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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