European Journal of Pharmacology 813 (2017) 10–16

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

A preclinical study on the combined effects of repeated eltoprazine and preladenant treatment for alleviating L-DOPA-induced dyskinesia in Parkinson's disease

Wai Kin D. Ko[a,*], Qin Li[a,b,1], Long Yun Cheng[a,b], Micaela Morelli[c], Manolo Carta[d], Erwan Bezard[a,b,e,f]

[a] Motac Neuroscience Ltd, Manchester, United Kingdom
[b] Institute of Laboratory Animal Sciences, China Academy of Medical Sciences, Beijing, China
[c] Department of Biomedical Sciences, Section of Toxicology, University of Cagliari, University Campus, SS554 km 4.5, 09042 Monserrato, Italy
[d] Department of Biomedical Sciences, Section of Physiology, University of Cagliari, University Campus, SS554 km 4.5, 09042 Monserrato, Italy
[e] Univ. de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, F-33000 Bordeaux, France
[f] CNRS, Institut des Maladies Neurodégénératives, UMR 5293, F-33000 Bordeaux, France

ARTICLE INFO

Key words:
Parkinson's disease
L-DOPA-induced dyskinesia
5-HT
Adenosine

ABSTRACT

Eltoprazine, a serotonergic $(5\text{-HT})_{1A/B}$ receptor agonist, is a potential treatment for L-DOPA-induced dyskinesia (LID) in Parkinson's disease (PD) but notably compromises the anti-parkinsonian effects of L-DOPA, as seen in rodent and monkey models of PD. Preladenant, a selective adenosine A_{2a} receptor antagonist, mediates modest anti-parkinsonian effects in parkinsonian monkeys. In a recent investigation, combined eltoprazine and preladenant treatment with a sub-threshold dose of L-DOPA acutely attenuated dyskinesia without exacerbating PD disability in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated macaques. The aim of this study was to investigate the daily repeated treatment effects of eltoprazine (1 mg/kg) alone, and in combination with preladenant (5 mg/kg), on the motor symptoms of PD and LID in MPTP-treated macaques. The anti-dyskinetic and –parkinsonian effects of combinative drug administration with a sub-threshold dose of L-DOPA were measured over 14 days. Eltoprazine treatment alone produced a near-complete suppression of dyskinesia but consistently increased parkinsonism. The administration of preladenant with eltoprazine prevented the increased severity of parkinsonian motor symptoms but was unable to maintain a reduced expression of dyskinesia with repeated administration. These data demonstrate the clinical utility of the modulation of the serotonergic and adenosine neurotransmitter systems with selective pharmacological agents for only acute treatment of LID. This multi-targeted approach is unsuitable as a long-term treatment regimen due to unsustainable therapeutic effects on dyskinesia.

1. Introduction

Chronic L-DOPA treatment in Parkinson's disease (PD) commonly leads to L-DOPA-induced dyskinesia (LID) (Bastide et al., 2015). Although a single non-dopaminergic treatment given in conjunction with L-DOPA presents a promising avenue for the management of PD and LID motor symptoms (Fox et al., 2008), simultaneous modulation of several neurotransmitter systems may enhance the clinical outcome. The serotonergic (5-HT) and adenosine neurotransmitter systems have been individually targeted and explored for developing treatments in PD and LID (Goetz et al., 2007; Olanow et al., 2004). As 5-HT neurons contribute to the unregulated efflux of dopamine, becoming a prime site for dopamine catabolism after L-DOPA administration in advanced PD (Carta et al., 2007, 2008a, 2008b; Carta and Bezard, 2011), the specific modulation of 5-HT autoreceptors (Bezard et al., 2013a, 2013b; Munoz et al., 2008) or associated catabolic pathways (Ko et al., 2014a; Tronci et al., 2013) dramatically alleviates expression of LID. However, recent reports have shown that this effect can take place at the expense of the anti-parkinsonian effects of L-DOPA (Bezard et al., 2013b, 2013a), which represents a major limitation for

Abbreviations: 5-HT, Serotoninergic; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NHP, Non-human primate; PD, Parkinson's disease; LID, L-DOPA-induced dyskinesia; L-DOPA, L-3,4-dihydroxyphenylalanine

* Correspondence to: Motac Neuroscience Ltd, PO Box 363, Manchester M15 6WE, United Kingdom.
E-mail address: d.ko@motac.com (W.K.D. Ko).

[1] Place of experimentation.

http://dx.doi.org/10.1016/j.ejphar.2017.07.030
Received 27 February 2017; Received in revised form 15 July 2017; Accepted 17 July 2017
Available online 21 July 2017
0014-2999/

the use of 5-$HT_{1A/B}$ agonists in the treatment of dyskinesia. In advanced PD patients, adenosine A_{2a} receptors antagonists can be utilised as an adjunct treatment to extend the therapeutic action of L-DOPA i.e. on-time (Factor et al., 2010; Hauser et al., 2011). The blockade of localised adenosine A_{2a} receptors that are intimately linked to dopamine D_2 receptors (Ferre et al., 1997) provides modulation of the indirect (striato-pallido-nigral) pathway of the basal ganglia (Schiffmann et al., 1991, 2007). While administration of adenosine A_{2a} antagonists potentiate the effects of dopamine through alleviation of the increased endogenous adenosine tone in PD, treatment with an optimal dose of L-DOPA exacerbates LID in L-DOPA-primed parkinsonian monkeys (Ko et al., 2016) and PD patients (Mizuno and Kondo, 2013; Pourcher et al., 2012).

We have previously shown that the combined 5-$HT_{1A/B}$ agonist (eltoprazine) and adenosine A_{2a} receptor antagonist (preladenant) treatment in animal models of PD and LID (Pinna et al., 2016) acutely abolishes LID without compromising anti-parkinsonian effects of L-DOPA. In this study, we investigate the effect of this treatment regimen over 14 days repeated administration to further evaluate its potential clinical utility.

2. Materials and methods

All experimental procedures were conducted under the regulations set by the European Communities Council Directive 24 November 1986 (86/609/EEC), approved by the Institute of Laboratory Animal Science Ethical Committee (Chinese Academy of Medical Sciences, Beijing, China), and completed in an AAALAC-accredited facility.

2.1. Animals

Captive bred female monkeys (*Macaca fascicularis;* mean weight: 3.7 kg; mean age: 9 years; Xieerxin, Beijing, China) were housed individually in cages with free access to food and water. Environmental conditions were controlled (humidity, temperature, and 12 h light/dark cycle with lights on from 8:00 a.m. to 8:00 p.m.) and animal care was provided by experienced veterinarians and technicians.

2.2. Experimental Parkinsonism and L-DOPA-induced dyskinesia

Monkeys received daily MPTP hydrochloride injections (0.2 mg/kg, intravenous [IV]) for the induction of PD motor symptoms, as previously described (Bezard et al., 2001). After stabilisation of PD motor signs, animals were treated once daily with L-DOPA (Madopar; L-DOPA/carbidopa, 4:1 ratio; range, 9–17 mg/kg; Roche, Indianapolis, IN). Treatment was individually titrated for the maximum reversal of PD motor symptoms, which was defined as the 100% L-DOPA dose. Daily treatment with L-DOPA lasted for up to 5 months for induction of reproducible dyskinesia, as previously described (Gold et al., 2007; Guigoni et al., 2005; Shen et al., 2015). Thereafter, MPTP-treated animals were treated with L-DOPA twice-weekly for maintaining established dyskinesia.

2.3. Drug treatment

Eltoprazine (Carbosynth, Compton, UK) was dissolved in sterile saline and administered by subcutaneous (SC) injection at a volume of 0.25 mL/kg. Preladenant (Carbosynth, Compton, UK) was suspended in 0.5% methylcellulose (Sigma-aldrich) and administered orally (per os, PO) at 3 mL/kg.

2.4. Experimental design

L-DOPA at threshold (100%) and sub-threshold (66%) doses, defined relative to the maximal reversal of PD motor symptoms from behavioural observations, were given for establishing baseline behavioural responses prior to repeated drug treatment regimen. Thereafter, drugs were given once daily over 14 days in combination with L-DOPA 66%. On treatment day 15, animals received L-DOPA 66% alone before final behavioural assessment.

2.5. Behavioural assessment

For 4 h post drug administration, animals were video recorded in observation cages (1.1 m × 1.5 m × 1.1 m). Locomotor activity was simultaneously measured from an automated actimetry system connected to infrared sensors (Excalibur; University of Manchester, Manchester, UK). Behaviours were scored from 10 min time bins, every 30 min, as previously described (Bezard et al., 2003). A behavioural analyst blinded to treatment conditions scored each animals PD motor signs according to an established non-parametric rating scale for (i) range of movement (motor), (ii) bradykinesia, (iii) posture, and (iv) tremor, as previously reported (Ko et al., 2014b). Dyskinesia, chorea and dystonia, were also rated according to the revised nhps dyskinesia rating scale (NHPDysR) (Fox et al., 2012) and scored according to criteria as previously described (Ko et al., 2014b). 'Good on-time' was calculated on a linear scale for the periods when bradykinesia was absent and when scores for chorea and dystonia were absent, mild or moderate (Fox et al., 2012).

2.6. Statistical analyses

Data analyses were carried out using GraphPad Prism (version 6.02, Graphpad Software Inc., La Jolla, CA). The median of time course and total scores for dyskinesia and PD disability scores are presented over 4 h. Summed scores were analysed across treatment days using a nonparametric one-way repeated measures ANOVA (Friedman's test) followed by Dunn's multiple comparison. Scores at day 0 and day 15 were compared using a Wilcoxon matched test. The time course of locomotor activity is presented as mean counts in 5 min time bins over 4 h. Total locomotor activity and good on-time data are presented as mean scores. The data across treatment days were analysed using a parametric one-way repeated-measures analysis of variance (ANOVA), followed by Dunnett's multiple comparison. Data from day 0 and day 15 were compared using a paired *t*-test. $P < 0.05$ was used for significance.

3. Results

3.1. Effect of repeated eltoprazine treatment combined with sub-threshold L-DOPA in MPTP-treated macaques

3.1.1. Dyskinesia

Following combined treatment of eltoprazine and 66% L-DOPA, scores for dyskinesia (Fr = 15.36; $P < 0.01$; Fig. 1A, B), chorea and dystonia (Fr = 15.07 and Fr = 15.36; $P < 0.05$ and $P < 0.01$; data not shown) were reduced compared to L-DOPA 100% on days 1 ($P < 0.05$ for all observations) and 7 ($P < 0.05$ for all observations), but not day 14 ($P > 0.05$). There was no significant difference between the first (day 0) and final (day 15) treatment after repeated eltoprazine and 66% L-DOPA administration for dyskinesia ($P > 0.05$; Fig. 1A,B), chorea or dystonia ($P > 0.05$ and $P > 0.05$; data not shown).

3.1.2. Parkinsonian motor symptoms

PD disability following eltoprazine and 66% L-DOPA administration (Fr = 14.70; $P < 0.05$; Fig. 1C,D) was increased compared to L-DOPA 100% on treatment days 1 ($P < 0.05$) and 7 ($P < 0.05$). There was no significant difference in PD scores following a comparison of day 0 and day 15 ($P < 0.05$; Fig. 1C,D).

Eltoprazine and 66% L-DOPA treatment had a significant effect on scores for motor and bradykinesia (Fr = 14.70 and Fr = 13.70; $P < 0.05$