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RESEARCH ARTICLE

Effect of the selective 5-HT_{2A} receptor antagonist EMD-281,014 on L-DOPA-induced abnormal involuntary movements in the 6-OHDA-lesioned rat

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Abstract

L-3,4-Dihydroxyphenylalanine (L-DOPA) is the most effective therapy for motor symptoms of Parkinson's disease (PD); however, with repeated administration, as many as 94% of PD patients develop complications such as L-DOPA-induced dyskinesia. We previously demonstrated that EMD-281,014, a highly selective serotonin 2A (5-HT_{2A}) receptor antagonist, reduces the severity of dyskinesia in the parkinsonian marmoset, without interfering with L-DOPA anti-parkinsonian benefit. Here, we assessed the effects of EMD-281,014 on L-DOPA-induced abnormal involuntary movements (AIMs) in the 6-hydroxydopamine (6-OHDA)-lesioned rat. We first determined the pharmacokinetic profile of EMD-281,014, to administer doses leading to clinically relevant plasma levels in the behavioural experiments. Dyskinetic 6-OHDA-lesioned rats were then administered EMD-281,014 (0.01, 0.03 and 0.1 mg/kg) or vehicle in combination with L-DOPA and AIMs severity was evaluated. We also assessed the effect of EMD-281,014 on L-DOPA anti-parkinsonian action with the cylinder test. We found that the addition of EMD-281,014 (0.01, 0.03 and 0.1 mg/kg) to L-DOPA did not reduce AIMs severity ($P > 0.05$), when compared to vehicle. EMD-281,014 did not compromise L-DOPA anti-parkinsonian action. Our results suggest that the highly selective 5-HT_{2A} receptor antagonist EMD-281,014 is well-tolerated by parkinsonian rats, but does not attenuate L-DOPA-induced AIMs. Our results highlight differences between rodent and primate models of PD when it comes to determining the anti-dyskinetic action of 5-HT_{2A} receptor antagonists.

Keywords Parkinson's disease · L-DOPA · Dyskinesia · Pharmacokinetics · EMD-281,014 · 5-HT_{2A} receptor

Introduction

L-3,4-Dihydroxyphenylalanine (L-DOPA) is the most effective symptomatic treatment for Parkinson's disease (PD) (Connolly and Lang 2014). However, with long-term

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administration and disease progression, severe complications such as dyskinesia develop (Rascol et al. 2000), which causes significant morbidity to as many as 94% of patients treated with L-DOPA for 15 years (Hely et al. 2005). Many factors contribute to the development and progression of dyskinesia, notably administration of high doses of L-DOPA and the severity of nigrostriatal degeneration (Jenner 2008; Cilia et al. 2014; PD Med Collaborative Group 2014).

Several studies have investigated the involvement of the serotonergic system in dyskinesia, and suggested that serotonergic raphe-striatal neurons play an important role in the emergence and maintenance of dyskinesia. Indeed, serotonergic terminals contain enzymes required to convert L-DOPA into dopamine (Arai et al. 1994, 1995). However, the lack of retroactive control results in a non-physiological and pulsatile dopamine release in the striatum, which is considered core to the pathophysiology of dyskinesia (Carta et al. 2007; Navailles et al. 2010).

Regarding the role of the serotonergic system in the aetiology of dyskinesia, several pre-clinical and clinical studies assessed the effect of modulating serotonin (5-HT) transmission as a way to reduce dyskinesia, mostly through activation of 5-HT_{1A} (Iravani et al. 2006; Goetz et al. 2008; Bishop et al. 2009) and blockade of 5-HT_{2A} receptors (Meco et al. 1988; Vanover et al. 2008). We have recently demonstrated that the 5-HT_{2A} receptor antagonist 7-[[4-[2-(4-fluorophenyl)ethyl]-1-piperazinyl]carbonyl]-1*H*-indole-3-carbonitrile (EMD-281,014, also referred to as pruvanserin or LY-2,422,347) reduces the severity of dyskinesia in the marmoset model of PD (Hamadjida et al. 2018). EMD-281,014 is a highly selective 5-HT_{2A} receptor neutral antagonist (half-maximal inhibitory concentration [IC_{50}] 0.35 nM) with ≈2000× greater affinity for 5-HT_{2A} receptors over other targets (Bartoszyk et al. 2003) and is therefore ideal to assess the effect of selective 5-HT_{2A} receptor blockade on dyskinesia. EMD-281,014 has been tested in the clinic (Mamo et al. 2004) and therefore has tangible translational potential. Here, we determined the pharmacokinetic (PK) profile of EMD-281,014 in the rat, following which we assessed its effect on dyskinesia in the 6-hydroxydopamine (6-OHDA)-lesioned rat model of PD.

Materials and methods

Animals

Female Sprague–Dawley rats (250–275 g, Charles River, Canada) were group-housed under conditions of controlled temperature (21 ± 1 °C), humidity (55%) and light (12 h light/dark cycle, lights on at 07.00), with unrestricted access to food and water. Upon arrival, rats were left undisturbed for one week to acclimatise. All procedures were approved by the Montreal Neurological Institute Animal Care Committee in accordance with the regulations defined by the Canadian Council on Animal Care.

Pharmacokinetic study

Eight female rats were used for these studies. Following sub-cutaneous (s.c.) injection of EMD-281,014 (0.01, 0.03 and 0.1 mg/kg, from Cedarlane Laboratories, Canada), we collected blood samples (150 µL) at each of the following time points: baseline, 5 min, 10 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h and 8 h, as previously described (Gaudette et al. 2017, 2018; Hamadjida et al. 2018). All animals received all treatments once. Blood samples were collected by jugular vein puncture, transferred into K_3-EDTA-coated tubes, gently inverted and centrifuged at 1500×*g* for 10 min at 4 °C and stored at − 80 °C until analysis.

EMD-281,014 plasma levels were determined using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS), as previously described (Hamadjida et al. 2018). Plasma PK parameters were determined from the mean concentration value at each time point by a non-compartmental analysis method using PKSolver (Rowland and Tozer 1995; Zhang et al. 2010). Area under the curve (AUC) was calculated using the linear and log-linear trapezoidal rule. AUC_{0-t}, $AUC_{0-\infty}$, maximal plasma concentration (C_{max}), time to C_{max} (T_{max}), elimination half-life ($T_{1/2}$), clearance (CL), bioavailability (*F*) and volume of distribution (V_d) were all calculated.

Induction of hemi-parkinsonism

Rats were pre-treated with pargyline and desipramine (5 and 10 mg/kg s.c., respectively, both from MilliporeSigma, Canada), to prevent damage to noradrenergic neurons (Ungerstedt 1968). Thirty minutes later, rats were anaesthetised using isoflurane (2–4%; MilliporeSigma, Canada) in 100% oxygen (1 L/min) and placed into a stereotaxic frame (David Kopf Instruments, USA) with the incisor bar set 3.3 mm below ear bars (Huot et al. 2015). Rats were then injected with 2.5 µL of 6-OHDA hydrobromide (7 µg/µL in 0.02% ascorbic acid dissolved in 0.9% NaCl; MilliporeSigma, Canada) in the right medial forebrain bundle (MFB) at the following coordinates, according to the Paxinos and Watson's rat brain Atlas (Paxinos and Watson 2017): (antero-posterior: − 2.8, medio-lateral: − 2.0, dorso-ventral: − 9.0) relative to Bregma. 6-OHDA was injected at a rate of 0.5 µL/min and the syringe was left in place for 5 min after injection before being drawn back. At the end of the surgery, rats received s.c. injections of carprofen (10 mg/kg) and 0.9% NaCl (10 mL), to minimise post-surgical pain and avoid dehydration.

Assessment of parkinsonism and induction of dyskinesia

Three weeks after surgery, the degree of parkinsonism was assessed using the cylinder test, in which use of the forelimb ipsilateral to the lesion in ≥ 70% of the rears is indicative of ≥ 88% striatal dopamine depletion, as described before (Schallert et al. 2000). The cylinder test was performed in a transparent cylinder (14 cm diameter × 28 cm height) for 10 min, during which animals were recorded for post hoc behavioural analysis. Briefly, during a rear, the first limb to contact the wall was scored as an independent wall placement for that limb. If a subsequent placement of the other limb on the wall occurred while the initial placement was maintained, a score of "bilateral" was attributed. A simultaneous placement of both forepaws on the walls was also scored as a "bilateral" movement. Only animals exhibiting preferential use of the un-lesioned forepaw in ≥ 70% of the rears were selected to undergo dyskinesia induction.

Following the cylinder test, parkinsonian rats were primed to elicit abnormal involuntary movements (AIMs) with once daily injections of L-DOPA/benserazide (10/15 mg/kg s.c., MilliporeSigma, Canada) for 14 days.

AIMs assessment

On experimental days, AIMs were scored at the beginning of behavioural experiments and every 20 min thereafter, starting after animals had received their respective treatments and had been placed in glass cylinders. Axial, limbs and oro-lingual (ALO) AIMs were rated by an observer blinded to treatment, according to a protocol described by Cenci and Lundblad (2007), which encompasses both time-based, i.e., "duration" and severity-based, i.e. "amplitude", assessment of abnormal movements. ALO AIMs were scored for 2 min, every 20 min for 180 min. ALO AIMs duration 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. Axial AIMs amplitude was rated according to the following scale: 1 = sustained deviation of the head and neck at ≈ 30° angle; 2 = sustained deviation of the head and neck at an angle between 30° and 60°; 3 = sustained twisting of the head, neck and upper trunk at an angle between 60° and 90° and 4 = sustained twisting of the head, neck and trunk at an angle ≥ 90°, causing the rat to lose balance from a bipedal position. Limbs AIMs amplitude was rated according to the following scale: 1 = tiny movements of the paw around a fixed position; 2 = movements leading to a visible displacement of the whole limb; 3 = large displacement of the whole limb with visible contraction of shoulder muscles and 4 = vigorous limb displacement of maximal amplitude, with concomitant contraction of shoulder and extensor muscles. Oro-lingual AIMs amplitude was rated according to the following scale: 1 = twitching of facial muscles accompanied by small masticatory movements without jaw opening; 2 = twitching of facial muscles accompanied by masticatory movements that result in jaw opening; 3 = movements with broad involvement of facial and masticatory muscles, with frequent jaw opening and occasional tongue protrusions and 4 = involvement of all of the above muscles to the maximal possible degree.

Integrated ALO AIMs was defined as the product of ALO AIMs amplitude × ALO AIMs duration, as previously described (Ohlin et al. 2011), while cumulative ALO AIMs indicates the sum of ALO AIMs duration or of ALO AIMs amplitude over different consecutive measurement time points.

Administration of EMD-281,014 in combination with L-DOPA

ALO AIMs assessment

On days of behavioural testing, rats ($N = 32$) were administered L-DOPA/benserazide (6/15 mg/kg s.c., from this point forward referred to as L-DOPA) in combination with EMD-281,014 (0.01, 0.03 and 0.1 mg/kg s.c) or vehicle (0.9% NaCl). They were then put in transparent glass cylinders where ALO AIMs were scored. Drug administration schedule was randomised according to a within-subject design and a minimum of 48 h was left between treatments.

Effect of EMD-281,014 on L-DOPA anti-parkinsonian action

Following a 3-day washout period, rats used in the dyskinesia study were administered acute challenges of a low dose of L-DOPA/benserazide (3/15 mg/kg s.c.), sufficiently high to produce an anti-parkinsonian effect but without triggering AIMs, in combination with vehicle or EMD-281,014 (0.01, 0.03 and 0.1 mg/kg s.c.), in a randomised within-subject design, after which they underwent the cylinder test, 45 min later, to determine the effect of EMD-281,014 on L-DOPA anti-parkinsonian action. At least 48 h separated each treatment.

Measurement of monoamines

At the end of the experiments, rats were euthanised by isoflurane anaesthesia (2–4%; MilliporeSigma, Canada), followed by trans-cardial perfusion of ice-cold 0.9% NaCl, after which brains were collected. Left and right striata were dissected, flash-frozen in 2-methyl-butane (− 56 °C) and stored

at − 80 °C until processing for determination of dopamine and homovanillic acid (HVA) by high-performance liquid chromatography (HPLC), as previously described (Huot et al. 2012b).

Briefly, striatum tissue was homogenised in 200–750 µL of 0.1 M trichloroacetic acid (TCA), which contained 10^{-2} M sodium acetate, 10^{-4} M EDTA and 10.5% methanol (pH 3.8), using a tissue dismembrator (Fisher Scientific, USA). Samples were centrifuged at 10,000×g_{max} for 20 min. The supernatant was removed and stored at − 80 °C. On the day of analysis, the supernatant was thawed and centrifuged at 10,000×g_{max} for 20 min. Supernatant samples were then analysed for dopamine and HVA. Analyte levels were determined by an HPLC assay using an Antec Decade II (oxidation: 0.5) electrochemical detector operated at 33 °C. 20 µL samples of supernatant were injected, using a Water 717+ autosampler, onto a Phenomenex Nucleosil C18 HPLC column (150 × 4.60 mm; 5 µm, 100 Å). Elution mobile phase consisted of 89.5% 100 mM trichloroacetic acid, 10 mM sodium acetate, 100 µM EDTA and 10.5% methanol (pH 3.8). The mobile phase was delivered at a flow rate of 0.8 mL/min using a Waters 515 HPLC pump. HPLC control and data acquisition were handled by Waters Empower software (Waters Corporation).

Statistical analysis

EMD-281,014 plasma PK parameters are presented as the mean ± standard deviation (SD). Dopamine and HVA levels are presented as mean ± standard error (SEM) and were analysed by unpaired Welch's unequal variances *t* test. Cylinder test data for animal selection are presented as the mean ± SEM and analysed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests. AIMs time course data are presented as the median and, following ranking of data in ascending order, were analysed by two-way repeated measures (RM) ANOVA followed by Bonferoni's post hoc test (Bland and Altman 1996; Howell 2006). Cumulative AIMs scores are presented as the median with semi-interquartile range and were analysed using Friedman test followed by Dunn's post hoc test. The effect of EMD-281,014 on L-DOPA anti-parkinsonian action is presented as the mean ± SEM and was analysed by one-way RM ANOVA followed by Tukey's post hoc test. Statistical significance was set to $P < 0.05$. Statistical analyses were computed using GraphPad Prism 7.0d (GraphPad Software Inc, USA).

Results

PK profile of EMD-281,014 in the rat

EMD-281,014 PK parameters in the rat following s.c. injection are summarised in Table 1. As displayed in Fig. 1, EMD-281,014 (0.01, 0.03 and 0.1 mg/kg) was detected in the plasma as early as 5 min after administration in healthy rats. T_{max} occurred 10 min following administration of EMD-281,014 (0.01 and 0.03 mg/kg) and after 5 min following administration of 0.1 mg/kg. C_{max} was dose-dependent and maximal plasma levels reached were 1.12 ng/mL, 5.70 ng/mL and 29.67 ng/mL, after administration of EMD-281,014 0.01, 0.03 and 0.1 mg/kg, respectively.

Table 1 Derived plasmatic PK parameters following s.c. administration of EMD-281,014 in the rat

Parameters	EMD-281,014		
	0.01 mg/kg	0.03 mg/kg	0.10 mg/kg
AUC_{0-t} (ng/mL h)	2.13	8.87	32.13
$AUC_{0-\infty}$ (ng/mL h)	2.40	9.09	32.42
C_{max} (ng/mL)	1.12	5.70	29.67
T_{max} (min)	10	10	5
$t_{1/2}$ (h)	1.95	1.72	1.22
CL/*F* (L/h)	4.17	3.30	3.08
V_d/F (L)	11.75	8.21	5.42
MRT (h)	1.55	1.92	1.47

AUC area under the curve, C_{max} maximal plasma concentration, T_{max} time to maximal plasma concentration, $t_{1/2}$ terminal half-life, *CL* clearance, *F* bioavailability, V_d volume of distribution, *MRT* mean residence time

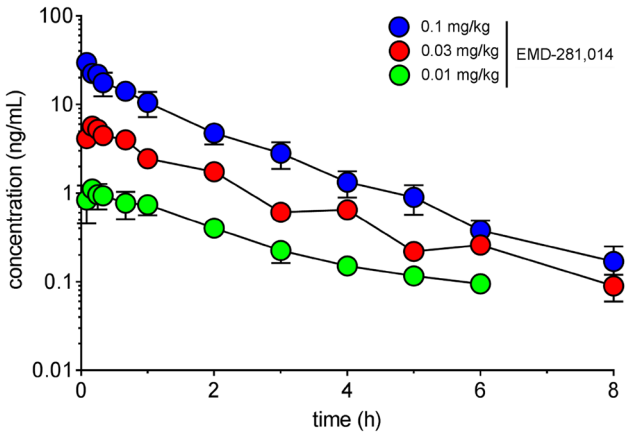

Fig. 1 Mean ± SD plasma concentrations of EMD-281,014 (0.01, 0.03 and 0.1 mg/kg) after s.c. administration in the rat ($N = 8$)

Extent of parkinsonism and dopaminergic denervation

As shown in Fig. 2a, 6-OHDA-lesioned rats displayed marked forelimb asymmetry [$F_{(2,39)} = 125$, $P < 0.0001$; one-way ANOVA] with preferential use of the right (un-lesioned) forepaw in 76.9 ± 4.4% of wall contacts when compared to

0.7% with the left (lesioned) forepaw and 22% with both forepaws, respectively, assessed by the cylinder test.

Accordingly, HPLC analysis (Fig. 2b) revealed significant reductions of dopamine and its metabolite HVA in the lesioned striata, when compared to the un-lesioned striata, by 95.7% [$t_{(13.12)} = 7.427$, $P < 0.0001$] and 86.2% [$t_{(17.71)} = 9.095$, $P < 0.0001$], respectively.

EMD-281,014 does not reduce ALO AIMs duration or amplitude

As illustrated in Fig. 3a, adding EMD-281,014 to L-DOPA had no effect on ALO AIMs duration [$F_{\text{time}(9,1116)} = 0$, $P > 0.05$; $F_{\text{treatment}(3,124)} = 1.011$, $P > 0.05$; $F_{\text{interaction}(27,1116)} = 0.8903$, $P > 0.05$; two-way ANOVA] or ALO AIMs amplitude [$F_{\text{time}(9,1116)} = 0$, $P > 0.05$; $F_{\text{treatment}(3,124)} = 1.481$, $P > 0.05$; $F_{\text{interaction}(27,1116)} = 0.9948$, $P > 0.05$; two-way ANOVA; Fig. 3b] throughout the behavioural sessions.

Furthermore, combining EMD-281,014 and L-DOPA did not reduce cumulative ALO AIMs duration [Friedman statistic (FS) = 1.382, $P > 0.05$; Fig. 4a], cumulative ALO AIMs amplitude (FS = 3.849, $P > 0.05$; Fig. 4b) or integrated cumulative ALO AIMs (FS = 0.7642, $P > 0.05$; Fig. 4c), when compared to vehicle. We also performed analyses of each of axial, limbs and oro-lingual components separately, but were equally unable to find AIMs reductions (data not shown).

EMD-281,014 does not affect L-DOPA anti-parkinsonian action

As shown in Fig. 5, L-DOPA, whether combined with vehicle or EMD-281.014, attenuated the severity of parkinsonism [$F_{(3.133,40.73)} = 6.153$, $P < 0.01$; one-way RM ANOVA]. Thus, L-DOPA/vehicle reduced right forepaw use by ≈29% ($P < 0.01$; Tukey's post hoc test) when compared to L-DOPA-untreated 6-OHDA-lesioned animals. The addition of EMD-281,014 (0.01, 0.03 and 0.1 mg/kg) did not hinder the anti-parkinsonian action of L-DOPA, as right forepaw use was similar across all treatments when compared to L-DOPA/vehicle (all $P > 0.05$; Tukey's post hoc test), and remained lower than when animals were not administered L-DOPA.

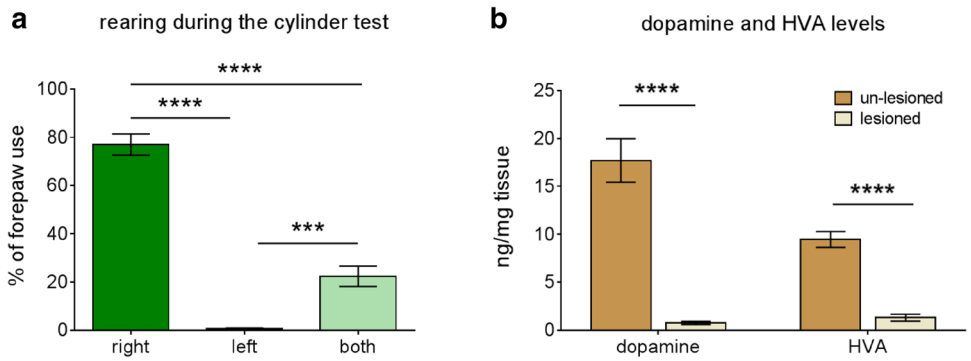

Fig. 2 Extent of dopaminergic denervation in 6-OHDA-lesioned rats. Rears using the right, left and both forepaws were assessed through the cylinder test (**a**). Dopamine and HVA levels (**b**) were quantified from the lesioned and un-lesioned hemispheres by HPLC ($N = 32$). In **a, b**, data are presented as the mean ± SEM (***$P < 0.001$, ****$P < 0.0001$)

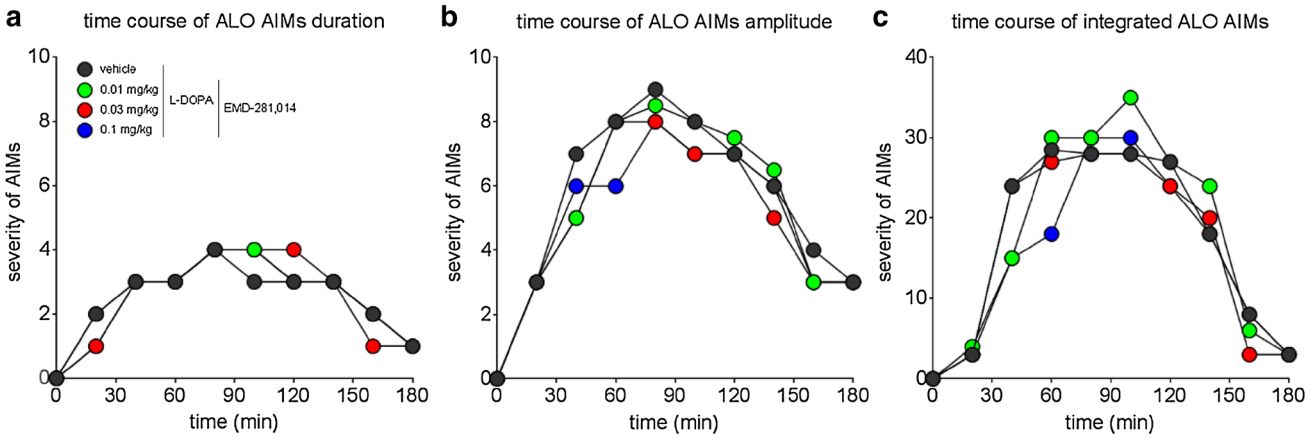

Fig. 3 Dyskinesia time course in 6-OHDA-lesioned rats ($N = 32$) treated with L-DOPA in combination with EMD-281,014 (0.01, 0.03 and 0.1 mg/kg) or vehicle. ALO AIMs duration (**a**), amplitude (**b**) and integrated (**c**) are presented. Data are expressed as the median

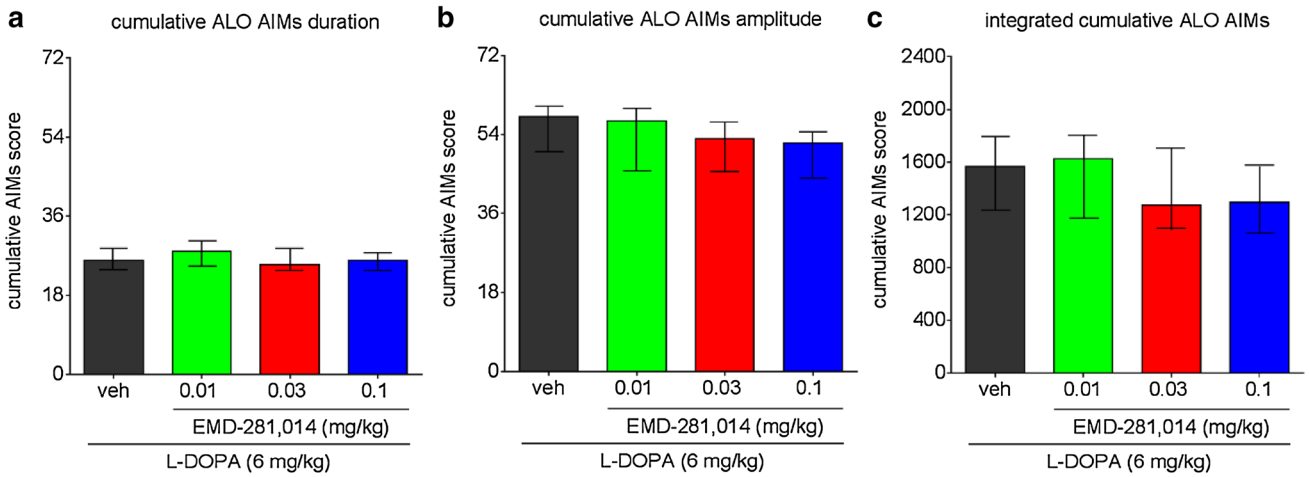

Fig. 4 Cumulative dyskinesia scores (over the 180 min experimental period) in 6-OHDA-lesioned rats ($N=32$) treated with L-DOPA in combination with EMD-281,014 (0.01, 0.03 and 0.1 mg/kg) or vehicle. Cumulative ALO AIMs duration (**a**), amplitude (**b**) and integrated (**c**) are presented. Dyskinesia scores are graphed as the median with semi-interquartile range

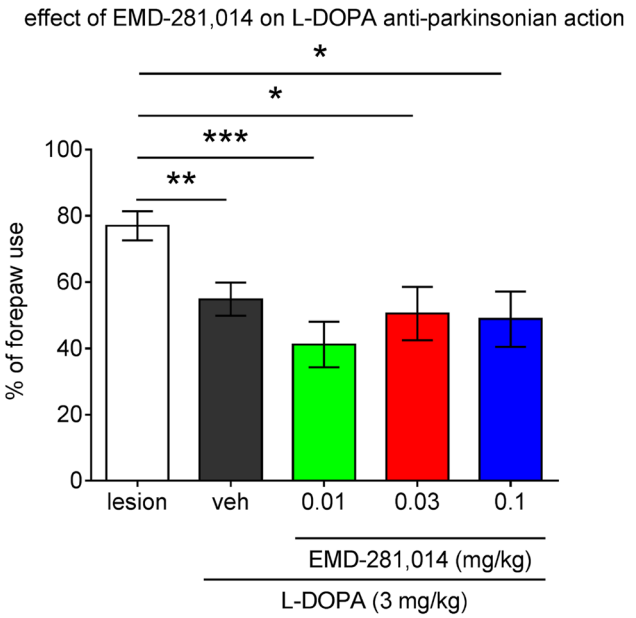

Fig. 5 Degree of parkinsonism in rats ($N=32$) after 6-OHDA lesion and administration of EMD-281,014 in combination with L-DOPA. Right forepaw use was assessed across treatments by the cylinder test. Data are presented as the mean ± SEM (*$P<0.05$, **$P<0.01$ and ***$P<0.001$)

Discussion

In this study, we first determined the PK profile of EMD-281,014 in the rat, and then administered doses leading to plasma levels comparable to those that were achieved in clinical settings (Mamo et al. 2004) to L-DOPA-treated hemi-parkinsonian rats, to determine whether highly selective 5-HT_{2A} receptor blockade would alleviate dyskinesia. To our surprise, and in contrast to results that we recently obtained with EMD-281,014 in the parkinsonian marmoset (Hamadjida et al. 2018), EMD-281,014 did not attenuate L-DOPA-induced dyskinesia in the rat model of PD.

If the current findings contrast with post-mortem (Riahi et al. 2011; Huot et al. 2012b) and pharmacological literature arguing in favour of an anti-dyskinetic effect following blockade of 5-HT_{2A} receptors (Vanover et al. 2008; Huot et al. 2011; Hamadjida et al. 2018), they are nevertheless in agreement with a previous study conducted in the 6-OHDA-lesioned rat with MDL-100,907 (also known as M-100,907 or volinanserin) (Taylor et al. 2006). MDL-100,907 is a highly selective 5-HT_{2A} antagonist, with ≈ 300-fold selectivity over 5-HT_{2C} receptors (Herth et al. 2009). In that previous study (Taylor et al. 2006), MDL-100,907 was ineffective against each individual component of ALO AIMs; however, it reduced rotations induced by stimulation of dopamine D_1, but not D_2 receptors, while being devoid of effects on L-DOPA-induced rotational behaviour. Here, we did not assess the effect of EMD-281,014 on L-DOPA-induced rotations and cannot rule out that it might, or not, have produced an effect on rotational behaviour, although this previous study with MDL-100,907 suggests that it might not have diminished the rotations. To the best of our knowledge, no study assessing the anti-dyskinetic effect of MDL-100,907 on dyskinesia in the non-human primate has been published and, as such, comparisons between rodent and primate cannot be made directly using this specific compound.

A possible explanation for the lack of efficacy at a dose of up to 0.1 mg/kg, here is that perhaps the doses that we administered were too low. However, the highest dose

administered here, 0.1 mg/kg, led to plasma levels close to 30 ng/mL, which is higher than these documented to be well-tolerated in the clinic, which are around 11 ng/mL (Mamo et al. 2004). Thus, if an anti-dyskinetic effect were achieved with higher doses, it would be of limited translational potential, as higher doses would lead to plasma levels unlikely to be tolerated by human subjects. Lastly, we performed the PK experiments in intact rats, while the behavioural studies were conducted in 6-OHDA-lesioned rats. Differences in metabolism of EMD-281,014 between intact and 6-OHDA-lesioned rats might exist and, were that the case, perhaps our choice of dose should have been slightly different.

Another explanation for this contrast in anti-dyskinetic effectiveness between rodent and primate might relate to difference in striatal anatomy and neuro-chemistry. For instance, there are dissimilarities between the neuronal organisation of the striatum between rodent and primate (Cicchetti et al. 2000). In addition, differences in the expression of 5-HT_{2A} receptors in parkinsonism between rat and primate might be at stake. Thus, in the adult rat, 6-OHDA lesion of the MFB led to increased levels of 5-HT_{2A} receptor messenger ribonucleic acid (mRNA) in the lesioned striatum (Numan et al. 1995; Zhang et al. 2007), while chronic treatment with L-DOPA prevented the up-regulation of striatal 5-HT_{2A} receptor mRNA (Zhang et al. 2007). In contrast to these findings, a study reported a reduction of 5-HT_{2A} receptor levels in the striatum following 6-OHDA lesion of the MFB in the adult L-DOPA-naïve rat (Li et al. 2010). The discrepancy may be due to the different doses of neurotoxin administered and the variable recovery period prior to assessment of 5-HT_{2A} receptor levels or mRNA. In contrast, in non-human primates, the levels of striatal 5-HT_{2A} receptor remained unchanged following administration of MPTP (Huot et al. 2012b). However, 5-HT_{2A} receptor levels were increased in the striatum of dyskinetic MPTP-lesioned primates.

Lastly, it is uncertain whether selective 5-HT_{2A} blockade would alleviate dyskinesia in clinical settings, as no drug harbouring selectivity for this target has been assessed with a dyskinesia-related end point. For instance, ritanserin, which effectively alleviated dyskinesia in clinical trials (Maertens de Noordhout and Delwaide 1986; Meco et al. 1988), harbours high affinity for dopamine D_2 receptors (Leysen et al. 1985). In addition, it cannot be ruled out that the anti-dyskinetic action of clozapine (Durif et al. 2004) is due to interaction(s) with receptor(s) other than 5-HT_{2A} receptors (Bymaster et al. 1996), despite preferential binding to this target at doses used in PD (Nordstrom et al. 1995).

In summary, in the current study, 5-HT_{2A} receptor blockade with the highly selective antagonist EMD-281,014 did not alleviate ALO AIMs, in the 6-OHDA-lesioned rat. EMD-281,014 is the second 5-HT_{2A} receptor antagonist that fails to alleviate dyskinesia in the hemi-parkinsonian rat. The translational implications of this finding are unclear because, as discussed above, there are post-mortem evidence suggesting that 5-HT_{2A}-mediated transmission may be over-active in dyskinesia and, while selective blockade of the 5-HT_{2A} receptor alleviated dyskinesia in the parkinsonian primate, it has yet to do so in the clinic.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest. PH has received speaker and travel fees from UCB.

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