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TC-8831, a nicotinic acetylcholine receptor agonist, reduces L-DOPA-induced dyskinesia in the MPTP macaque

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ABSTRACT

Long-term L-DOPA treatment for Parkinson's disease (PD) is limited by motor complications, particularly L-DOPA-induced dyskinesia (LID). A therapy with the ability to ameliorate LID without reducing anti-parkinsonian benefit would be of great value. We assessed the ability of TC-8831, an agonist at nicotinic acetylcholine receptors (nAChR) containing α6β2/α4β2 subunit combinations, to provide such benefits in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine- (MPTP) lesioned macaques with established LID.

Animals were treated orally for consecutive 14-day periods with twice-daily vehicle (weeks 1–2) or TC-8831 (0.03, 0.1 or 0.3 mg/kg, weeks 3–8). L-DOPA was also administered, once-daily, (weeks 1–12, median-dose 30 mg/kg, *p.o.*). For the following two-weeks (weeks 9–10), TC-8831 was washed out, while once-daily L-DOPA treatment was maintained. The effects of once-daily amantadine (3 mg/kg, *p.o.*) were then assessed over weeks 11–12. LID, parkinsonism, duration and quality of ON-time were assessed weekly by a neurologist blinded to treatment.

TC-8831 reduced the duration of 'bad' ON-time (ON-time with disabling dyskinesia) by up to 62% and decreased LID severity (median score 18 *cf.* 34 (vehicle), 0.1 mg/kg, 1–3 h period). TC-8831 also significantly reduced choreiform and dystonic dyskinesia (median scores 6 and 31 *cf.* 19 and 31 respectively (vehicle), both 0.03 mg/kg, 1–3 h). At no time did TC-8831 treatment result in a reduction in anti-parkinsonian benefit of L-DOPA. By comparison, amantadine also significantly reduced dyskinesia and decreased 'bad' ON-time (up to 61%) but at the expense of total ON-time (reduced by up to 23%).

TC-8831 displayed robust anti-dyskinetic actions and improved the quality of ON-time evoked by L-DOPA without any reduction in anti-parkinsonian benefit.

1. Introduction

Long-term dopamine replacement therapy in Parkinson's disease (PD) is typically accompanied by motor side-effects of treatment, including L-DOPA-induced dyskinesia (LID) (Fabbrini et al., 2007; Poewe, 2009). LID can be troublesome and impact significantly on quality of life in PD (Chapuis et al., 2005) and remain a significant unmet clinical need (Meissner et al., 2011). Current pharmacological strategies for reducing dyskinesia in the clinical setting include the adjunctive administration of amantadine, to suppress dyskinesia, or reduction of L-DOPA dose to reduce the expression of dyskinesia, although the latter strategy is compromised by a reduction in anti-parkinsonian benefits. However, the former approach may not be effective or suitable in many patients, while the latter reduces anti-parkinsonian benefit (Goetz et al., 2005; Pahwa et al., 2006).

The last 20-years have seen an emerging appreciation of the potential for non-dopaminergic adjunct therapies to address these

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issues (Buck and Ferger, 2010). Recently, the role of nicotinic acetylcholine receptors (nAChR) in mediating the side-effects of dopamine replacement therapy has gained much ground (Quik et al., 2008). Nicotine reduces established L-DOPA-induced motor complications in both rodent (Bordia et al., 2008; Huang et al., 2011b) and primate (Quik et al., 2007) PD models, as well as in humans (Inc., 2010). However, as a candidate for therapeutic development, nicotine is limited by gastrointestinal and cardiovascular side effects due to interaction with α3β4 nAChR in the peripheral autonomic ganglia (Holladay et al., 1997). As such, investigations have focused on specific nicotinic receptor assemblies that, because of anatomical and functional specificity, might mediate the beneficial actions of nicotine to reduce expression of established LID with limited side effect liability.

nAChRs are found on both dopaminergic and non-dopaminergic neurons in the striatum (Kaiser and Wonnacott, 2000; Salminen et al., 2004). nAChRs containing the β2 subunit are expressed on a majority of nigrostriatal neurons (Gotti et al., 2010), the most frequently encountered combination of subtypes may include α4β2* or α6β2* nAChRs (the asterisk denotes the possible presence of other subunits in the pentameric receptor complex) (Letchworth and Whiteaker, 2011; Quik et al., 2012). Examination of the dynamics of striatal dopamine release in knock-out animals show that nAChR assemblies containing the α4, α6 or β2 subunits are considered essential for normal physiological function of dopamine neurons (Huang et al., 2011b; Perez et al., 2010; Quik et al., 2012). In addition, mice that lack either the α6 or β2 subunit do not fully develop LID and do not demonstrate the anti-dyskinetic response to nicotine, described above for wild-type animals (Huang et al., 2011b; Quik et al., 2012). Whereas the expression of α6β2* nAChR is limited to dopaminergic terminals within the striatum, α4β2* nAChR are also found on non-dopaminergic neurons (Quik et al., 2005). Recently, it was shown that animals with severe dopaminergic lesions (with near-complete loss of α6β2* in the striatum) still exhibit an anti-dyskinetic effect in response to compounds with α4β2* and α6β2* activity (Quik et al., 2013a), indicating that α4β2* receptors also play a role in LID. These observations thus form the rationale for development of small molecule agonists with selectivity for α6β2* and α4β2* nAChRs (Quik and Wonnacott, 2011) Such findings also indicate utility for compounds targeting both subtypes for treating LID over varying degrees of dopaminergic loss during the course of PD. Of the compounds tested in the rodent study, only TC-8831 improved abnormal involuntary movements on all three endpoints (oral-lingual, axial and forelimb) and was chosen for further study.

In the current study, we assessed, for the first time in a primate species, the effects of a subtype-selective nAChR agonist on L-DOPA-induced motor complications. Specifically, we evaluated TC-8831, an α6β2*/α4β2* nAChR agonist, with less affinity for the α3β4 nAChR subtype (Quik et al., 2013a). TC-8831 was administered in combination with L-DOPA and evaluated for effects on dyskinesia and parkinsonism, as well as duration and quality of ON-time in the MPTP macaque model of PD. We also evaluated plasma exposure of TC-8831 throughout the study.

2. Methods

2.1. Animals

Seven female cynomolgus monkeys (*Macaca fascicularis)* (3.5 ± 0.3 kg, 8.2 ± 0.4 years, Suzhou Xishan-Zhongke Laboratory Animal Company, PRC) were housed two per-cage in caging (192 × 152 × 136 cm) exceeding Council of Europe, UK, NIH and CCAC minimum size recommendations. After completing the TC-8831 assessment portion of the experiment, one animal was excluded from further testing due to non-study related health concerns. Cages were equipped with a variety of environmental enrichment (including perch, fruit and toys) and subject to a 12-h light–dark cycle (lights on 7:00a.m.) and controlled temperature (22±3 °C), humidity (51% ± 1%) and light (12-h light–dark cycle, lights on at 7:00a.m.). Fruit, primate pellets and water were available *ad libitum* except on days when observation cage behaviour was assessed. On these days, food was withheld from 4:00p.m. the day before until the 6 h observation had commenced post treatment administration (9.30a.m.). 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).

2.2. 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 22.4 ± 10.4 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. Dose-finding observations were conducted (data not shown) to individually titrate L-DOPA dose (range 20–35 mg/kg, mean L-DOPA dose, 29±2 mg/kg) for each animal to allow optimal anti-parkinsonian benefit lasting up to 3 h, but which was compromised by disabling dyskinesia. The responses to these doses of L-DOPA were assessed to ensure stability and reproducibility within each animal on successive L-DOPA administrations. The extent of lesion was confirmed by positron emission tomography of striatal VMAT2 sites (see Supplementary materials).

2.3. Treatments

During the first eight weeks of the study, the effects of vehicle and three doses of TC-8831 (0.03, 0.1 and 0.3 mg/kg, *p.o.*) were assessed. Dose selection was based upon plasma exposures at efficacious doses from a previous rodent efficacy study (Quik et al., 2013a) and those below the No Observable Adverse Event Level (NOAEL) from a 14-day exploratory toxicology study in cynomolgus macaques (data not shown). Each treatment was administered twice-daily (at approximately 9:00a.m. in conjunction with L-DOPA and 6:00p.m. without L-DOPA) for a period of 14-days in a non-randomised, escalating dose design (weeks 1–8). Animals then received two weeks of wash-out (weeks 9–10), during which time once-daily treatment with L-DOPA was continued but treatment with TC-8831 was ceased. After this time, as a reference condition, the effects of repeated once-daily administration of amantadine (3 mg/kg, *p.o.*) in combination with L-DOPA, were assessed over a period of 2-weeks (weeks 11–12). Throughout the entire study, L-DOPA (oral Madopar™) was administered at a dose of L-DOPA defined, for each animal, as one that provided reversal of parkinsonian symptoms but which also elicited marked to severe dyskinesia (this dose is referred to as L-DOPA$_{dyskinesia}$ or LDd), as described above. Parkinsonian disability and dyskinesia were assessed directly following morning treatments on the 7th and 14th day of each treatment period. Thus, behaviour was assessed weekly for 8-weeks during the TC-8831 dosing period (weeks 1–8) and twice during the amantadine dosing phase (weeks 11–12).

2.4. Assessment of stability of response to L-DOPA

To demonstrate the stability of the model, ensure that any reductions in dyskinesia seen over time were due to TC-8831 treatment and not time-dependent changes in L-DOPA sensitivity, and to provide an independent vehicle-L-DOPA treatment group for the amantadine arm (weeks 11–12), after one week of washout (week-9), animals were challenged with a sentinel administration of L-DOPA. Dyskinesia, parkinsonism and ON-time were assessed and compared to the L-DOPA/vehicle responses obtained at either week-2 (TC-8831 comparisons) or week-9 (amantadine comparisons).

2.5. Assessment of dyskinesia, parkinsonism, quality and duration of ON-time

Following administration of treatments, animals were transferred immediately to individual observation cages (1.5 × 1.0 × 1.1 m) and their behaviour recorded on HD-video. Ratings of behaviour were made, blinded to treatment, by *post-hoc* analysis of recordings by a movement disorders neurologist. A measure of total parkinsonian disability as described previously (Johnston et al., 2010a) 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 (Visanji et al., 2009). Parkinsonian disability and dyskinesia were assessed for 5-min every 10-min, the score given being most representative of each 5-min observation period.

Scores were summed for each 30-min across the entire 6-h of observations for time-course analyses and during the period of peak-effect (1–3 h). The duration of

anti-parkinsonian action, i.e. ON-time, was defined as the number of minutes for which bradykinesia was absent i.e. score = 0. In addition, the duration of ON-time associated with dyskinesia of varying severity was defined as follows; "*good*" quality ON-time represents the number of minutes for which bradykinesia was zero whilst dyskinesia was either absent or of mild or moderate severity. Meanwhile, "*bad*" quality ON-time represents the number of minutes for which bradykinesia was zero whilst dyskinesia was either marked or severe (Johnston et al., 2010a).

2.6. Assessment of plasma TC-8831 levels following oral administration

Alongside behavioural observations, the plasma levels of drug associated with repeated oral administration of TC-8831 (0.03–0.3 mg/kg) were assessed. Thus, on every 9th day of treatment within a given 2-week block (day 23; 0.03 mg/kg, day-37; 0.1 mg/kg and day-51; 0.3 mg/kg) 1 ml samples of venous blood were taken from the cephalic vein of each animal, immediately pre-dosing (approximately 9:30a.m.), then at +15-min, 30-min and at 1, 2, 4, 6, 7 and 8-h post dose.

Blood samples were placed into K_2-EDTA tubes (Becton Dickinson, Mississauga, ON, Canada) and mixed by gently inverting the closed tube 8-times. The blood was centrifuged at 4 °C for 5-min at 1500g_{ave} and plasma analysed for TC-8831 by LC/MS/MS.

2.7. Statistical analysis and data presentation

2.7.1. Continuous data

Continuous data derived calculations of ON-time and quality of ON-time in terms of presence or absence of non-disabling or disabling dyskinesia ('bad' or 'good' ON-time) were plotted as mean ± s.e.m. Statistical analyses for time-course data were carried out using a parametric repeated measures two-way ANOVA followed by a Bonferroni Multiple Comparison's test. Summed activity data (for the 1–3 h period) were analysed using a one-way analysis of variance (RM-ANOVA) followed by Dunnett's Multiple Comparison Test.

2.7.2. Discontinuous, categorical data

Categorical, discontinuous data for parkinsonian disability, dyskinesia, chorea and dystonia were graphed as median scores alone (time course) with individual values (1–3 h cumulated totals). Time course data for parkinsonian disability dyskinesia, chorea and dystonia were analysed according to previously published methods (Huot et al., 2013). First, transformed before being subjected to statistical analysis using a parametric non-matched 2-way ANOVA (with time and treatment as variables) followed by multiple Bonferroni Multiple Comparison tests. Summed data (for the 1–3 h period) were analysed non-parametrically using a Friedman test followed by a Dunn's Multiple Comparisons test. For all analysis, significance was set at $P \leq 0.05$. Analyses were performed using GraphPad Prism® v.5.2.

3. Results

Orally administered TC-8831 in combination with L-DOPA was well tolerated at all doses of TC-8831 assessed (0.03–0.3 mg/kg). While not formally assessed, the movement-disorder neurologist assessing the animals in a blinded fashion observed no signs of abnormal behaviour or sedation.

3.1. Effect of TC-8831 on quality and duration of ON-time

TC-8831 significantly improved the quality of ON-time such that there was a significant reduction in duration of 'bad' ON-time (ON-time with disabling dyskinesia, by up to 62%) and an increase in the duration of 'good' ON-time (ON-time without disabling dyskinesia, by up to 82%). Following treatment with L-DOPA in combination with vehicle, MPTP-lesioned macaques displayed a mean total duration of 'bad' ON-time with disabling dyskinesia (where dyskinesia was either marked or severe) of 109 ± 20 min (60% of total ON-time, week-2). There was a significant effect of TC-8831 treatment on ON-time with disabling dyskinesia ($F_{7,42} = 4.0$, $P < 0.01$, one-way, ANOVA, Fig. 1A). *Post-hoc* Dunnett's analysis revealed that, following treatment with L-DOPA in combination with TC-8831, duration of 'bad' ON-time was significantly reduced at 0.03 mg/kg (week-4, by 49%, 56 ± 15 min, $P < 0.05$), 0.1 mg/kg (week-5, by 46%, 59 ± 19 min, $P < 0.01$ and week 6, by 62%, 41 ± 15 min, $P < 0.01$) and 0.3 mg/kg (week-7, by 53%, 51 ± 20 min, $P < 0.01$), compared to vehicle alone (week-2).

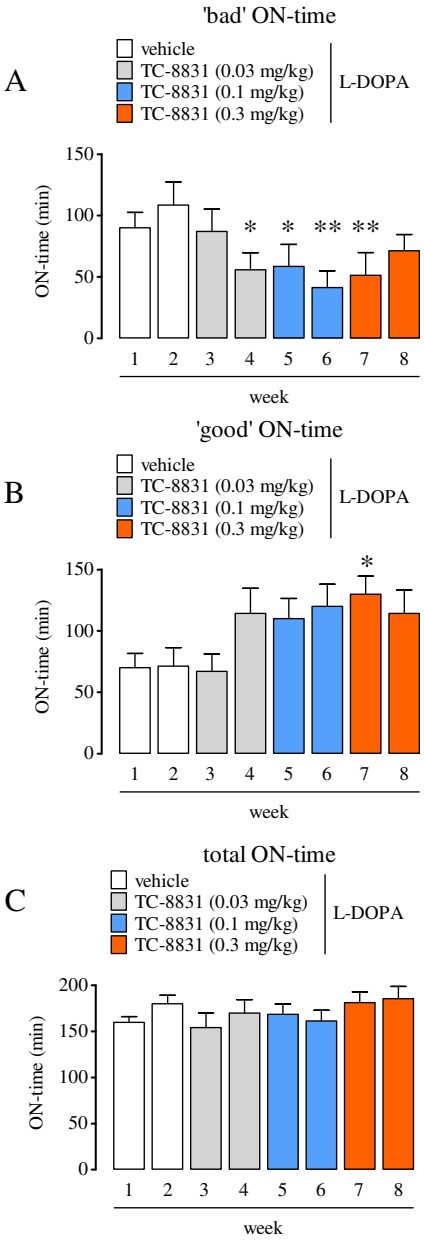


Fig. 1. Effect of TC-8831 in combination with L-DOPA on duration and quality of ON-time in MPTP-lesioned primates. MPTP-lesioned cynomolgus monkeys received daily L-DOPA treatment (median dose 30 mg/kg, range 20–35 mg/kg, *p.o.* once-daily) in addition to either two weeks of consecutive daily treatment with vehicle (weeks 1 and 2; 1 ml/kg, *p.o., b.i.d.*) followed sequentially by TC-8831 at 0.03 mg/kg (weeks 3 and 4), 0.1 mg/kg (weeks 5 and 6) and 0.3 mg/kg (weeks 7 and 8). On days 7, 13, 21, 28, 35, 42, 49 and 56 immediately following a.m. treatments, 'bad' quality ON-time (**A**, that compromised by marked or severe dyskinesia), 'good' quality ON-time, (**B**, characterized by either no, mild or moderate dyskinesia) or total ON-time (**C**), was assessed for the duration of the 6 h time-course. Data are mean ± s.e.m.. $N = 7$ for all treatment groups. */** represents $P < 0.05/P < 0.01$ *cf.* week 2 (vehicle-treatment; 1-way, RM-ANOVA with Dunnett's Multiple Comparison test).

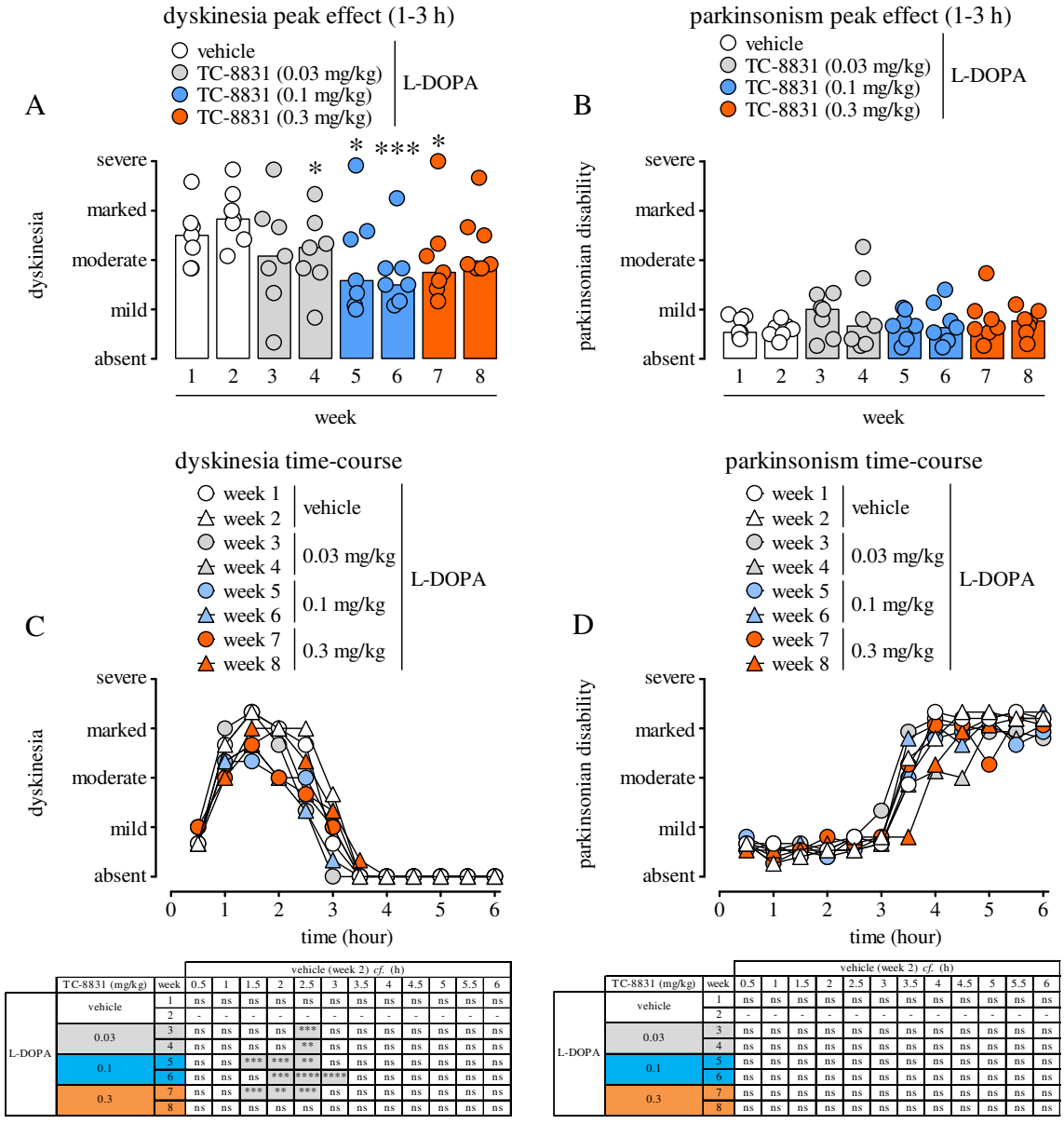
Following treatment with L-DOPA, MPTP-lesioned macaques displayed a mean total duration of 'good' ON-time without disabling dyskinesia (where dyskinesia was either absent or non-disabling) of 71 ± 16 min (40% of total ON-time). There was a significant effect of TC-8831 treatment on duration of 'good' ON-time ($F_{7,42} = 3.8$, $P < 0.01$, one-way, RM-ANOVA, Fig. 1B) and following treatment with L-DOPA in combination with TC-8831 (0.3 mg/kg), duration of

'good' ON-time was significantly increased (by 82%) at week-7 (130 ± 16 min, $P < 0.05$) compared to vehicle alone (week-2).

MPTP-lesioned macaques treated with L-DOPA in combination with vehicle, displayed a mean total duration of total ON-time of 180 ± 10 min (week-2). There was no significant effect of TC-8831 treatment on total ON-time ($F_{7,42} = 2.2$, $P > 0.05$, Fig. 1C).

3.2. Effect of TC-8831 on dyskinesia and parkinsonism

In examining the source of the improvement in quality of ON-time, we observed that TC-8831 administration significantly decreased dyskinesia evoked by L-DOPA yet at no time during the study did treatment with TC-8831 exacerbate parkinsonism or reduce duration of total ON-time observed following L-DOPA. Thus, in MPTP-lesioned macaques, median levels of dyskinesia assessed during the peak-effect period 1–3 h post administration of L-DOPA and vehicle were in the moderate to marked range (week-2; median = 34, range 25–46). Friedman analysis revealed a significant effect of TC-8831 treatment (Friedman Statistic (FS) = 22.3, $P < 0.01$, Friedman test, Fig. 2A). Dunn's *post-hoc* analysis revealed that levels of L-DOPA-induced dyskinesia were significantly reduced in animals following co-administration of TC-8831 at 0.03 mg/kg (week-4; median score = 27, $P < 0.05$), 0.1 mg/kg (week-5; median score = 19, $P < 0.05$ and week-6; median score = 18, $P < 0.001$) and 0.3 mg/kg (week-7; median score = 21, $P < 0.05$), compared to that seen following vehicle (week-2; median



vehicle (week 2) *cf.* (h)

L-DOPA	TC-8831 (mg/kg)	week	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
	vehicle	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		2	-	-	-	-	-	-	-	-	-	-	-	-
	0.03	3	ns	ns	ns	ns	***	ns	ns	ns	ns	ns	ns	ns
		4	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
	0.1	5	ns	ns	***	***	**	ns	ns	ns	ns	ns	ns	ns
		6	ns	ns	ns	***	****	****	ns	ns	ns	ns	ns	ns
	0.3	7	ns	ns	***	**	***	ns	ns	ns	ns	ns	ns	ns
		8	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

vehicle (week 2) *cf.* (h)

L-DOPA	TC-8831 (mg/kg)	week	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
	vehicle	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		2	-	-	-	-	-	-	-	-	-	-	-	-
	0.03	3	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	0.1	5	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		6	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	0.3	7	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		8	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Fig. 2. Effect of TC-8831 on L-DOPA-induced dyskinesia and parkinsonism in MPTP-lesioned primates. MPTP-lesioned cynomolgus monkeys received daily L-DOPA treatment (median dose 30 mg/kg, range 20–35 mg/kg, *p.o.* once-daily) in addition to either two weeks of consecutive daily treatment with vehicle (weeks 1 and 2; 1 ml/kg, *p.o., b.i.d.*) followed sequentially by TC-8831 at 0.03 mg/kg (weeks 3 and 4), 0.1 mg/kg (weeks 5 and 6) and 0.3 mg/kg (weeks 7 and 8). On days 7, 13, 21, 28, 35, 42, 49 and 56, immediately following a.m. treatments, dyskinesia and parkinsonism were assessed every 10 min for a 5 min period and cumulated over the period of peak-effect (1–3 h, **A** and **B** respectively) or into 30 min epochs for the duration of the 6 h time-course (**C** and **D** respectively). Data are median (time-course) with individual values (peak-effect only). $N = 7$ for all treatment groups. */**/*** represents $P < 0.05$, $P < 0.01$ or $P < 0.001$ *cf.* vehicle-treatment. Friedman test with Dunn's Multiple Comparison test (peak-effect totals) or ranked data followed by 2-way RM-ANOVA with Bonferroni Multiple Comparison test (time-courses).

score = 34). Examining the time-course of effect on treatments (Fig. 2C), we found that L-DOPA evoked dyskinesia lasting approximately 3 h that reached peak intensity (marked to severe levels) in the period 1–1.5 h following administration. Evaluation of the whole 6 h time-course period of observation revealed a significant effect of TC-8831 treatment and the interaction of treatment and time, but not time alone, on dyskinesia ($F_{time\ 11,528} = 0.0$, $P > 0.05$; $F_{treatment\ 7,528} = 4.7$, $P < 0.001$; $F_{interaction\ 77,528} = 2.0$, $P < 0.001$; two-way, RM-ANOVA). *Post-hoc* Bonferroni analysis revealed that, following treatment with TC-8831, median levels of dyskinesia seen following co-administration of L-DOPA were significantly decreased during the 2–2.5 h (0.03 mg/kg, weeks 3–4), 1–2.5 and 1.5–3 h (0.01 mg/kg, weeks 5–6) and 1–2.5 h (0.3 mg/kg, week-7) periods post-administration, respectively, compared to the response exhibited following vehicle treatment (all $P < 0.01$).

With regard to parkinsonian disability, during the peak-effect period (1–3 h), vehicle-treated MPTP-lesioned animals expressed disability of moderate–marked levels (median = 18, range 10–25, not shown), whereas L-DOPA treatment reduced disability to absent-mild levels (median = 18, range 10–25, week-2, Fig. 2B). During this period, there was no significant effect of TC-8831 treatment on disability (FS = 3.4, $P > 0.05$, Fig. 2B). Over the 6 h period of observation (Fig. 2D), there was no significant effect of either treatment, interaction between time and treatment, or time alone, on levels of parkinsonian disability ($F_{time\ 11,528} = 0.0$, $P > 0.05$; $F_{treatment\ 7,528} = 1.4$, $P > 0.05$; $F_{interaction\ 33,528} = 1.0$, $P > 0.05$; two-way, RM-ANOVA).

3.3. Effect of TC-8831 on chorea and dystonia

Examining the phenomenology of dyskinesia evoked by L-DOPA in the current MPTP-macaque cohort distinguished differential effects of treatment on chorea and dystonia. Median levels of chorea assessed during the peak-effect period 1–3 h in L-DOPA treated animals following administration of vehicle were in the mild to moderate range (median = 19, range 2–26) and while Friedman analysis was not significant (FS = 13.4, $P = 0.06$, Fig. 3A), *Post-hoc* Dunn's analysis did show a significant decrease in median levels of chorea in animals treated with low-dose TC-8831 (0.03 mg/kg, week-3; median score = 6). Indeed, examining the time-course of effect (Fig. 3C), revealed levels of L-DOPA evoked chorea lasting approximately 3 h that reached peak intensity (mild to moderate levels) in the period 1–1.5 h following administration, which, across the whole 6 h time-course period of observation, revealed a significant effect of TC-8831 treatment and the interaction of treatment and time, but not time alone, on chorea ($F_{time\ 11,528} = 0.0$, $P > 0.05$; $F_{treatment\ 7,528} = 3.1$, $P < 0.01$; $F_{interaction\ 77,528} = 1.4$, $P < 0.05$; two-way, RM-ANOVA). *Post-hoc* Bonferroni analysis revealed that following treatment with TC-8831, median levels of chorea seen following co-administration of L-DOPA were significantly decreased during the 1–1.5 and 2–2.5 h (0.03 mg/kg, week-4), 1–2.5 h (0.1 mg/kg, week-6) and 1–2.5 h (0.3 mg/kg, week-8) periods post-administration, respectively, compared to the response exhibited following vehicle treatment (all $P < 0.05$).

By contrast, median levels of dystonia assessed during the peak-effect period 1–3 h in L-DOPA treated animals following administration of vehicle were in the moderate to marked range (median level = 31, range 12–46). Friedman analysis revealed a significant effect of TC-8831 treatment (FS = 17.2, $P = 0.02$, Fig. 3B) on levels dystonia with a decrease in median levels evident in TC-8831-treated animals at week-6 (0.1 mg/kg; median score = 17). Examining the time-course of effect (Fig. 3D) revealed levels of L-DOPA evoked dystonia lasting approximately 3 h that reached peak intensity (moderate to marked levels) in the period 1–1.5 h following administration. Across the whole 6-h time-course period of observation, there was a significant effect of TC-8831 treatment and the interaction of treatment and time, but not time alone, on dystonia ($F_{time\ 11,528} = 0.0$, $P > 0.05$; $F_{treatment\ 7,528} = 3.8$, $P < 0.01$; $F_{interaction\ 77,528} = 1.9$, $P < 0.001$; two-way, RM-ANOVA). *Post-hoc* Bonferroni analysis revealed that, following treatment with TC-8831, median levels of dystonia seen following co-administration of L-DOPA were significantly decreased during the 2.5–3 h (0.03 mg/kg, weeks 3–4), 1–2 h and 1.5–3 h (0.1 mg/kg, weeks 5–6) and 1–2 h (0.3 mg/kg, week-7) periods post-administration, respectively, compared to the response exhibited following vehicle treatment (all $P < 0.05$).

3.4. Sentinel response to L-DOPA pre- and post- TC-8831 treatment

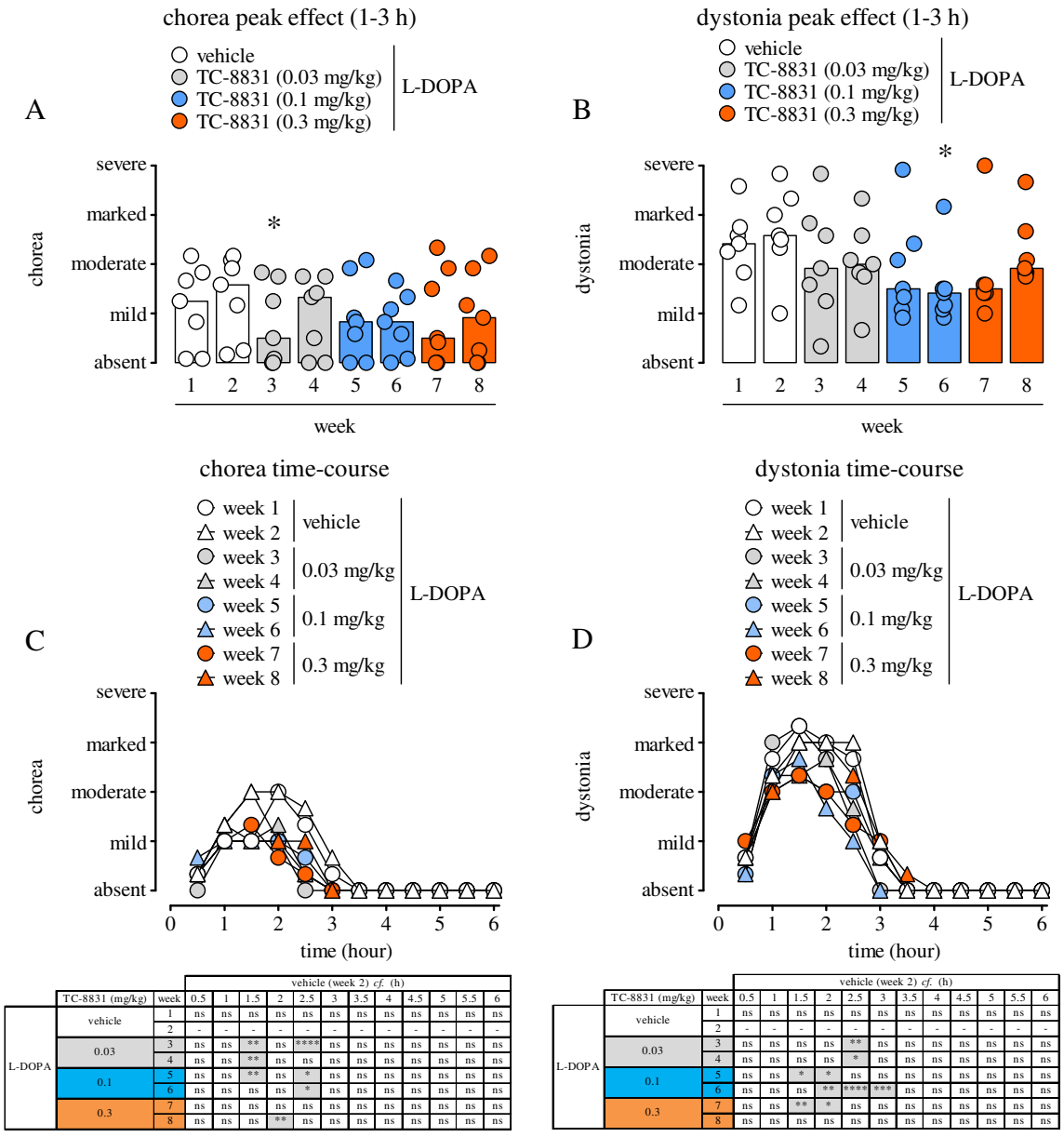
A sentinel L-DOPA challenge administered 1 week following cessation of all TC-8831 treatments (week-9) elicited dyskinesia that was not significantly different to that observed with L-DOPA alone during week-2. Thus, the reduction in L-DOPA-induced dyskinesia observed during the week 3–8 period of TC-8831 administration could not be attributed to any change in baseline expression in L-DOPA induced dyskinesia and likely represent an effect of TC-8831. Specifically, comparing the effects of L-DOPA on levels of dyskinesia over the whole 6 h time-course period of observation during week-2 and week-9 (1-week following cessation of TC-8831 treatment) revealed identical median peak levels of dyskinesia (marked-severe) which occurred at the same time (1–1.5 h) after administration of treatments. Indeed, there was no significant difference between the levels of dyskinesia (cumulated over the period 1–3 h post administration) evoked by L-DOPA during week-2 and that observed in week-9 ($P > 0.05$, Wilcoxon matched-pairs signed rank test, data not shown).

3.5. Effect of amantadine on ON-time, parkinsonism, dyskinesia, chorea and dystonia

Amantadine significantly improved the quality of ON-time and afforded a significant reduction in duration of 'bad' ON-time (by up to 61%) and an increase in the duration of 'good' ON-time (by up to 40%). At week-9, following treatment with L-DOPA in combination with vehicle, MPTP-lesioned macaques displayed a mean total duration of 'bad' ON-time of 102 ± 10 min (56% of total ON-time, week-9). There was a significant effect of TC-8831 treatment on 'bad' ON-time with disabling dyskinesia ($F_{2,10} = 18.3$, $P < 0.001$, one-way, ANOVA, Fig. 4A). *Post-hoc* Dunnett's analysis revealed that, following treatment with L-DOPA in combination with amantadine (3 mg/kg, *p.o.*), duration of 'bad' ON-time was significantly reduced after both one (week-11, by 49%, 52 ± 13 min, $P < 0.01$) and two (week-12, by 61%, 40 ± 23 min, $P < 0.001$) of treatment, compared to vehicle alone (week-9).

MPTP-lesioned macaques displayed a mean total duration of L-DOPA-induced 'good' ON-time of 80 ± 20 min (44% of total ON-time). There was a significant effect of amantadine (3 mg/kg, *p.o.*) treatment on duration of 'good' ON-time ($F_{2,10} = 7.3$, $P < 0.01$, one-way, RM-ANOVA, Fig. 4B) and following treatment with L-DOPA in combination with amantadine, duration of 'good' ON-time was significantly increased (by 40%) at week-11 (112 ± 16 min, $P < 0.05$) compared to vehicle alone (week-9).

MPTP-macaques treated with L-DOPA in combination with vehicle, displayed a mean total duration of total ON-time of 182 ± 15 min (week-9). There was a significant effect of amantadine (3 mg/kg, *p.o.*) treatment on duration of total ON-time ($F_{2,10} = 7.5$, $P < 0.05$, one-way, RM-ANOVA, Fig. 4C) and following treatment with L-DOPA in combination with amantadine, duration of total ON-time was significantly decreased (by 23%) at week-12 (140 ± 15 min, $P < 0.01$) compared to vehicle alone (week-9).



vehicle (week 2) *cf.* (h)

L-DOPA	TC-8831 (mg/kg)	week	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
	vehicle	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		2	-	-	-	-	-	-	-	-	-	-	-	-
	0.03	3	ns	ns	**	ns	****	ns	ns	ns	ns	ns	ns	ns
		4	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns
	0.1	5	ns	ns	**	ns	*	ns	ns	ns	ns	ns	ns	ns
		6	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
	0.3	7	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		8	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns

vehicle (week 2) *cf.* (h)

L-DOPA	TC-8831 (mg/kg)	week	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
	vehicle	1	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		2	-	-	-	-	-	-	-	-	-	-	-	-
	0.03	3	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
		4	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
	0.1	5	ns	ns	*	*	ns	ns	ns	ns	ns	ns	ns	ns
		6	ns	ns	ns	**	****	***	ns	ns	ns	ns	ns	ns
	0.3	7	ns	ns	**	*	ns	ns	ns	ns	ns	ns	ns	ns
		8	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Fig. 3. Effect of TC-8831 on L-DOPA-induced chorea and dystonia in MPTP-lesioned primates. MPTP-lesioned cynomolgus monkeys received daily L-DOPA treatment (median dose 30 mg/kg, range 20–35 mg/kg, *p.o.* once-daily) in addition to either two weeks of consecutive daily treatment with vehicle (weeks 1 and 2; 1 ml/kg, *p.o., b.i.d.*) followed sequentially by TC-8831 at 0.03 mg/kg (weeks 3 and 4), 0.1 mg/kg (weeks 5 and 6) and 0.3 mg/kg (weeks 7 and 8). On days 7, 13, 21, 28, 35, 42, 49 and 56, immediately following a.m. treatments, chorea and dystonia were assessed every 10 min for a 5 min period and cumulated over the period of peak-effect (1–3 h, **A** and **B** respectively) or into 30 min epochs for the duration of the 6 h time-course (**C** and **D** respectively). Data are median (time-course) with individual values (peak-effect only). $N = 7$ for all treatment groups. */**/*** represents $P < 0.05$, $P < 0.01$ or $P < 0.001$ *cf.* vehicle-treatment. Friedman test with Dunn's Multiple Comparison test (peak-effect totals) or ranked data followed by 2-way RM-ANOVA with Bonferroni Multiple Comparison test (time-courses).

Amantadine administration significantly decreased dyskinesia evoked by L-DOPA yet at no time during the study did treatment with amantadine exacerbate levels of parkinsonism observed following L-DOPA. Thus, in MPTP-lesioned macaques, median levels of dyskinesia assessed during the peak-effect period 1–3 h post administration of L-DOPA and vehicle were in the moderate to marked range (median = 33, range 28–46). Friedman analysis revealed a significant effect of amantadine (3 mg/kg, *p.o.*) treatment (Friedman Statistic (FS) = 8.9, $P < 0.001$, Friedman test, Fig. 5A). Dunn's *post-hoc* analysis revealed that levels of L-DOPA-induced dyskinesia were significantly reduced in animals following two weeks co-administration of amantadine (week-12; median score = 17, $P < 0.05$) compared to that seen following vehicle (week-9; median score = 33). Examining the 6 h time-course of effect on treatments (Fig. 5B) also revealed a significant effect of amantadine treatment and the interaction of treatment and time, but not time alone, on dyskinesia ($F_{time\ 11,165} = 0.0$, $P > 0.05$; $F_{treatment\ 2,165} = 30.4$, $P < 0.001$; $F_{interaction\ 22,165} = 5.5$, $P < 0.001$; two-way, RM-ANOVA). *Post-hoc* Bonferroni analysis revealed that, following treatment with amantadine, median levels of dyskinesia seen following co-administration of L-DOPA were significantly decreased during the 0.5–2.5 h (week-11) and 0–3 h (week-12) periods post-administration, respectively, compared to vehicle treatment (all $P < 0.05$).

With regard to parkinsonian disability, during the peak-effect period (1–3 h), there was no significant effect of amantadine

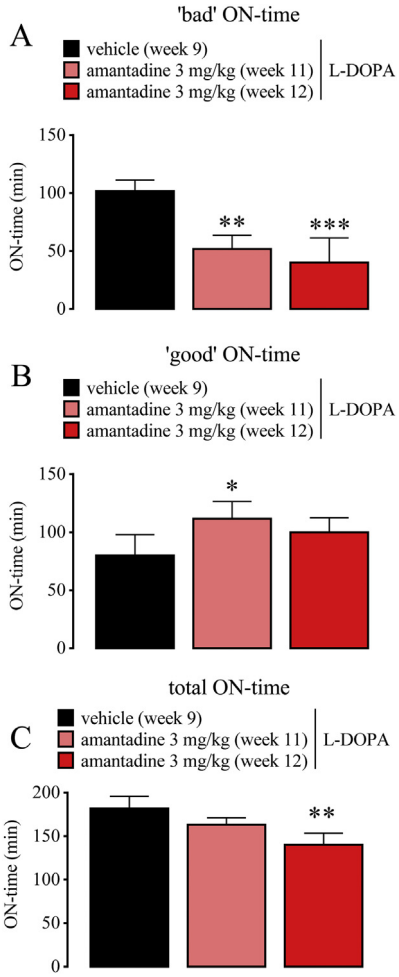


Fig. 4. Effect of amantadine in combination with L-DOPA on duration and quality of ON-time in MPTP-lesioned primates. MPTP-lesioned cynomolgus monkeys received daily L-DOPA treatment (median dose 30 mg/kg, range 20–35 mg/kg, *p.o.* once-daily) in addition to either two weeks of consecutive daily treatment with vehicle (week 9; 1 ml/kg, *p.o., b.i.d.*) followed by two-weeks of treatment with amantadine (3 mg/kg, *p.o.*) during weeks 11 and 12. Immediately following a.m. treatments, 'bad' quality ON-time (**A**, that compromised by marked or severe dyskinesia), 'good' quality ON-time, (**B**, characterized by either no, mild or moderate dyskinesia) or total ON-time (**C**), was assessed for the duration of the 6 h time-course. Data are mean ± s.e.m.. $N = 6$ for all treatment groups. */** represents $P < 0.05$/$P < 0.01$ *cf.* week 9 (vehicle-treatment; 1-way, RM-ANOVA with Dunnett's Multiple Comparison test).

treatment on disability (FS = 5.5, $P > 0.05$, Fig. 5C). Over the 6 h period of observation (Fig. 5D), there was a significant effect of the interaction between time and treatment, but neither treatment nor time alone, on levels of parkinsonian disability ($F_{time\ 11,165} = 0.0$, $P > 0.05$; $F_{treatment\ 2,165} = 1.1$, $P > 0.05$; $F_{interaction\ 22,165} = 2.4$, $P > 0.001$; two-way, RM-ANOVA).

Median levels of chorea assessed during the peak-effect period 1–3 h in L-DOPA treated animals following administration of vehicle were in the absent to mild range (median = 9, range 0–35) and Friedman analysis showed there to be no significant effect of treatment (FS = 3.1, $P = 0.18$, Fig. 5E). However, examining the 6 h time-course of effect (Fig. 5F), revealed a small but significant effect of amantadine treatment and the interaction of treatment and time, but not time alone, on chorea ($F_{time\ 11,165} = 0.0$, $P > 0.05$; $F_{treatment\ 2,165} = 5.4$, $P < 0.05$; $F_{interaction\ 22,165} = 1.7$, $P < 0.05$; two-way, RM-ANOVA). *Post-hoc* Bonferroni analysis revealed that following two-weeks of amantadine treatment, median levels of chorea seen following co-administration of L-DOPA were significantly decreased during the 2–3 h period (week-12) post-administration compared to the response exhibited following vehicle treatment (all $P < 0.05$).

Median levels of dystonia assessed during the peak-effect period (1–3 h) in L-DOPA treated animals following administration of vehicle were in the moderate to marked range (median level = 29, range 28–46). Friedman analysis revealed a significant effect of amantadine treatment (FS = 8.8, $P = 0.008$, Fig. 5G) on levels dystonia with a decrease in median levels evident following two weeks of amantadine-treatment (week-12; median score = 15). Examining the 6 h time-course of effect (Fig. 5H) revealed a significant effect of amantadine treatment and the interaction of treatment and time, but not time alone, on dystonia ($F_{time\ 11,165} = 0.0$, $P > 0.05$; $F_{treatment\ 2,165} = 29.9$, $P < 0.001$; $F_{interaction\ 22,165} = 7.5$, $P < 0.001$; two-way, RM-ANOVA). *Post-hoc* Bonferroni analysis revealed that, following treatment with amantadine, median levels of dystonia seen following co-administration of L-DOPA were significantly decreased during the 0.5–3 h (week-11) and 0–3 h (week-12) periods post-administration, respectively, compared to the response exhibited following vehicle treatment (all $P < 0.05$).

3.6. Pharmacokinetic profile

In L-DOPA-treated, MPTP-lesioned cynomolgus macaques, TC-8831 was readily detectable in plasma following oral administration of the drug (Figure S1). Times to peak levels of drug (t_{max}) among the three dose groups averaged from 1.2 to 3.0 h after dosing and were associated with maximum plasma concentrations ranging from 13.7 to 114 ng/ml and an $AUC_{0\text{-}8}$ of 56–516 h.ng/ml (0.03 and 0.3 mg/kg, respectively). The elimination phase half-life, $t_{1/2}$, for TC-8831 in the MPTP-macaque averaged 2.3–3.3 h for each dose group. TC-8831 concentrations were still above lowest quantifiable limits at 8 h post-administration. On each of the three days of analysis (day 23; 0.03 mg/kg, day 37; 0.1 mg/kg and day 51; 0.3 mg/kg) small amounts of TC-8831 were detectable in the pre-treatment sample, indicating that the second dose of TC-8831 from the previous day had not been completely cleared from the system after approximately 16 h. Thus, residual TC-8831 was detectable at levels of up to 5% of C_{max} (0.3 mg/kg; pre-sample; 4.7 ± 2.2 ng/ml *cf.* 100.5 ng/ml at $t = +2$ h).

4. Discussion

TC-8831 is a nicotinic agonist with selectivity for α6β2*/α4β2* nAChR over other nAChR subtypes such as α7, ganglion-type (α3β4) and muscle-type receptors (Quik et al., 2013a). In MPTP-lesioned macaques, TC-8831 reduced L-DOPA induced dyskinesia without compromising the ability of L-DOPA to alleviate parkinsonian symptoms. Thus, in combination with L-DOPA, all doses of TC-8831, to varying extents, significantly reduced dyskinesia. The effects of TC-8831 on dyskinesia were expressed as reductions in both chorea and dystonia. Furthermore, TC-8831 significantly improved the quality of ON-time such that there was a reduction in duration of 'bad' ON-time (ON-time with disabling dyskinesia). Moreover, at no time during the study did any dose of TC-8831 reduce any measure of anti-parkinsonian benefit of L-DOPA, produce side-effects or impair alertness. Together with the reduction in dyskinesia, these findings form the basis of a clinical application of TC-8831.

4.1. Technical considerations

Cynomolgus macaques were rendered parkinsonian using protocols and cumulative doses of MPTP in line with those that we, and others, have previously employed. After chronic, repeated L-DOPA therapy, all animals developed L-DOPA-induced dyskinesia. Furthermore, *in vivo* PET imaging with the ligand [^{18}F]-AV-133

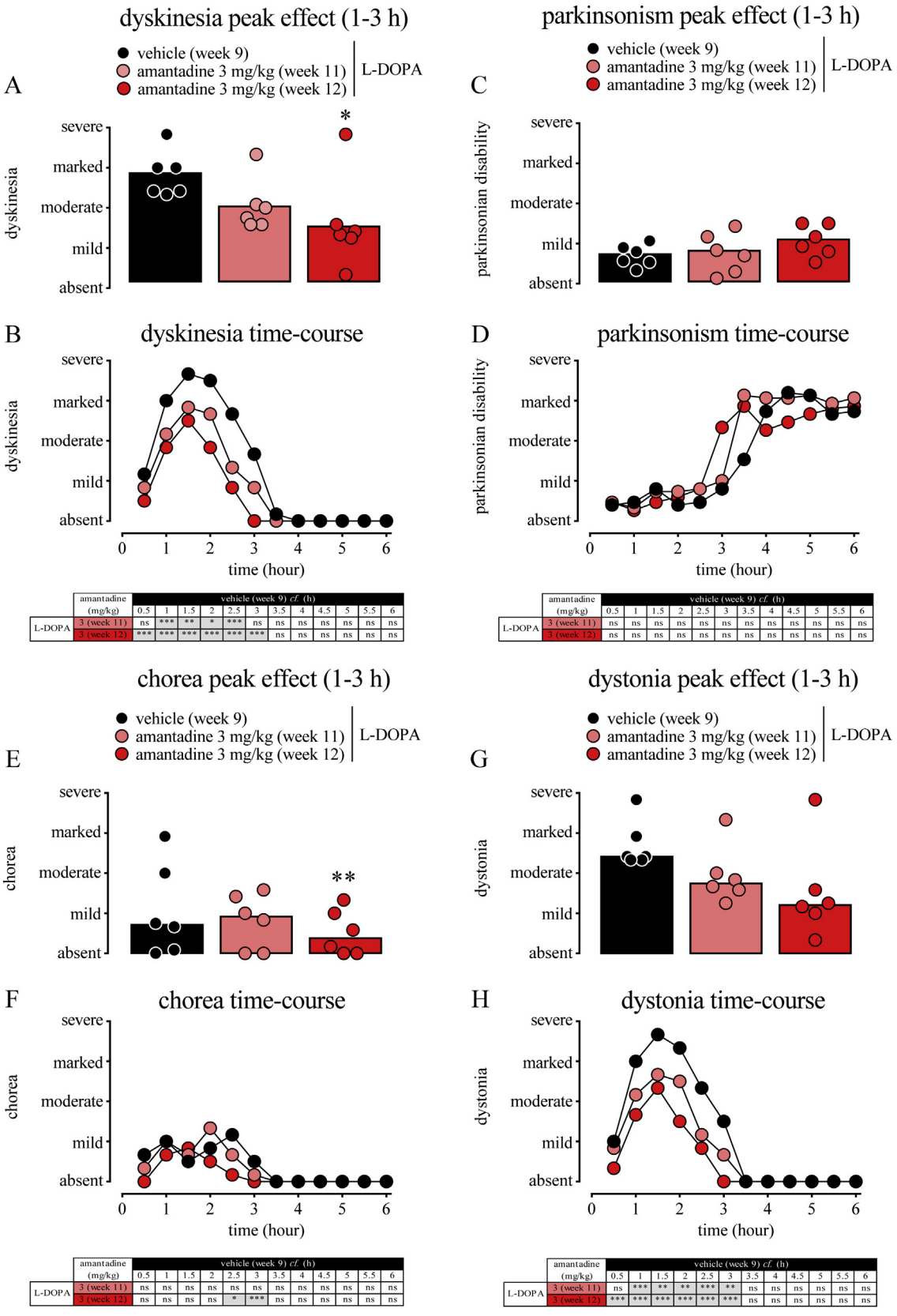


Fig. 5. Effect of amantadine on dyskinesia, parkinsonism, chorea and dystonia in L-DOPA-treated MPTP-lesioned primates. MPTP-lesioned cynomolgus monkeys received daily L-DOPA treatment (median dose 30 mg/kg, range 20–35 mg/kg, *p.o.* once-daily) in addition to either two weeks of consecutive daily treatment with vehicle (week 9; 1 ml/kg, *p.o.*, *b.i.d.*) followed by two-weeks of treatment with amantadine (3 mg/kg, *p.o.*) during weeks 11 and 12). Immediately following a.m. treatments, dyskinesia, parkinsonism,/chorea and dystonia were assessed every 10 min for a 5 min period and cumulated over the period of peak-effect (1–3 h, **A, C, E** and **G** respectively) or into 30 min epochs for the duration of the 6 h time-course (**B, D, F** and **H** respectively). Data are median (time-course) with individual values (peak-effect only). $N = 7$ for all treatment groups. */**/*** represents $P < 0.05$, $P < 0.01$ or $P < 0.001$ *cf.* vehicle-treatment. Friedman test with Dunn's Multiple Comparison test (peak-effect totals) or ranked data followed by 2-way RM-ANOVA with Bonferroni Multiple Comparison test (time-courses).

(supplemental materials) revealed an average 85% reduction in striatal VMAT2 sites in MPTP-lesioned animals compared to a normal control. This extent of lesion is comparable to that observed in advanced Parkinson's patients and typical of MPTP-lesioned animals with robust parkinsonism. Interestingly, recent work points to an enhanced ability of nicotine to alleviate LID in animals with partial, rather than complete, lesions of the nigrostriatal tract (Huang et al., 2011a). Thus, in L-DOPA-treated rats with virtually a complete 6-OHDA-induced lesion (>99% loss of specific striatal dopamine transporter (DAT) binding), the α4β2/α6β2 subtype selective ligand A-85380 reduced AIMs by 20%. By contrast, A-85380 reduced L-DOPA-induced AIMs by 40–50% in rats with a partial striatal dopamine lesion (~67% loss of DAT). Similarly, nicotine effectively decreased LID in moderately-lesioned, but not severely-lesioned, non-human primates (Quik et al., 2013b). TC-8831 produced a 24% reduction of total AIMs when previously tested for its anti-dyskinetic action in rodents with 99% loss of DAT (Quik et al., 2013a). Although based on examination of an alternative marker of terminal striatal function (VMAT2), our data suggest that the extent of lesion in the current MPTP macaque cohort (85% reduction *cf.* normal) has permitted a sufficiency of striatal dopamine terminals to remain with which to robustly permit the anti-dyskinetic actions of TC-8831. In contrast to the previous rodent study, TC-8831 reduced total dyskinesias by up to 47% in the current study. This difference is likely due to the anti-dyskinetic contribution of α6β2* receptors, which are expressed exclusively in the striatum on dopamine terminals, in addition to α4β2* receptors, which are located on dopaminergic and non-dopaminergic neurons (Quik et al., 2013a).

The doses of L-DOPA employed for the current study provided maximal anti-parkinsonian benefit, typically of ~3 h duration but which was compromised by disabling dyskinesia (greater than moderate levels). Although therapeutically-active doses of L-DOPA administered to the MPTP-lesioned macaque are higher on a mg/kg basis than those administered in clinical settings, we have recently shown that they deliver similar plasma pharmacokinetic profiles to those achieved with clinically relevant L-DOPA doses employed in the clinic (Huot et al., 2012). Time to peak plasma levels (t_{max}) of L-DOPA were previously determined to be approximately 1.5 h after dosing using a similar experimental paradigm and dosing regimen as in the current study (Huot et al., 2012). This corresponds very well with the time to peak plasma levels for TC-8831, which averaged from 1.2 to 3.0 h after dosing among the three dose groups. This finding indicates that both TC-8831 and L-DOPA reach t_{max} within a similar time frame, and supports adjunctive co-dosing of the two compounds in a clinical setting.

In this study, macaques, as with patients in the clinic, developed dyskinesia with an idiosyncratic mix of chorea and dystonia. On balance, the group was somewhat dystonia-dominant but the presence of both chorea and dystonia allowed the study to address the potential impact of TC-8831 on dyskinesia of either phenomenology. This is an important consideration as several drugs have been shown to have differential effects on chorea and dystonia (Fox et al., 2002; Gomez-Ramirez et al., 2006). Thus, while all patients with either type of dyskinesia might benefit from treatment with TC-8831, the data suggest that close attention should be paid to clinical trial design and a focus on the need to analyse chorea and dystonia separately.

The sentinel L-DOPA challenge performed one week after completing treatments with the test compound evoked levels of dyskinesia, provided reversal of parkinsonian disability and increases in ON-time that were indistinguishable to those seen before commencing TC-8831 treatment, at week-2 (vehicle). Thus, the ability of L-DOPA to elicit dyskinesia did not alter over the course of the experiment and any effect observed over the course of the treatment period can, most likely, be attributed to the actions of test drug and not drift, over time, in baseline behavioural response to L-DOPA administration.

The final component of the study assessed the effects of amantadine (3 mg/kg, once-daily, *p.o.*), in combination with L-DOPA. Amantadine reduced both choreiform and dystonic dyskinesia, the magnitude of which increased with duration of treatment and was comparable to that previously described in MPTP-primates as well as in Phase II clinical studies (Blanchet et al., 1998; Verhagen Metman et al., 1998) thus demonstrating that the study was powered sufficiently to demonstrate anti-dyskinetic actions of TC-8831. In clinical use amantadine is often poorly-tolerated by many patients and its use can often be compromised by tachyphylaxis and cognitive problems (Thomas et al., 2004). It is possible that after chronic (14 days) treatment in the current study, the effect of amantadine to reduce total L-DOPA-evoked ON-time may relate to these side-effects of treatment, elements that were not observed at any time with TC-8831. While no overt indications of poor tolerance to amantadine were noted in the present study, effects such as loss of cognitive ability or mild psychosis might often go un-noticed in any animal model while impacting greatly on the PD patient, leading to immediate withdrawal of the therapy. The dose of amantadine employed (3 mg/kg) was selected from prior experience as one that demonstrated anti-dyskinetic effects in MPTP-lesioned macaques. However, we had not undertaken exhaustive investigation of the effect of chronic repeat dosing paradigms for this dose and therefore it may well be that a lower dose would not reduce ON-time and be more in keeping with current clinical experiences. Regardless, the real potential for differentiating the window of benefit for TC-8831 from that of amantadine is in helping the 50–60% of patients who obtain little or no benefit from amantadine.

The current study did not examine the acute behavioural effects of TC-8831. This design was driven by previous studies in rat that showed a requirement for repeated treatment with TC-8831 for it to reduce L-DOPA-induced AIMs (Quik et al., 2013a). This is in keeping with a lack of anti-dyskinetic effect of acute nicotine administration and the concept that nicotine mediates its anti-dyskinetic effects via desensitisation of nAChR populations rather than an increase in function effect (Bordia et al., 2010).

In assessing the relevance of the actions of TC-8831 described herein, it should be noted that, to date, the effects of TC-8831 in alleviating L-DOPA-induced dyskinesia in non-human primates have only been measured following treatment of up to 14 days of any dose. It is possible that the anti-dyskinetic benefits of TC-8831 would not remain stable with longer treatment; they might become greater over time or, on the other hand, show tachyphylaxis. Indeed, at week 8, the overall actions of 0.3 mg/kg TC-8831 in reducing dyskinesia were apparently less than those of 0.1 mg/kg in week 6. This observation might represent tachyphylaxis or that there is an inverted U-shaped dose response relationship between efficacy and dose, although the escalating dose design of the study, and the resulting inter-relationship between duration of treatment and dose, makes it difficult to attribute either to this finding. With regard to tachyphylaxis, TC-8831 demonstrated a stable anti-dyskinetic effect in rodents, with a similar reduction in AIMs 6-weeks after the initial assessment, even with the last 4 of those weeks at a lower dose (Quik et al., 2013a). In addition, nicotine has demonstrated a stable anti-dyskinetic effect when tested for several months in non-human primates (Quik et al., 2013b). With regard to the potential for an inverted U-shaped dose–response curve, studies in L-DOPA-treated rats with chronically administered nicotine, whether given intermittently via injection or in a more continuous paradigm via osmotic pump delivery, showed a similar effect of nicotine to ameliorate expression of established orolingual and forelimb AIMs over a ten-fold range of doses (Bordia et al.,

2010). Given that the decrease in peak dyskinesia at week 8, although not statistically significant, was qualitatively similar to changes reported for other weeks that were significant (in the range between week 2 and week 6, which were both significant), it is possible that the outcome for week 8 reflects greater variability in the study at that time point.

4.2. Clinical potential

While patients with Parkinson's disease would benefit from a reduction in dyskinesia, they are typically not prepared to do so at the expense of loss of anti-parkinsonian benefit. Thus, the lack of any negative effect of TC-8831 in combination with these higher L-DOPA doses is critical for supporting the concept that TC-8831 might reduce dyskinesia in a manner that might be useful in the clinic.

The impact of a nicotinic receptor agonist on dyskinesia may be greater than other compounds which are currently in, or being considered for, clinical development. Thus, it was notable that TC-8831 was able to reduce both choreic and dystonic dyskinesia. In this respect, TC-8831 differs from some other classes of compound which have been shown to reduce L-DOPA-induced dyskinesia in the MPTP-lesioned monkey. Thus, some agents, for instance alpha$_2$ adrenergic antagonists, reduce chorea with no effect on dystonia (Johnston et al., 2010b). In contrast, others reduce chorea and exacerbate dystonia such as certain NMDA antagonists (Papa and Chase, 1996; Rupniak et al., 1992), while histamine H3 receptor agonists reduce chorea but with lesser effect on dystonia (Gomez-Ramirez et al., 2006) and muscarinic cholinergic antagonists reduce dystonia (Lubarr and Bressman, 2011) while exacerbating chorea (Nomoto et al., 1987). This broad action of TC-8831 on both chorea and dystonia suggests a central role for the nicotinic receptor in the mechanisms of expression of dyskinesia, whatever the phenomenology.

Because TC-8831 was able to reduce dyskinesia, there was an improvement in the quality of ON-time produced by L-DOPA. Thus, 'bad' ON-time, i.e. ON-time with troubling dyskinesia, was reduced by up to 62%, and conversely 'good' ON-time, i.e. ON-time without disabling dyskinesia, was increased by up to 82% for L-DOPA. This finding could have implications for clinical development of TC-8831 beyond Phase II. Although positive data from the MPTP-lesioned primate showing reduction in dyskinesia have been translated into successful Phase II studies to show the same in equivalent parameters (Johnston et al., 2010b; Lewitt et al., 2012), to date, no product has been approved, by either FDA or EMEA, as adjunctive therapy to reduce dyskinesia. Indeed, there have been Phase III failures of compounds that were anti-dyskinetic in monkeys and at Phase II (e.g. sarizotan with the PADDY I (Rascol et al., 2006) and PADDY II (Muller et al., 2006) trials). Thus, candidates for a dyskinesia indication face the challenge of charting new territory at Phase III. However, a path to approval based around improvement in amount and quality of ON-time has been defined, e.g. by the LARGO and PRESTO studies, as secondary endpoints for rasagiline. The actions of TC-8831 in increasing the amount of 'good' ON-time support the concept that trials for a product based upon TC-8831 or similar molecules with end-points similar to LARGO/PRESTO, such as ON-time without troublesome dyskinesia, may provide a route to approval.

In conclusion, TC-8831 was able to provide a significant alleviation of L-DOPA induced dyskinesia in the MPTP-lesioned macaque model of Parkinson's disease without negatively impacting on the anti-parkinsonian actions of L-DOPA.

Author roles

1. *Research project*: A. Conception, B. Organization, C. Execution;
2. *Statistical Analysis*: A. Design, B. Execution, C. Review and Critique;
3. *Manuscript Preparation*: A. Writing of the first draft, B. Review and Critique;

TJ – 1BC, 2AB, 3A
PH – 1C, 2C.
SHF – 1C, 2C, 3B
JBK – 1C, 3B
KTS – 1AB, 2C, 3B
JWJ – 1ABC, 2C, 3B
JDG – 1AB, 2C, 3B
SRL – 1AB, 2C, 3B
KGJ – 1AB, 2C, 3B
MPH – 1BC, 2AC, 3B
JMB – 1BC, 2AC, 3B

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THJ – has received consultancy fees from and holds shares in Atuka Inc.

PH – has received consultancy fees from Atuka Inc.

SHF – has received consultancy fees from Merck, Merck Serono and Teva.

JBK – has received consultancy fees from and holds shares in Atuka Inc.

KTS – is a shareholder in, and former employee of, Targacept, Inc.

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SRL – is a shareholder in, and former employee of, Targacept, Inc.

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Appendix A. Supplementary data

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