containing 1% (w/v) methyl cellulose (Sigma-Aldrich, M0430, Oakville, ON, Canada) in 95% sterile water (v/v) and 5% (v/v) DMSO (Sigma-Aldrich, 472301, Oakville, ON, Canada) given at a dose volume of between 1 (rat) and 10 (primate) ml/kg.

2.4. Behavioural assessment in the 6-OHDA-lesioned rat

2.4.1. Unilateral 6-OHDA lesion surgery

Female Sprague-Dawley rats (Charles River, Senneville, QC, Canada, 275–300 g at time of surgery, N = 24), housed 2 per cage at standard temperature (21 ± 2 °C) and with access to food (Teklad 7912, Harlan, Madison, WI) and water *ad libitum*, were acclimatised for at least one week prior to the start of any procedures. All procedures were approved by the University Health Network Animal Care Committee (Toronto) and were in accordance with the regulations defined by the Canadian Council on Animal Care. Briefly, 30 min prior to surgery, animals were administered pargyline (5 mg/kg, s.c., Sigma-Aldrich, Oakville, ON, Canada) to enhance 6-OHDA toxicity. Under isofluorane anaesthesia (2% with 2 L/min oxygen flow rate, Pharmaceutical Partners of Canada Inc., Richmond Hill, Ontario, Canada) and after confirmation of loss of tail-pinch and corneal reflexes, rats were placed in a Kopf small animal stereotaxic frame with the incisor bar set 3.3 mm below the ear bars (interaural line). An incision (~2 cm) was then made with a sterile scalpel blade in an anteroposterior direction along the midline. After exposure of Bregma, using a cotton-bud, a burr hole was drilled in the skull above the right median forebrain bundle at co-ordinates: 2.8 mm posterior, and 2 mm lateral to Bregma (according to the atlas of Paxinos and Watson, 1986). A 1 inch, 26G Hamilton needle with 45-degree bevel was lowered 8.6 mm below the skull. Injection (0.5 μl/min) of 6-OHDA (12.5 μg in 2.5 μl) was then made. The needle was left in place for 5 min to ensure complete absorption of the solution. After slow retraction of the injection needle, the incision was closed by means of wound clips and animals administered saline (50 ml/kg, s.c.) and an analgesic (Ketoprofen (Anafen, Merial Montreal, QC, Canada); 0.5 mg/kg, s.c.). Finally, animals were removed from the frame and placed in a recovery cage, positioned atop a thermostatically-controlled pad, and monitored until conscious. Those animals displaying the most robust levels of forelimb asymmetry were included in the study and divided into three equal groups (Groups A-C, each N = 8) such that mean levels of asymmetry were comparable between them.

2.4.2. Assessment of extent of 6-OHDA lesion

Three weeks following stereotaxic surgery, forelimb asymmetry was assessed in order to gauge the extent of surgical 6-OHDA lesion success. Each animal was placed in a clear glass cylinder and the type of forelimb contact assessed. Following a 21-day recovery period, the degree of parkinsonism was assessed using the cylinder test, in which 70% use of the forelimb ipsilateral to the lesion is indicative of more than 88% striatal dopamine depletion (Schallert et al., 2000).

The number of times each paw touched the side of the cylinder during an individual rear was determined from post hoc analysis of video by an observer blinded to the treatments given. The first limb in any rear to touch the wall was scored a single point. If both limbs contacted within 0.4s of each other then a 'both' was scored. A minimum of ten independent rears were required in order for data for a given behavioural observation to be included in analyses. Asymmetry scores as a percentage were calculated using the formula: 100*[(ipsilateral - contralateral)/(ipsilateral + contralateral)] and animals lacking overt behavioural asymmetry (>85% ipsilateral forelimb use) were excluded from the Study.

2.4.3. Drug treatments

Six weeks after surgery animals received once-daily treatment for 15 days. Those in Group A received $vehicle_1$ (that used to formulate L-DOPA, *i.p.*) and $vehicle_2$ (that used to formulate DPI-289, *p.o.*). Animals in Group B received L-DOPA (6 mg/kg in combination with benserazide, 15 mg/kg, *i.p.*) and $vehicle_2$ (*p.o.*) and those in Group C received $vehicle_1$ (1 ml/kg, *i.p.*) and DPI-289 (3 mg/kg, *p.o.*). The dose of DPI-289 was chosen as that representing the minimally anticipated biological effect level (MABEL) in having produced a small but significant alleviation of haloperidol-induced catalepsy in the rat.

2.4.4. Abnormal involuntary movements (AIMs)

Baseline Abnormal Involuntary Movements (AIMs) were determined at this moment, after which the animals were injected with their respective treatments. Axial, limb and orolingual (ALO) AIMs were rated by an observer blinded to treatment, according to a protocol described by Cenci and Lundblad (2007) which encompasses both 'severity' and 'amplitude' of the abnormal movements. AIMs were rated for 1 min at baseline, and then every 20 min for a period of 3 h. AIMs severity was rated according to the following scale: 0 = no dyskinesia; 1 = occasional signs of dyskinesia, present for less than 50% of the observation period; 2 = frequent signs of dyskinesia, present for more than 50% of the observation period; 3 = dyskinesia present during the entire observation period, but suppressible by external stimuli and 4 = continuous dyskinesia not suppressible by external stimuli.

2.5. Pharmacokinetic profiling of DPI-289 in rat and macaque

2.5.1. Blood sampling

Twelve female Sprague Dawley rats (276–300 g) were used. Rats were fasted from 5.00 p.m. on the preceding day. Groups of three animals were administered DPI-289 either orally (1 or 3 mg/kg), or via i.v. or intraportal infusion (1 mg/kg). On each day of sampling, six blood samples for drug level analysis (N = 3 animals per 3 time-points) were collected at pre-dose and then at 15, 30 min and at 1, 2 and 4 h post drug administration. Macaques (N = 3, see behavioural assessment section for details) were fasted from 5.00 p.m. on the preceding day. For primate sampling, on the day of treatment administration and plasma sampling, macaques were transferred from their home cages and seated in individual primate chairs. Samples were collected pre-dose (0 min) and then at 15, 30 min and at 1, 2, 4, 8 and 24 h post drug administration. Samples (0.15 ml or 0.5 ml; rat and macaque respectively) were placed into K_2-EDTA tubes (Becton Dickinson, Mississauga, ON, Canada) and centrifuged at 4 °C for 5 min at $1500g_{ave}$ and plasma analysed for DPI-289 via LC/MS/MS.

2.5.2. Bioanalysis of DPI-289 in rat and macaque plasma

An aliquot of 20 μl of plasma was added to 80 μl of acetronitrile containing 1–10 ng/ml of the IS. After centrifuging at 13000 rpm for 8 min, 70 μl of supernatant was isolated and added to 70 μl of sterile water. Finally, an aliquot of 1–10 μl of the mixture was injected into the LC-MS/MS system. For all bioanalytical work EPI-102 (d2-DPI-289) was used as the internal standard. In brief, 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 0.02 ng/ml to 10 ng/ml (primate) and 1 ng/ml to 200 ng/ml (rat). All PK parameters were calculated according to nominal time, that is, within ±5% from schedule time-point by non-compartmental modelling using WinNonlin 6.3. Plasma concentration-time data were processed by linear trapezoidal linear log interpolation regression analysis.

2.6. Behavioural assessment in the MPTP-lesioned macaque

2.6.1. Animals

Eight cynomolgus monkeys (*Macaca fascicularis*, 7–13 years of age, 3–4 kg, Suzhou Xishan-Zhongke Laboratory Animal Company, PRC) were used in this study. Animals were housed two or three per cage in the same housing room with cage sizes exceeding Council of Europe, UK, EU, NIH and CCAC minimum size recommendations. Cages were equipped with a variety of environmental enrichment (including perch, fruit and toys). The housing rooms were subject to a 12-h light-dark cycle (lights on 7 a.m.), temperature 20–25 °C in a room containing only animals of the same sex. Fresh fruit, primate pellets and water were available *ad libitum* other than at times of overnight fasting (from 5 p.m.) prior to days of behavioural assessment. All efforts were made to reduce to a minimum the number of animals necessary for statistically valid analyses and to minimise animal suffering. All studies were performed with local IACUC approval and in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the NIH (Institute of Laboratory Animal Resources (U.S.). Committee on Care and Use of Laboratory Animals (1996)) (National Research Council Institute for Laboratory Animal, 1996).

2.6.2. MPTP administration and development of motor complications

Animals received once-daily subcutaneous injection of MPTP (0.2 mg/kg in 0.9% sterile-saline, Sigma-Aldrich, Oakville, ON, Canada) for 8–30 days. A parkinsonian syndrome was then allowed to develop over at least a 90-day period, during which time additional MPTP administrations were given as necessary, until animals reached moderate to marked levels of disability. Average cumulative MPTP dose was 33.3 mg. MPTP lesions were allowed to stabilise for a minimum of a further 60-day prior to commencing induction of L-DOPA-induced motor complications. LID, including both choreiform and dystonic dyskinesia, were evoked by chronic L-DOPA treatment (25 mg/kg, Madopar™, Roche, L-DOPA: benserazide, ratio 4:1) for at least 4-months. During this same period animals were acclimatised to the experimental setting, trained to provide blood samples (while restrained in chair) and to receive administration of treatment by oral, intravenous or subcutaneous routes. During this period, animals were handled by technical staff and transferred from home caging to observation caging on a regular basis. Animal ID were identified via individually inscribed metal collar tags and also by subcutaneously implanted transponders encoded with the animal ID (Plexx, Elst, Netherlands; model IPTT-300).

2.6.3. L-DOPA dose-finding

Dose-finding observations were conducted to identify two L-DOPA doses. Thus, animals were administered a range of L-DOPA doses (10, 15, 20, 25, 30 and 35 mg/kg) and effects on levels of parkinsonian disability and dyskinesia assessed for a period of 6 h as described in Section 2.6.5. From these data, a 'low' dose of L-DOPA (LDl) was selected that was optimised for each animal to produce sub-optimal anti-parkinsonian actions (range 10–15 mg/kg, mean 10.6 mg/kg) and non-disabling dyskinesia. Similarly, a higher dose (LDh) was selected for each animal that was intended to produce optimal anti-parkinsonian actions but which was compromised by disabling dyskinesia (range 30–35 mg/kg, mean 30.6 mg/kg). The responses to these doses of L-DOPA were assessed to ensure stability and reproducibility within each animal on successive L-DOPA administrations.

2.6.4. Treatments

For efficacy assessments, in an acute challenge randomized design consisting of two successive blocks, animals were treated either with monotherapy (vehicle or DPI-289; 1, 10 or 20 mg/kg, PO) or one of four combination treatments; $vehicle_1/vehicle_2$ (vehicles for DPI-289 and L-DOPA respectively), $vehicle_1$/LDl, DPI-289/LDl, or $vehicle_1$/LDh. The choice of dose of DPI-289 (20 mg/kg) for the combination experiment was selected based on the outcome of the previous monotherapy block. DPI-289 (or $vehicle_1$) was administered 1 h prior to L-DOPA (or $vehicle_2$) and observations were carried out for a period of 6 h.

2.6.5. Assessment of parkinsonian disability, dyskinesia and activity

Animals were transferred to individual observation cages (1.5 × 1.0 × 1.1 m) and their behaviour recorded on HD-video. Rating scales for parkinsonism and dyskinesia adapted from their clinical counterparts (UPDRS pt. III and UDysRS respectively) were used to assessed recordings via *post-hoc* analysis by a movement disorders neurologist blinded to treatment. A measure of total parkinsonian disability as described previously (Johnston et al., 2013) was derived by adding scores for range of movement (score 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-min every 10-min of the 6 h observation period, the score given being most representative of each 5-min observation period.

Scores were summed for each hour for time-course analyses and across the entire observation period (0–6 h). Thus, for measures of parkinsonian disability and dyskinesia, the maximum scores possible (equating to severe) over the 0–6 h period were 360 and 144 respectively. A quantitative assessment of activity was attained using computer-based activity monitors. Movement of the animal was assessed by passive infra-red detectors. Activity counts were cumulated in each minute of analysis. These data provide information on total activity.

2.7. Statistical analyses

Data derived from assessment of mouse tail-pinch, rodent forelimb asymmetry and primate activity were plotted as mean ± s.e.m. Statistical analyses for these data were performed using parametric repeated measures one- or two-way ANOVA as appropriate, followed by Holm-Sidak multiple comparison's tests. Data for rat AIMs data and primate measures of parkinsonian disability and dyskinesia were graphed, where appropriate, as median scores alone (time course) or box and whisker plots (cumulated totals). Time course data for parkinsonian disability and dyskinesia were first ranked within each animal across all treatments using Excel's RANKAVG function. These transformed data were then analysed in GraphPad Prism (v 7.02) and subjected to non-repeated measures 2-way ANOVA followed by Holm-Sidak multiple comparison tests. Cumulated AIMs, disability and dyskinesia data were analysed using a Friedman test followed by a Dunn's Multiple Comparisons test.

3. Results

3.1. DPI-289 is a delta agonist/mu antagonist (DAMA) compound

DPI-289 (Fig. 1A; 4-((alpha-R)-alpha-((2S, 5R)-2,5-dimethyl-4-(3-fluorobenzyl)-1-piperazinyl)-3-hydroxybenzyl)-N,N-diethylbenzamide) is a new chemical entity with a dimethyl benzhydrylpiperazine backbone. DPI-289 combines both delta agonist and mu antagonist activity (Fig. 1B). DPI-289 displays affinity for both the delta and mu receptor with K_i: 0.73 and 11.6 nM respectively. Studies assessing the actions of DPI-289 in both the guinea pig ileum (containing mu and kappa receptors (Lord et al., 1977; Ward and Takemori, 1976) and mouse vas deferens (containing delta-receptors in addition to mu- and kappa sites (Lord et al., 1977) showed DPI-289 to possess intrinsic activity at, the delta receptor (EC_{50}: 0.75 nM) but lacked any intrinsic activity for mu (Fig. 2A). Moreover, in the mouse tail-pinch assay, DPI-289 (10 mg/kg, p.o.) antagonised the analgesic action of fentanyl (100 μg/kg, s.c.) by up to 45% compared to vehicle (AUC; 2433 ± 54 cf. 1345 ± 53 MPE.min respectively, Fig. 2B).

3.2. DPI-289 provides sustained anti-parkinsonian benefit without evoking motor-complications in the 6-OHDA-lesioned rat model of PD

In treatment-naïve, unilateral 6-OHDA-lesioned animals, DPI-289 (3 mg/kg, p.o.) produced a robust anti-parkinsonian response as evidenced by a significant reduction in forelimb asymmetry, specifically a reduction in the over-use of the forelimb ipsilateral to the 6-OHDA lesion, over 15 days of treatment (Fig. 3). On Day 1, there was an acute effect of treatment (1-way ANOVA; $F(2, 17) = 4.035$, $P < 0.05$) with DPI-289-treated animals displaying a decrease in asymmetry of 59% compared to that observed in vehicle-treated animals (Fig. 3A; Holm-Sidak *post-hoc* test, $P = 0.0368$). The extent of anti-parkinsonian effect of L-DOPA (6 mg/kg, i.p.) on Day 1 was equivalent to that of DPI-289 (Fig. 3A: 69% reduction cf. vehicle-treated animals, $P < 0.05$ 1-way ANOVA with Holm-Sidak post-hoc test). The anti-parkinsonian benefit of DPI-289 was maintained throughout the fifteen-day repeat-treatment period (Figure 3B, 2-way ANOVA with Holm-Sidak post-hoc test, all $P < 0.05$ cf. vehicle-treatment on observation days 4, 8, 11 and 15). DPI-289 evoked no significant Abnormal Involuntary Movements (Fig. 3C and D: AIMs), a rodent correlate of dyskinesia, either acutely ($P > 0.05$, Dunn's post-hoc test) or across the entire fifteen days of repeat treatment ($P > 0.05$, Friedman's test). In contrast, following repeat-treatment, the quality of anti-parkinsonian response to L-DOPA, unlike that of DPI-289, was compromised by the emergence of AIMs (Fig. 3C: acute and 3D: chronic). Thus, once-daily L-DOPA evoked significant levels of AIMs by the fourth day of treatment (median score = 5.5, $P < 0.001$ cf. vehicle) increasing to mild-moderate levels by day fifteen (median score = 7.5, Days 8, 11 and 15; all $P < 0.001$ cf. vehicle). These AIMs, along with the emergence of sensitization of the L-DOPA rotational response and increased variability compromised the assessment of asymmetry, and hence the anti-parkinsonian benefit of L-DOPA (Fig. 3B). These negative effects caused a stark decrease in the number of animals able to perform in the cylinder test. For example, at Day 11, only a single L-DOPA-treated animal was able to rear a sufficient number of times to be included in the analysis. By contrast, at least seven of eight animals in the DPI-289-treated group completed the test over each of the days of assessment. Post-mortem confirmation of extent of lesion demonstrated that all animals showed ipsilateral loss of specific dopamine transporter binding greater than 90% of that observed on the contralateral side (95.2% ± 0.5%) and were thus appropriate for inclusion in the final analyses.

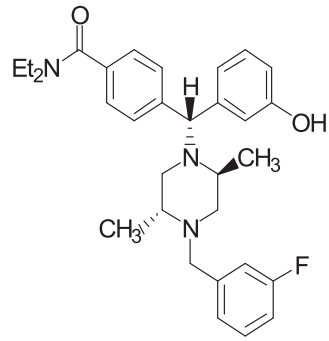
3.3. Pharmacokinetic profile of DPI-289 in the rat and macaque

In rat, administration of the dose of DPI-289 with demonstrated behavioural efficacy (3 mg/kg), was associated with peak plasma levels, C_{max}, of 33.8 ng/ml and a plasma exposure, AUC_{0-4h}, of 76.4 h ng/ml. Fig. 3E shows the profile of plasma concentrations of DPI-289 following an acute dose, there was little decay over the duration of the sampling period up to 4 h. Comparison of $AUC_{0\text{-}t}$ values following oral, i.v. and intraportal vein administration, showed oral bioavailability values for DPI-289 of 13–14% (Fig. 3F) indicating that first-pass hepatic elimination appears to be the main barrier to systemic uptake of DPI-289.

A pharmacokinetic study in MPTP-lesioned macaques then identified a range of oral doses whose corresponding drug plasma exposure levels bracketed the efficacious rat C_{max} exposure. Oral doses in the macaque of 1, 5, 10 and 20 mg/kg were associated with mean C_{max} values of 2.4, 17.4, 36.5 and 49.9 ng/ml and mean AUC_{0-4h} values of 5.8, 43.5, 87.6 and 140.8 h ng/ml (Fig. 4). Based on these data, doses of 1, 10 and 20 mg/kg were chosen for subsequent

DPI-289 structure

A



B

DPI-289 opioid receptor pharmacology

Opioid receptor	Binding affinity		Intrinsic activity	
	Assay system	Ki	Assay system	IC50
Delta (δ)	Rat brain	0.73 nM	Mouse vas deferens	0.75 nM
Mu (μ)	Rat brain	12.0 nM	Guinea pig ileum	>1000 nM
Kappa (κ)	Guinea pig cerebellum	567 nM	Mouse vas deferens	>1000 nM

Fig. 1. Structure and key opioid pharmacology of DPI-289.

assessment of acute anti-parkinsonian efficacy.

3.4. DPI-289, as monotherapy, provides anti-parkinsonian benefit in MPTP-lesioned macaques with previously established L-DOPA-induced motor complications

Orally administered DPI-289 was well tolerated at all doses assessed. The effects of acute monotherapy with DPI-289 on parkinsonian disability, dyskinesia and activity are shown in Fig. 5. Levels of parkinsonian disability observed following treatment with vehicle alone were moderate (median value 179) over the 0–6 h period of observation. During this period, DPI-289 produced a modest but significant reduction in parkinsonian disability (0–6 h; Friedman Statistic (FS) = 16.15, P = 0.0011, Friedman test, Fig. 5A) with post-hoc Dunn's analysis showing significant decreases in disability of 6% and 19% (10 and 20 mg/kg, $P < 0.05$ and $P < 0.001$) respectively, after DPI-289 administration compared to that seen following vehicle. At no time did treatment with DPI-289 evoke relevant dyskinesia (0–6 h; Friedman Statistic (FS) = 6.0, P = 0.116, Friedman test; Fig. 5C). Analysis of disability levels across the whole 6 h time-course period of observation revealed a significant effect of DPI-289 treatment ($F(3, 28) = 7.555$, $P = 0.0007$) and the interaction of treatment and time ($F(15, 140) = 3.114$, $P = 0.0002$) but not time alone ($F(5, 140) = 0.0$, $P > 0.9999$) on levels of disability (2-way, RM-ANOVA, Fig. 5B). Post-hoc Holm-Sidak's analysis revealed a significant decrease in parkinsonian disability in the first (20 mg/kg, by 12%), second (10 and 20 mg/kg, by 11% and 18% respectively), third (10 and 20 mg/kg, by 21% and 31% respectively) and fourth (1, 10 and 20 mg/kg, by 20%, 23% and 24% respectively) hours after administration compared to that seen following vehicle treatment (all $P < 0.05$). In terms of activity levels, examination of the whole 6 h time-course period revealed a significant effect of time ($F(5, 35) = 7.928$, $P < 0.0001$) but not of treatment ($F(4, 28) = 0.67$, $P = 0.5779$) nor of the interaction of time and treatment ($F(15, 105) = 1.295$, $P = 0.2184$; 2-way, RM-ANOVA, Fig. 5E). Indeed, there was a dose-dependent increase in activity during the first 4 h (10 and 20 mg/kg, by up to 54% and 67% respectively) of observation (up to 5 h after administration) compared to that seen following vehicle treatment (all $P < 0.05$, Holm-Sidak's post-hoc test).

3.5. DPI-289 enhances the anti-parkinsonian benefit of L-DOPA without exacerbating dyskinesia, in the MPTP-lesioned macaque with motor complications

There was a significant effect of treatments on levels of parkinsonian disability cumulated over the 6-h period of analysis (0–6 h; Friedman Statistic (FS) = 15.9, P = 0.0012, Friedman test, Fig. 6A). A low dose of L-DOPA (LDl) had a significant effect on total parkinsonian disability only in combination with DPI-289 (20 mg/kg). Moreover, the LDl/DPI-289 combination was broadly equivalent in terms of total anti-parkinsonian benefit over 6 h to that observed following a high dose of L-DOPA (LDh) with parkinsonian disability being significantly reduced by 35% and 50% respectively, compared to vehicle alone (both $P < 0.05$, post-hoc Dunn's analysis).

In examining the time-course of behaviour over the individual hour periods, we observed a mild, short duration, anti-parkinsonian effect of LDl alone that was significant only in the first and second hour. In contrast, the combination of LDl with DPI-289 greatly extended the duration of action, providing a significant anti-parkinsonian benefit to the end of the study at 6 h. The high dose L-DOPA provided an anti-parkinsonian benefit that was only significant for 3 h but, at peak, was of greater magnitude than the LDl/DPI-289 combination (Fig. 6B).

In terms of parkinsonian disability, examining the whole 6 h time-course period of observation revealed a significant effect of treatment ($F(3, 28) = 15.25$, $P < 0.0001$), the interaction of time and treatment ($F(15, 140) = 4.137$, $P < 0.0001$) but not time alone ($F(5, 140) = 0.0$, $P > 0.9999$, 2-way, RM-ANOVA, Fig. 6B). Thus, LDl alone

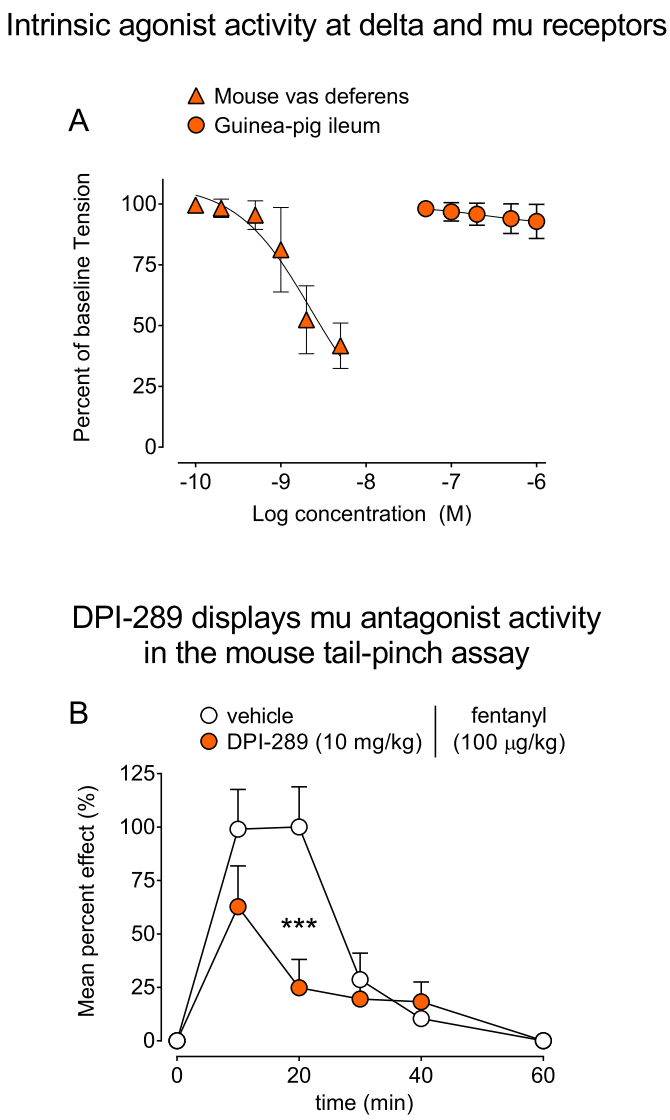


Fig. 2. Intrinsic delta activity and mu antagonist properties of DPI-289.
(A) Tension development in isolated mouse vas deferens or guinea pig ileum was measured in response to bath application of DPI-289 (0.1 nM–1 μM). (B) Male C57BL/6 mice were administered a subcutaneous bolus of fentanyl (100 μg/kg) 30 min after receiving an oral dose of either vehicle (5% dextrose) or DPI-289 (10 mg/kg). Tail pinch reaction time was determined at 10-min intervals from the time of fentanyl dosing for 60 min. Analgesic effects of fentanyl were expressed as the Mean Percent Effect; (response time − basal time)/(maximum time − basal time). Data are mean ± SEM. *** represents $P < 0.0001$ cf. vehicle (2-way RM ANOVA with Holm-Sidak's test.

provided only threshold anti-parkinsonian benefit during the first 2 h following administration (Fig. 5B, $P < 0.05$ cf. vehicle, Holm-Sidak post-hoc test). By contrast, the combination of LDl with DPI-289 (20 mg/kg) evoked greater and more sustained decreases in disability, parkinsonism being significantly reduced during the first, second, fourth, fifth and sixth hours after treatment (by 37%, 50%, 43%, 30% and 25% respectively). LDh evoked significant anti-parkinsonian benefits, somewhat greater at peak effect than those of the LDl/DPI-289 combination, but the benefits were of shorter duration being significant only in the first, second and third hours (by 77%, 67% and 51% respectively). There was a significant effect of treatment on total dyskinesia cumulated over the 6-h period of analysis (0–6 h; Friedman Statistic (FS) = 20.2, $P = 0.0002$, Friedman test, Fig. 6C). Neither the low dose of L-DOPA (LDl) alone nor LDl in combination with DPI-289 elicited significant dyskinesia when cumulated over the 0–6 h period. However, the higher dose of L-DOPA alone was associated with significant dyskinesia over 6 h, ($P < 0.001$, post-hoc Dunn's analysis, compared to vehicle alone: Fig. 6C and D).

With respect to duration, LDl elicited dyskinesia over the first 2 h though this was only mild and non-disabling (Fig. 6C and D). Addition of DPI-289 (combination) did not increase or prolong this in that there was no statistically difference in dyskinesia scores between the two treatments (LDl cf. combination: all $P > 0.05$). In contrast, LDh elicited dyskinesia for 3 h, at peak being severe and disabling (Fig. 6D). The dyskinesia exhibited in the LDh group mirrored the time course of the reduction in parkinsonian disability conferred by this high dose (Fig. 6D and E).

The anti-parkinsonian benefit observed following LDl/DPI-289 combination was not associated with any increased dyskinesia, compared to LDl alone. Thus, comparing to vehicle-vehicle treatment, there were mild increases in dyskinesia during the first 2-h after treatment in response to either LDl alone or in combination with DPI-289 (20 mg/kg) with median levels below mild (score of 6, i.e. non-disabling). By contrast, high-dose L-DOPA evoked robust and significant increases in dyskinesia across the first 3-h period reaching median marked to severe (i.e. disabling; score of 18–24) levels (all $P < 0.001$).

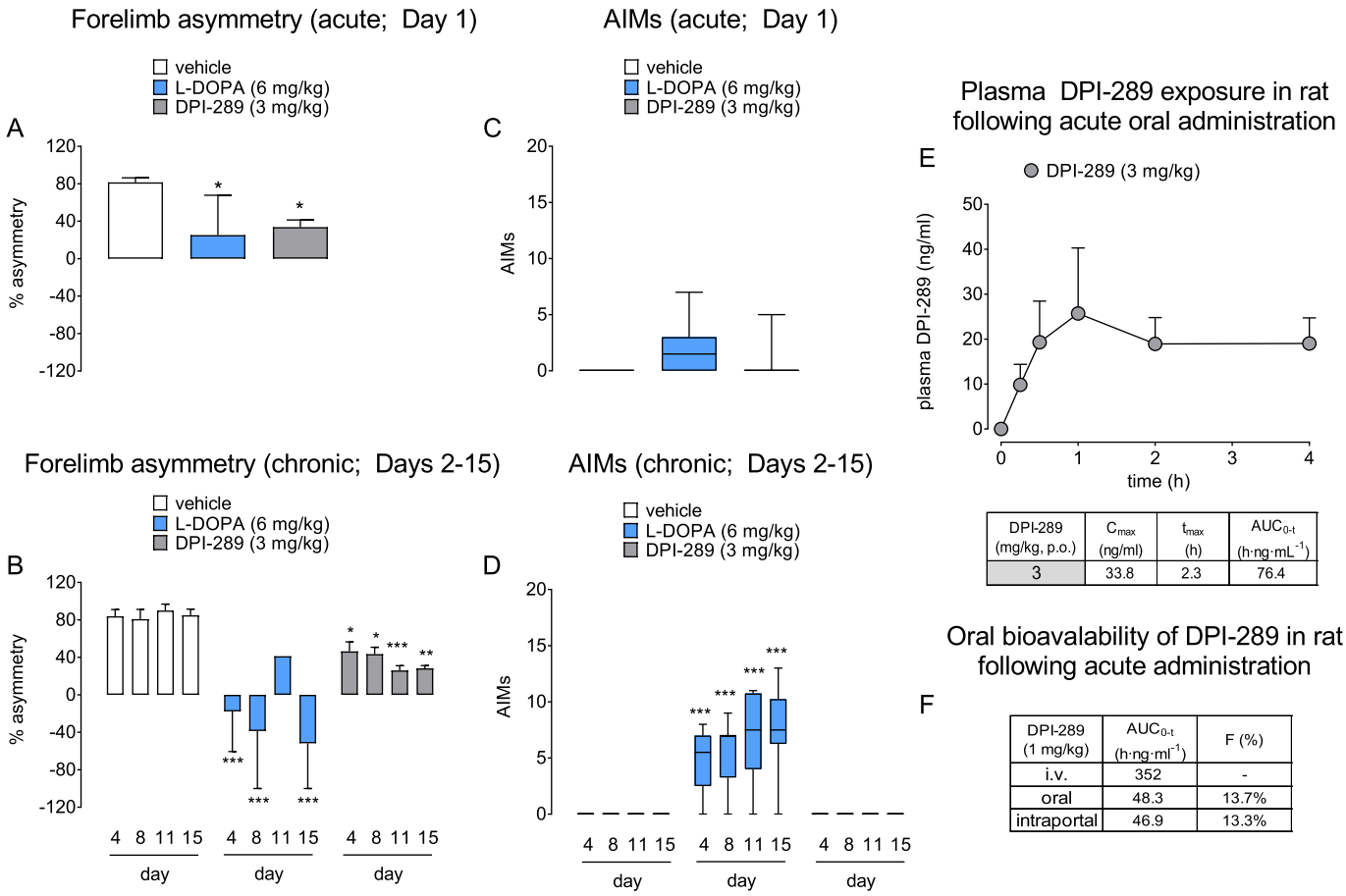
ANOVA revealed a significant effect of treatment on activity cumulated over the 0–6 h period (0–6 h; $F(1.663, 11.64) = 13.03$, $P = 0.0015$, Fig. 6E) such that LDl in combination with DPI-289, but not LDl alone, evoked a significant increase in activity (by 90%, $P < 0.05$, compared to vehicle) which in turn was not significantly different to that evoked by LDh (by 150%, $P > 0.05$ compared to LDl/DPI-289).

With respect to activity levels, there was a significant effect of time ($F(5, 35) = 21.82$, $P < 0.0001$), treatment ($F(3, 21) = 13.21$, $P < 0.0001$) and the interaction of the two ($F(15, 105) = 8.304$, $P < 0.0001$) on levels of activity over the 6 h period (Fig. 6F). Compared to vehicle-vehicle treatment, post-hoc Holm-Sidak's analysis revealed a significant increase in activity in just the first hour after treatment in response to LDl (by 175%). By contrast, the combination of LDl with DPI-289 evoked significant increases in activity during the first, second and fourth hours after treatment (by 123%, 148 and 78% respectively). High-dose L-DOPA evoked significant increases in activity across the first 4 h of treatment (by 230%, 338%, 269% and 6% respectively).

4. Discussion

This study represents the first report of demonstrable efficacy of a delta-agonist, mu-antagonist (DAMA) compound in providing anti-parkinsonian benefit in rodent and NHP models of PD. Thus, DPI-289 shows affinity for, and intrinsic activity at, the delta opioid receptor (K_i: 0.73 and EC_{50}: 0.75 nM respectively) and affinity (K_i) for the mu opioid receptor of 11.62 nM but lacking any intrinsic activity at this site. Further examination of the mu receptor in a mouse tail-pinch model of fentanyl-induced analgesia demonstrated that DPI-289 at 10 mg/kg p.o. produced a 45% decrease in the Mean Percent Effect of fentanyl in this assay system, indicating functional mu antagonist activity. As a preliminary characterisation of the basic pharmacology of DPI-289 these studies will benefit from more conclusive interrogations of the functional activity of DPI-289 by expanding the range of doses applied to the fentanyl tail-pinch assay (mu) and confirming delta receptor involvement in the actions of DPI-289 in guinea pig-ileum with a combination delta antagonist experiment.

We have further shown that DPI-289 is an orally bioavailable compound and established its pharmacokinetic profile in both rat and primate. Indeed, oral administration of DPI-289 produced a



DPI-289 (mg/kg, p.o.)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-t} ($h \cdot ng \cdot mL^{-1}$)
3	33.8	2.3	76.4

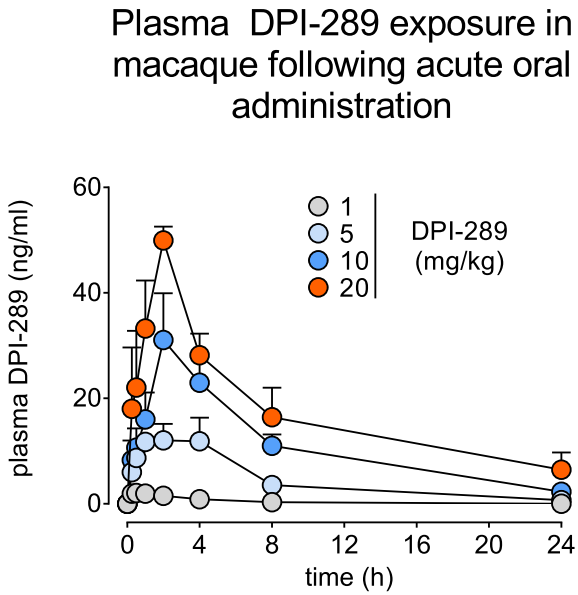
DPI-289 (1 mg/kg)	AUC_{0-t} ($h \cdot ng \cdot ml^{-1}$)	F (%)
i.v.	352	-
oral	48.3	13.7%
intraportal	46.9	13.3%

Fig. 3. Pharmacokinetic profile of DPI-289 and effects on forelimb asymmetry and AIMs in the 6-OHDA-lesioned rat.
Three groups of 6-OHDA-lesioned rats received once-daily treatment for 15 days with either vehicle, L-DOPA (6 mg/kg in combination with benserazide, 15 mg/kg, i.p.) or DPI-289 (3 mg/kg, p.o.) with associated vehicle controls. Extent of forelimb asymmetry (Panels A/B) and AIMs (Panels C/D) were assessed on Day 1 (A and C) and thereafter on Days 4, 8, 11 and 15 (B and D). Data are mean ± s.e.m (A/B) or median (bar) with interquartile range (box) and maximum and minimum (whiskers) (C/D). */**/*** represents $P < 0.05$, $P < 0.01$ or $P < 0.001$ cf. vehicle-vehicle values for same day of assessment. Pharmacokinetic profile of DPI-289 in rat following acute oral administration (3 mg/kg) is shown in Panel E. Oral bioavailability of DPI-289 is described in panel F.

robust anti-parkinsonian response in the 6-OHDA-lesioned rat model of PD following acute administration. This anti-parkinsonian benefit was maintained with repeated treatment. Importantly, DPI-289 elicited no AIMs (a corollary of dyskinesia) either acutely or with repeated treatment. Thus, comparing the actions of DPI-289 to those of L-DOPA revealed that while the extent of anti-parkinsonian benefit of L-DOPA was equivalent on Day 1 to that of DPI-289, because L-DOPA led to the development of AIMs, scoring of its anti-parkinsonian benefit became difficult to interpret after Day 1. Indeed, L-DOPA resulted in significant levels of AIMs by the fourth day and these AIMs increased with repeated treatment. The decreased quality of anti-parkinsonian response in L-DOPA-treated animals was evident not only as an increased incidence of AIMs but also as an increased variability in asymmetry evaluated in the cylinder test which resulted by a stark decrease in the number of animals able to perform in the cylinder test. Therefore, these data in the rat demonstrate the utility of repeated administration of DPI-289, as monotherapy, to deliver anti-parkinsonian actions, with almost no propensity to develop dyskinesia, compared to L-DOPA, when treatment is commenced *de novo*.

To inform our efforts to translate the anti-parkinsonian effect in rodents to NHP, we first determined the plasma exposures associated with the anti-parkinsonian efficacy observed in the 6-OHDA-lesioned rat. Oral administration of 3 mg/kg DPI-289 in rat was found to be associated with a peak plasma concentration, C_{max}, of ~34 ng/ml and a plasma exposure, AUC_{0-4h}, of 76.4 h ng/ml. In macaques, oral administration of DPI-289 (10 mg/kg) provided similar PK plasma characteristics (C_{max} of 36.5 ng/ml, AUC_{0-4h} of 87.6 h ng/ml) to that obtained in rats following administration of DPI-289 (3 mg/mg). However, the pharmacokinetic profiles in the rat and macaque were slightly different, with rats reaching T_{max} sooner than macaques and exhibiting higher plasma DPI-289 levels compared to macaques up to 1 h post-dose and lower levels from 2 to 4 h post-dose. The highest dose of DPI-289 employed in the NHP PK study, 20 mg/kg, was chosen for use in the subsequent primate experiments as it was found to deliver plasma levels of DPI-289 that exceeded levels seen in the rat experiment at all comparable time points (up to 4 h: Figs. 3E and 4). Higher doses/exposures could not be achieved with the current oral formulation. Further examination of the PK profile of DPI-289 in the macaque determined plasma T_{max} to occur, on average, in the 2–2.5 h range (Fig. 4). As L-DOPA T_{max} was previously determined to be approximately 1 h after administration (Huot et al., 2012), for NHP efficacy studies we opted to administered DPI-289 (or vehicle) 1 h prior to L-DOPA in order to provide optimal overlap of their respective plasma exposures.

The cynomolgus macaques employed in this study were rendered parkinsonian using protocols of cumulative doses of MPTP in line with those that we, and others, have previously employed. The extent of lesion produced by this regimen (Johnston et al., 2013) is comparable to that observed in advanced Parkinson's



DPI-289 (mg/kg, p.o.)	C_{max} (ng/ml)	t_{max} (h)	AUC_{0-t} ($h \cdot ng \cdot mL^{-1}$)	AUC_{0-4h} ($h \cdot ng \cdot mL^{-1}$)
1	2.4	1.1	8.2	5.8
5	17.4	2.3	108.4	43.5
10	36.5	2.7	261.8	87.6
20	49.9	2.0	413.0	140.8

Fig. 4. Plasma exposure levels of DPI-289 following oral administration in the MPTP-lesioned macaque.
Three MPTP-lesioned female macaques each received DPI-289 (1, 5, 10 and 20 mg/kg) formulated in acidified 5% DMSO/95% methylcellulose (v/v) and plasma samples prepared at times up to 24 h following administration. Key pharmacokinetic data are also shown. Data are mean ± s.e.m.

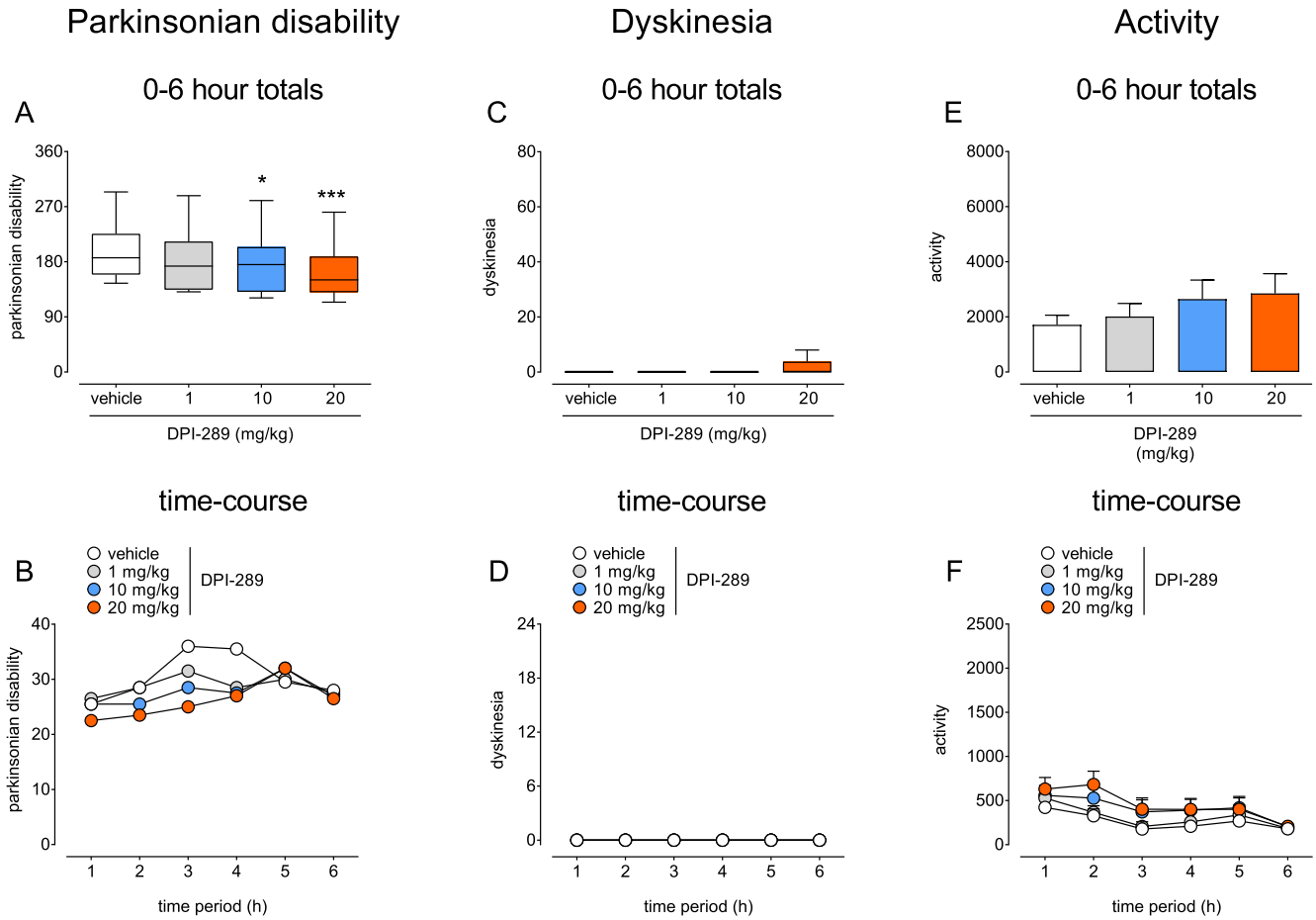
patients and typical of MPTP-lesioned animals with robust parkinsonism. The doses of L-DOPA employed as part of the current study provided maximal anti-parkinsonian benefit, typically with a duration of ~3 h but this was compromised by disabling dyskinesia (greater than moderate levels). Indeed, the duration of efficacy was mirrored by the duration of dyskinesia. Although those doses of L-DOPA administered in clinical settings are generally lower, on a mg/kg basis than those administered to the MPTP-lesioned macaque even corrected for human equivalent dosing, we have shown that they deliver equivalent plasma pharmacokinetic profiles to those achieved with clinically relevant L-DOPA doses as given to PD patients (Dizdar et al., 1999; Huot et al., 2012).

In the MPTP-lesioned primate with established LID, DPI-289 provided modest anti-parkinsonian benefits as monotherapy but without causing dyskinesia. This contrasts with previous observations of the use of the pure delta agonist, SNC80, in a similar cohort of MPTP-lesioned macaques with established LID. Thus, SNC80 exhibited anti-parkinsonian benefit similar to DPI-289 in the current study but, in stark contrast, elicited disabling levels of dyskinesia (Johnston et al., 2004). The data presented above suggests that DPI-289 as monotherapy may have a role in treating PD patients but it is likely to have greater utility in combination therapy to prolong "on-time" and reduce dyskinesia in an L-DOPA sparing strategy. Of clearer translational significance were the findings that the combination of DPI-289 and low dose L-DOPA enhanced the anti-parkinsonian benefits of low dose L-DOPA without exacerbating dyskinesia. The extension of duration of benefit was most remarkable with low dose L-DOPA providing anti-parkinsonian benefits only within the first 2 h after administration whereas the combination therapy provided benefits for at least 6 h. While some dyskinesia was associated with low dose L-DOPA, that dyskinesia was only mild and never reached disabling levels. The addition of the DAMA compound did not increase the level of dyskinesia over monotherapy with low dose L-DOPA. The clinical potential of these benefits likely lies in its application as an L-DOPA-sparing strategy, either to be given in early stage PD to slow the development of LID or in advanced PD, with existing complications, to allow down-titration of L-DOPA dose, without loss of anti-parkinsonian efficacy. The mechanism underlying the enhancement of L-DOPA actions by DPI-289 was not addressed in the study but may simply involve synergy, at the cellular and/or network level, between an action of dopamine, synthesised from L-DOPA, on D2 dopamine receptors on cell bodies of the indirect pathway, in the striatum, with an action on delta receptors, on terminals of the same neurons in the globus pallidus, as described above. As with the monotherapy, we were unable to probe the full dose-response relationship due to limitations of the oral formulation, so we cannot draw conclusions as to whether higher doses of DPI-289 might have conferred even greater efficacy. The combination effects we report are robust and likely to reflect an effect size that would translate into clinical benefit. While the total benefit, over 6 h, of the low dose L-DOPA/DPI-289 combination was equivalent to that of the high dose L-DOPA, we note that the peak anti-parkinsonian action of the combination was never as great as that of high dose L-DOPA alone. It is possible that with greater systemic exposure of DPI-289, an equivalent peak may be possible. This, in addition to a longer duration of effect without induction of dyskinesia, would further increase the attractiveness of DPI-289 for development of an L-DOPA-sparing strategy. We feel it is unlikely that the enhancement of L-DOPA effects observed here are due to a reduction, by DPI-289, in L-DOPA metabolism since while such an interaction would increase anti-parkinsonian benefit, it would also exacerbate LID and this was not observed. Nevertheless, future studies that assessed the impact of DPI-289 on L-DOPA pharmacokinetics should be undertaken to rule out this possibility.

Though the systemic exposures of DPI-289 in rat and NHP were equivalent, these animal models are quite different. There may be differences in the opioid system between the two species, whether in terms of receptor number, location and the affinity for and potency of DPI-289 at each receptor site. In addition, the types of dopaminergic lesion and the behavioural measures employed were also different.

Given the pharmacology of DPI-289, we propose that the anti-parkinsonian benefits of DPI-289, alone or in combination with L-DOPA, are likely to emerge from its delta opioid agonist activity. Such actions have previously been described for selective delta agonists in haloperidol and reserpine-treated rats and the MPTP-lesioned NHP (Hille et al., 2001). This effect could arise from a reduction of GABAergic transmission in the "indirect" striatal output pathway (Dewar et al., 1987). The lack of propensity for DPI-289-treated rats to develop/express AIMs following chronic treatment may reflect an inherent benefit of delta agonists, over L-DOPA, perhaps due to selective targeting of the "indirect", over "direct" pathway. That L-DOPA exerts anti-parkinsonian actions by providing stimulation of dopamine receptors on both the direct and indirect pathways has been suggested as critical for the development of dyskinesia (Picconi et al., 2003). Alternatively, the mu antagonist activity of DPI-289 may suppress the development or expression of dyskinesia should such arise from repeated delta opioid receptor agonist treatment. Indeed, mu antagonists do attenuate the expression of dyskinesia in the MPTP-lesioned NHP (Koprich et al., 2011).

Experiments to better define the mechanism of action of DPI-



DPI-289	vehicle cf. (h) 1	2	3	4	5	6
1 mg/kg	ns	ns	ns	*	ns	ns
10 mg/kg	ns	*	*	*	ns	ns
20 mg/kg	*	***	***	**	ns	ns

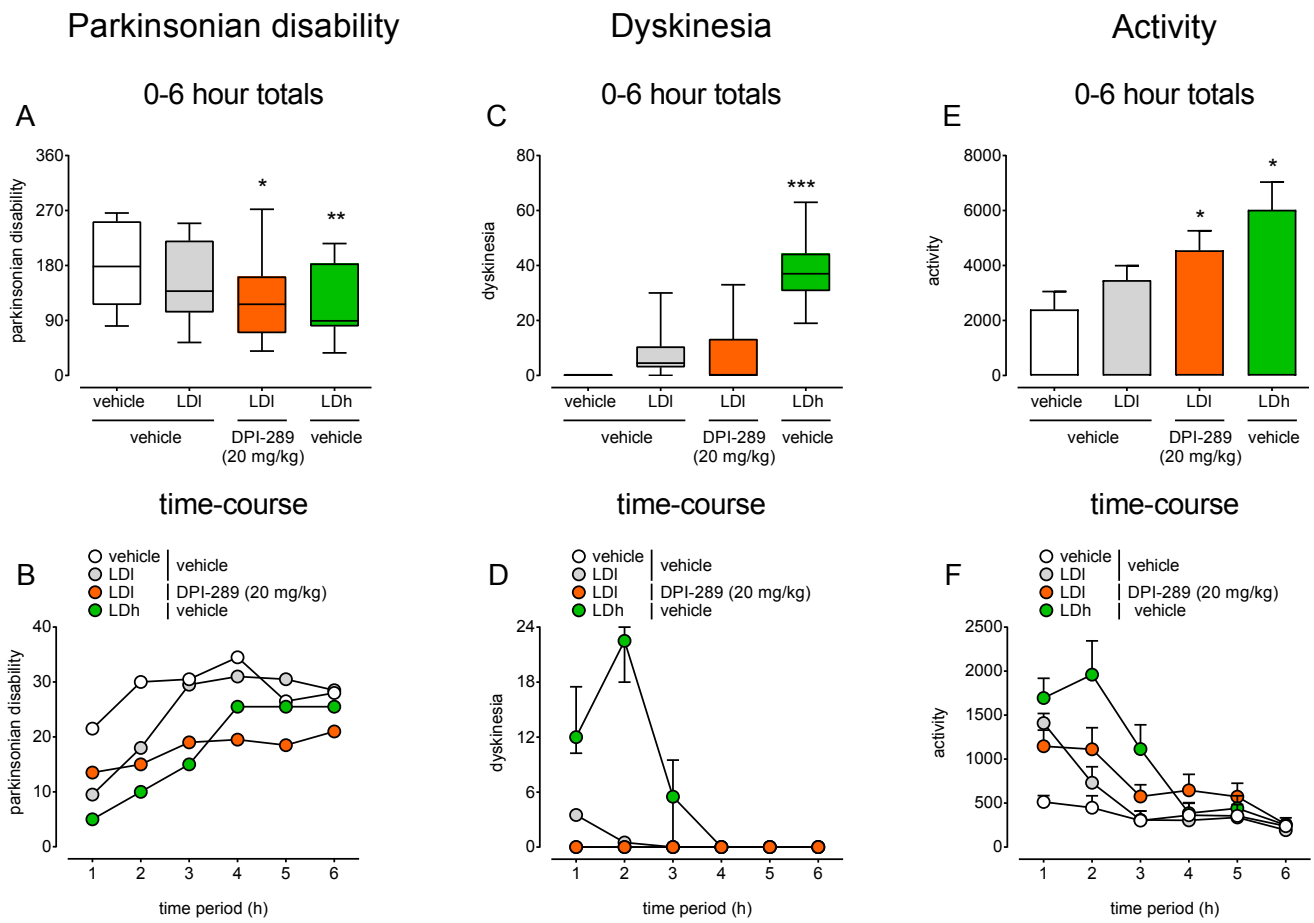
DPI-289	vehicle cf. (h) 1	2	3	4	5	6
1 mg/kg	ns	ns	ns	ns	ns	ns
10 mg/kg	ns	ns	ns	ns	ns	ns
20 mg/kg	ns	ns	ns	ns	ns	ns

DPI-289	vehicle cf. (h) 1	2	3	4	5	6
1 mg/kg	ns	ns	ns	ns	ns	ns
10 mg/kg	ns	*	*	*	ns	ns
20 mg/kg	*	***	**	*	ns	ns

Fig. 5. Effect of acute DPI-289 treatment on time-course of parkinsonian disability, dyskinesia and activity in L-DOPA-naïve MPTP-lesioned primates.
MPTP-lesioned cynomolgus monkeys received acute oral administration of either vehicle or DPI-289 (1, 10 or 20 mg/kg). Commencing 1 h later, levels of parkinsonian disability, dyskinesia and activity were assessed over a 6 h period and cumulated in either 1 h epochs (A, C, E respectively) or cumulated across the whole observation period (B, D, F respectively). Data are median (bar) with interquartile range (box) and maximum and minimum (whiskers) (A and C), median and interquartile range (B and D) or mean ± s.e.m. (E–F). N = 8 for all treatment groups. */**/*** represents $P < 0.05$, $P < 0.01$ or $P < 0.001$ cf. vehicle-treatment. Friedman test with Dunn's test (A, C), 1-way ANOVA (E) or 2-way RM ANOVA (B, D and F) with Holm-Sidak's test.

289 were not included in the current report as the focus was to highlight the clinical potential of the compound, irrespective of mechanism. Regardless, such studies would be of interest, and important in developing follow-up compounds. That said, the use of the relevant opposing pharmacological agents (delta antagonist/mu agonist) to selectively block each of the purported mediators of DPI-289, when given in combination with DPI-289 would not necessarily be straightforward or give a definitive answer. Thus, since the opioidergic system has well-described endogenous tone, the use of antagonists, for example, to explore a purported delta-agonist component will potentially result in changes in behaviour independent of any blockade of DPI-289 action. For example, it has previously been shown that the selective delta antagonist, naltrindole, can reduce L-DOPA induced dyskinesia in the MPTP-lesioned macaque ((Henry et al., 2001). Therefore, while one could confirm, if blocked by naltrindole, that the enhanced anti-parkinsonian actions of L-DOPA observed in combination with DPI-289 are delta-mediated, the effects or lack thereof, of DPI-289 on dyskinesia evoked by L-DOPA would be difficult to interpret. Functional blockade of the mu-antagonist properties of DPI-289 using a mu-agonist such as fentanyl suffers from practical considerations of using drugs with overt behavioural properties of their own, particularly sedation.

All the rodent and NHP studies noted in this report employed oral administration of DPI-289. However, we showed that the oral bioavailability of DPI-289 in the rat is low at ~13%. This was also the case when the drug was introduced into the hepatic portal vessel implying that low systemic exposure was due to a first pass hepatic metabolism effect. In the MPTP-lesioned macaque experiments reported here, oral administration of DPI-289 was associated with bioavailability of only 1–2%. It is not clear why there should be this species difference but such poor oral bioavailability usually precludes clinical development without resorting to a mode of delivery that would bypass the first-pass effect. The fact that statistically significant efficacy was noted despite such poor oral bioavailability suggests a robust effect. This also implies that our dosing regimen did not fully explore the dose response range. Therefore, it is possible that with higher systemic exposure, greater efficacy might



		vehicle-vehicle cf. (h)					
L-DOPA	DPI-289	1	2	3	4	5	6
LDl	vehicle	**	**	ns	ns	ns	ns
LDl	20 mg/kg	*	*	ns	***	*	**
LDh	vehicle	***	***	**	ns	ns	ns

		vehicle-vehicle cf. (h)					
L-DOPA	DPI-289	1	2	3	4	5	6
LDl	vehicle	***	**	ns	ns	ns	ns
LDl	20 mg/kg	***	**	ns	ns	ns	ns
LDh	vehicle	***	***	***	ns	ns	ns

		vehicle-vehicle cf. (h)					
L-DOPA	DPI-289	1	2	3	4	5	6
LDl	vehicle	***	ns	ns	ns	ns	ns
LDl	20 mg/kg	***	***	ns	*	ns	ns
LDh	vehicle	***	***	***	*	ns	ns

Fig. 6. Effect of acute DPI-289 treatment in combination with L-DOPA on parkinsonian disability, dyskinesia and activity in MPTP-lesioned primates with established motor complications.

MPTP-lesioned cynomolgus monkeys received acute oral administration of either vehicle/vehicle, vehicle/LDl, DPI-289/LDl, or vehicle/LDh. Commencing 1 h later, levels of parkinsonian disability, dyskinesia and activity were assessed over a 6 h period and cumulated in either 1 h epochs (A, C, E respectively) or cumulated across the whole observation period (B, D, F respectively). Data are median (bar) with interquartile range (box) and maximum and minimum (whiskers) (A and C), median and interquartile range (B and D) or mean ± s.e.m. (E–F). N = 8 for all treatment groups. */**/*** represents $P < 0.05$, $P < 0.01$ or $P < 0.001$ cf. vehicle-treatment. Friedman test with Dunn's test (A, C), 1-way ANOVA (E) or 2-way RM ANOVA (B, D and F) with Holm-Sidak's test.

be obtained. That noted, the present data does suggest that lower exposures are needed to be effective for combination therapy with L-DOPA in contrast to DPI-289 monotherapy.

In the current study, limitations of the oral formulation precluded employing higher doses of DPI-289 in NHP, and thus for now we cannot draw any firm conclusions as to the magnitude of anti-parkinsonian benefits that might be accrued from DPI-289 monotherapy, nor the exposure levels that should be targeted in clinical development.

The current study was designed to assess the L-DOPA sparing properties of DPI-289. Future studies will be required to evaluate the ability of DPI-289 to reduce disabling LID (marked or severe) elicited by high-dose L-DOPA. Additional studies will also include in-vivo positron-emission tomography (PET) imaging to validate the binding of DPI-289 to both delta and mu opioid receptors. The study will be performed under conditions resembling, as closely as possible, those used for the current efficacy studies reported. This will be necessary to confirm that DPI-289's beneficial anti-parkinsonian and anti-dyskinetic effects are brought on by the purported DAMA mechanism of action.

In conclusion, the current studies demonstrate that DPI-289, in combination with L-DOPA, provides improvement in overall activity without increasing dyskinesia. In terms of benefit/risk, this improvement was superior to high dose L-DOPA alone thus suggesting an L-DOPA-sparing strategy for clinical development.

Contributions

THJ, EV, PAH, MPH, RC and JMB designed and managed the studies. THJ performed the rat and primate studies and wrote the manuscript. PR and SHF analysed data from the primate behavioural studies. All authors helped revise and edit the manuscript.

Competing financial interests

THJ, PAH, PR, SHF, MPH, BER and JMB declare no competing financial interests.

EV and RC own shares of Dina Pharmaceuticals Inc.

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References

Brotchie, J., Fitzer-Attas, C., 2009. Mechanisms compensating for dopamine loss in early Parkinson disease. Neurology 72, S32–S38.
Cenci, M.A., Lundblad, M., 2007. Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice. Curr. Protoc. Neurosci. Chapter 9, Unit. 9 25.
Dewar, D., Jenner, P., Marsden, C.D., 1987. Effects of opioid agonist drugs on the in vitro release of 3H-GABA, 3H-dopamine and 3H-5HT from slices of rat globus pallidus. Biochem. Pharmacol. 36, 1738–1741.
Dizdar, N., Kullman, A., Norlander, B., Olsson, J.E., Kagedal, B., 1999. Human pharmacokinetics of L-3,4-dihydroxyphenylalanine studied with microdialysis. Clin. Chem. 45, 1813–1820.
Gerfen, C.R., McGinty, J.F., Young 3rd, W.S., 1991. Dopamine differentially regulates dynorphin, substance P, and enkephalin expression in striatal neurons: in situ hybridization histochemical analysis. J. Neurosci. 11, 1016–1031.
Henry, B., Fox, S.H., Crossman, A.R., Brotchie, J.M., 2001. Mu- and delta-opioid receptor antagonists reduce levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Exp. Neurol. 171, 139–146.
Hille, C.J., Fox, S.H., Maneuf, Y.P., Crossman, A.R., Brotchie, J.M., 2001. Antiparkinsonian action of a delta opioid agonist in rodent and primate models of Parkinson's disease. Exp. Neurol. 172, 189–198.
Huot, P., Johnston, T.H., Koprich, J.B., Fox, S.H., Brotchie, J.M., 2012. L-DOPA pharmacokinetics in the MPTP-lesioned macaque model of Parkinson's disease. Neuropharmacology 63, 829–836.
Johnston, T.H., Fox, S.H., Gomez-Ramirez, J., Lee, J., Brotchie, J.M., 2004. Antiparkinsonian effects of delta opioid receptor stimulation are accompanied by dystonia in MPTP-lesioned non-human primates previously treated with L-DOPA. In: 8th International Congress of Parkinson's Disease and Movement Disorders. Movement Disorder Society, Rome, Italy, p. P1166.
Johnston, T.H., Huot, P., Fox, S.H., Koprich, J.B., Szeliga, K.T., James, J.W., Graef, J.D., Letchworth, S.R., Jordan, K.G., Hill, M.P., Brotchie, J.M., 2013. TC-8831, a nicotinic acetylcholine receptor agonist, reduces L-DOPA-induced dyskinesia in the MPTP macaque. Neuropharmacology 73, 337–347.
Koprich, J.B., Fox, S.H., Johnston, T.H., Goodman, A., Le Bourdonnec, B., Dolle, R.E., DeHaven, R.N., DeHaven-Hudkins, D.L., Little, P.J., Brotchie, J.M., 2011. The selective mu-opioid receptor antagonist ADL5510 reduces levodopa-induced dyskinesia without affecting antiparkinsonian action in MPTP-lesioned macaque model of Parkinson's disease. Mov. Disord. 26, 1225–1233.
Kumar, N., Van Gerpen, J.A., Bower, J.H., Ahlskog, J.E., 2005. Levodopa-dyskinesia incidence by age of Parkinson's disease onset. Mov. Disord. 20, 342–344.
Lord, J.A., Waterfield, A.A., Hughes, J., Kosterlitz, H.W., 1977. Endogenous opioid peptides: multiple agonists and receptors. Nature 267, 495–499.
Manson, A., Stirpe, P., Schrag, A., 2012. Levodopa-induced-dyskinesias clinical features, incidence, risk factors, management and impact on quality of life. J. Park. Dis. 2, 189–198.
National Research Council Institute for Laboratory Animal, 1996. Guide for the Care and Use of Laboratory Animals. National Academies Press (US). Copyright 1996 by the National Academy of Sciences. All rights reserved., Washington (DC).
Negus, S.S., Gatch, M.B., Mello, N.K., Zhang, X., Rice, K., 1998. Behavioral effects of the delta-selective opioid agonist SNC80 and related compounds in rhesus monkeys. J. Pharmacol. Exp. Ther. 286, 362–375.
Paxinos, G., Watson, C.R., 1986. The rat brain in stereotaxic coordinates. Academic Press.
Picconi, B., Centonze, D., Hakansson, K., Bernardi, G., Greengard, P., Fisone, G., Cenci, M.A., Calabresi, P., 2003. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat. Neurosci. 6, 501–506.
Poewe, W., Mahlknecht, P., 2009. The clinical progression of Parkinson's disease. Park. Relat. Disord. 15 (Suppl. 4), S28–S32.
Schallert, T., Fleming, S.M., Leasure, J.L., Tillerson, J.L., Bland, S.T., 2000. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. Neuropharmacology 39, 777–787.
Ward, A., Takemori, A.E., 1976. Studies on the narcotic receptor in the Guinea-pig ileum. J. Pharmacol. Exp. Ther. 199, 117–123.