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The adenosine A_{2A} receptor antagonist, istradefylline enhances anti-parkinsonian activity induced by combined treatment with low doses of L-DOPA and dopamine agonists in MPTP-treated common marmosets

Shin-ichi Uchida [a], Kazuhiro Soshiroda [b], Eri Okita [b], Mika Kawai-Uchida [b], Akihisa Mori [c], Peter Jenner [d], Tomoyuki Kanda [a,*]

[a] Central Nervous System Research Laboratories, Central Nervous System Research & Development Unit, Research & Development Division, Kyowa Hakko Kirin Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan
[b] Research Core Function Laboratories, Research Functions Unit, Research & Development Division, Kyowa Hakko Kirin Co., Ltd., Shizuoka, 411-8731, Japan
[c] Strategic Product Portfolio Department, Kyowa Hakko Kirin Co., Ltd., Tokyo 100-8185, Japan
[d] Neurodegenerative Diseases Research Group, Institute of Pharmaceutical Sciences, Faculty of Life Sciences and Medicine, King's College, London SE1 1UL, UK

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ABSTRACT

The adenosine A_{2A} receptor antagonist, istradefylline improves motor function in patients with advanced Parkinson's disease (PD) optimally treated with a combination of L-DOPA and a dopamine agonist without increasing the risk of troublesome dyskinesia. However, the effects of istradefylline on motor function when administered in combination with low dose of L-DOPA and dopamine agonists as occurs in early PD are unknown. We investigated whether istradefylline enhances the combined anti-parkinsonian effects of a suboptimal dose of L-DOPA and a threshold dose of either the non-ergot dopamine agonist, ropinirole or the ergot dopamine agonist, pergolide in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated common marmoset.

Threshold doses of ropinirole (0.025–0.075 mg/kg p.o.) and pergolide (0.01 mg/kg p.o.) produced a weak anti-parkinsonian effect. Co-administration of a suboptimal dose of L-DOPA (2.5 mg/kg p.o.) with threshold doses of the dopamine agonists enhanced their anti-parkinsonian effect that led to increased 'ON' time without dyskinesia appearing. Administering istradefylline (10 mg/kg p.o.) with the threshold doses of dopamine agonists and the suboptimal dose of L-DOPA in a triple combination caused a further enhancement of the anti-parkinsonian response but dyskinesia was still absent.

In early PD, dopamine agonists are often used as first-line monotherapy, but efficacy is usually lost within a few years, at which time L-DOPA is added but with the risk of dyskinesia appearance. These results show that istradefylline is effective in improving motor function in combination with low dose dopaminergic drug treatment without provoking dyskinesia.

1. Introduction

Dopamine agonists are often used as first-line monotherapy for Parkinson's disease (PD), particularly in younger early stage patients (Bonuccelli et al., 2009) but within 2–5 years, their efficacy declines and additional dopamine replacement therapy is required. Higher dosage levels of dopamine agonists can be used but these are often associated with the onset of impulse control disorders (ICDs), vascular change, hallucinations and psychosis (Perez-Lloret and Rascol, 2010; Raja and Bentivoglio, 2012). In the majority of individuals, effective control of motor function is achieved by the addition L-3,4-dihydroxyphenylalanine (L-DOPA) to dopamine agonist therapy (Blandini and Armentero, 2014). However, adding L-DOPA carries the risk of the development of motor response fluctuations and complications such as 'wearing-off' and dyskinesia notably as its dose is increased (Ahlskog and Muenter, 2001; Nutt, 2001). This was emphasized recently, in the results of STRIDE-PD study showing the relationship between L-DOPA dose and age and the risk of developing 'wearing-off' or dyskinesia in early PD (Warren Olanow et al., 2013). These results suggest that when L-DOPA is added on to treatment with a low

* Corresponding author. Fax: +81 55 986 7430.
E-mail address: tomoyuki.kanda@kyowa-kirin.co.jp (T. Kanda).

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dose of dopamine agonists in early PD, the lowest L-DOPA dose required to achieve efficacy should be used. This strategy will initially provide adequate clinical effect but inevitably with time, the L-DOPA dose will need to be increased with disease progression so raising the risk of 'wearing off' and dyskinesia appearance.

Istradefylline (KW-6002) is a selective adenosine A_{2A} receptor antagonist approved for use in PD in Japan. In Phase IIB/III clinical trial, istradefylline reduced 'OFF' time in late stage PD patients receiving optimized dopaminergic therapy but who were exhibiting 'wearing off' and long 'OFF' periods and importantly, it did not worsen established dyskinesia (Hauser et al., 2008; LeWitt et al., 2008; Mizuno et al., 2013). However, these studies did not show the relationship between the dose of dopaminergic medication used and the effect of istradefylline as all patients were already receiving high levels of dopamine replacement therapy and they do not reflect events in early stage PD where lower doses of L-DOPA and dopamine agonists are used. This is important because in both rodent and primate models of PD, adenosine A_{2A} antagonists produce the most marked improvement in motor function when administered in conjunction with a sub-optimal dose of L-DOPA (Koga et al., 2000; Uchida et al., 2014). Similarly, adenosine A_{2A} antagonists enhance the anti-parkinsonian activity of a threshold dose of dopamine agonists in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated common marmosets (Uchida et al., 2015). However, there has not been any investigation whether adding istradefylline to low dose dopamine agonists and L-DOPA treatment in a triple combination therapy further improves motor function and avoids dyskinesia induction.

In this study, we have investigated whether istradefylline enhances the reversal of motor disability when administered in combination with low doses of L-DOPA and two clinically used dopamine agonist drugs, namely the ergot derivative, pergolide and the non-ergot compound, ropinirole in MPTP-treated common marmosets that show dyskinesia when challenged with high doses of L-DOPA.

2. Materials and methods

2.1. Animals

All experiments on animals were performed in accordance with Standards for Proper Conduct of Animal Experiments at Kyowa Hakko Kirin Co., Ltd. under the approval of the company's Institutional Animal Care and Use Committee. Fuji Research Park of Kyowa Hakko Kirin co., Ltd. is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. Common marmosets (*Callithrix jacchus*) obtained from CLEA Japan, Inc. (Tokyo, Japan) and were of either sex and over 2 years of age at the beginning of the study. The animals were housed under standard conditions (27 ± 2 °C, 20–70% humidity, 12 h light-dark cycle) and had *ad libitum* access to a pelleted diet (CMS-1M, CLEA Japan, Inc.) and to fresh water.

2.2. MPTP treatment of common marmosets

MPTP treatment was carried out using the methodology previously reported (Uchida et al., 2015). MPTP hydrochloride (Sigma-Aldrich, St Louis, USA) was dissolved in physiological saline and subcutaneously administered at 2.0 mg/kg daily for 5 consecutive days to the animals. About 8 weeks after the first exposure to MPTP, animals exhibiting consistent and marked motor deficits were selected for the study.

2.3. Drug preparation and administration

Istradefylline (KW-6002: (E)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methyl-3,7-dihydro-1 H-purine-2,6-dione; Kyowa Hakko Kirin Co., Ltd.) was suspended in 0.5 w/v% MC (Methyl cellulose 400 cP; Wako pure chemicals Co., Ltd., Osaka, Japan), 10 w/v% sucrose solution and administered in a final volume of 2.0 ml/kg body weight by oral gavage. Istradefylline was given at a single dose of 10 mg/kg (Kanda et al., 1998; Uchida et al., 2015). L-DOPA (L-3,4-dihydroxyphenylalanine; Kyowa Hakko Kirin Co., Ltd.) and benserazide (benserazide HCl; Kyowa Hakko Kirin Co., Ltd.) were also suspended in 0.5 w/v% MC, 10 w/v% sucrose solution and administered in a final volume of 2.0 ml/kg body weight by oral gavage. Ropinirole (ropinirole HCl: Sigma-Aldrich, St Louis, USA) and pergolide (pergolide mesylate; Sigma-Aldrich, St Louis, USA) were dissolved in 0.5 w/v% MC, 10 w/v% sucrose solution and administered in a final volume of 2.0 ml/kg body weight by oral gavage. In a pilot study, the dose responses to ropinirole (0.01–0.1 mg/kg p.o.) were tested to determine the doses in individual animals that produced a threshold effect on motor function. The individual threshold dose (0.025–0.075 mg/kg p.o.) was defined as the minimum dose of ropinirole which produced a detectable degree of anti-parkinsonian effect in each individual MPTP-treated common marmoset (data not shown). The threshold doses of ropinirole used in individual animals are described in Table 1. Pergolide was given at a single dose of 0.01 mg/kg. This dose produced a small non-significant anti-parkinsonian effect in a pilot study (data not shown). MPTP-treated common marmosets were treated with vehicle on Day 1, a single dose of L-DOPA or dopamine agonist or istradefylline on Day 2 and a single dose of double or triple combination with drug on Day 3. The animals were allowed at least a 5-day drug-free washout period between drug treatments. Basal locomotor activity and motor disability scores were reassessed on Day 1. Basal motor function of the individual animals was consistent between experiments.

2.4. Behavioral assessment

2.4.1. Rating of motor disability

The animals were continuously monitored by trained observers by observation through a one-way mirror. Basal motor disability was scored during the acclimatization period and once every 10 min after drug treatment for 6 h using an established motor disability rating scale (Smith et al., 1997). These values were summed with a maximum score of 17 indicating severe motor disability and a minimum score of 0 indicating normal motor function. "ON' time of the animals was defined by assessment of the numbers of 10 min periods in which motor disability scores were less than 9 (Uchida et al., 2015).

2.4.2. Measurement of locomotor activity

Locomotor activity was measured using aluminum cages ($50 \times 60 \times 70$ cm^3) with stainless steel grid doors (50×70 cm^2) equipped with eight horizontally orientated photoelectric emitters/detectors (light beams). Locomotor activity was assessed as the number of light beam interruptions accumulated in 10 min

Table 1
The threshold dose of ropinirole used in individual animals.

Animal ID	Threshold dose of ropinirole (mg/kg)	Animal ID	Threshold dose of ropinirole (mg/kg)
I2687 (M)	**0.025**	I2670 (F)	**0.075**
I3429 (M)	**0.025**	I3430 (M)	**0.075**
I3442 (M)	**0.05**	I3448 (F)	**0.075**
I3122 (F)	**0.05**	I2691 (M)	**0.075**