Parkinsonism and Related Disorders 19 (2013) 508–514

Contents lists available at SciVerse ScienceDirect

Parkinsonism and Related Disorders

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

Safinamide reduces dyskinesias and prolongs L-DOPA antiparkinsonian effect in parkinsonian monkeys

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ARTICLE INFO

Article history:
Received 16 August 2012
Received in revised form
18 December 2012
Accepted 13 January 2013

Keywords:
Safinamide
Amantadine
Dyskinesias
MPTP monkey
Parkinson's disease
L-DOPA
Motor behavior

ABSTRACT

Introduction: Safinamide is a compound under investigation for use in the treatment of Parkinson's disease for combination with pharmacological therapy currently available. The objective of this study was to test the effects of safinamide in an animal model of L-DOPA-induced dyskinesias (LID), the MPTP lesioned dyskinetic macaque monkey, in comparison to and in combination with amantadine.
Methods: LID and parkinsonian symptoms were measured in dyskinetic monkeys treated with L-DOPA with and without several dose levels of safinamide, amantadine, and the two in combination. Safinamide plasma levels were monitored during the experiments.
Results: Safinamide pre-treatment (3, 10, 20 and 30 mg/kg) dose-dependently reduced LID scores, in two acute and one semi-chronic experiment. Intensity and duration of LID were reduced and inversely correlated with safinamide blood levels. All doses of safinamide tested prolonged the duration of the beneficial antiparkinsonian effect of L-DOPA. Amantadine (5 and 20 mg/kg) reduced LID, but reduced duration of antiparkinsonian response to L-DOPA. When added to amantadine (5 mg/kg), safinamide showed no (3 mg/kg) or modest (20 mg/kg) additional beneficial effects on LID while the combined treatment prevented the reduction of the duration of the L-DOPA antiparkinsonian effect observed with amantadine only.
Conclusions: Safinamide and amantadine reduced LID in this primate model while only safinamide increased the duration of the antiparkinsonian response of L-DOPA, suggesting that safinamide may have effects on LID that are pharmacologically distinct from amantadine, which is in current clinical use for control of LID.

1. Introduction

Safinamide is an α-aminoamide compound with dopaminergic and non-dopaminergic activities being studied for use as add-on to L-3,4-dihydroxyphenylalanine (L-DOPA) or dopamine agonist therapy for patients with Parkinson's disease (PD) [1]. Clinical testing has shown that safinamide daily dosing increased on-time free of troublesome dyskinesias in early- and late-stage PD patients [2]. Safinamide's pharmacological activities include selective and reversible inhibition of monoamine oxidase-type B (MAO-B), use- and frequency-dependent blockade of voltage-gated sodium channels, calcium channel inhibition and reduction of induced presynaptic glutamate release *in vitro* [3-5].

Both dopaminergic and non-dopaminergic components (including but not limited to glutamate transmission imbalance) are implicated in L-DOPA-induced dyskinesias (LID), a common treatment complication in PD patients with no currently approved therapeutic options [6–11]. LID consists of involuntary, aimless movements resulting from repeated dopaminergic stimulation in PD patients [12]. Although MAO-B inhibition, considered a pro-dopaminergic action, is one of safinamide's activities, a small open-label study suggested that it may reduce LID [13]. This suggests that the mechanisms of action of safinamide in PD therapy may not be limited to inhibition of MAO-B. Dyskinesias are associated with changes in several neurochemical systems, including excessive glutamatergic activity [14]. Amantadine, with antagonist activity on NMDA receptors, reduces LID in PD patients [15].

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http://dx.doi.org/10.1016/j.parkreldis.2013.01.009

The aims of this study were to characterize the effects of safinamide on the expression of dyskinesias and parkinsonian symptoms in an animal model widely used to study mechanisms and drug actions against LID, and to compare its effects with amantadine, to help in the understanding of safinamide's effects in PD patients.

2. Methods

2.1. Animals

Twelve female ovariectomized cynomolgus monkeys (*Macaca fascicularis*) weighing 2.8–4.4 kg were used for these experiments. The primates were handled in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures were reviewed and approved by the Institutional Animal Care Committee of Laval University. The animals were rendered parkinsonian by continuous infusion of MPTP (Sigma–Aldrich, Canada) using subcutaneous osmotic minipumps (Alzet, 0.5 mg/24 h) until they developed a stable parkinsonian syndrome. After one to three months of stabilization, animals were treated daily with L-DOPA/benserazide 100/25 capsule p.o. (Prolopa, Hoffmann-La Roche) until clear and reproducible dyskinesias developed.

2.2. Test compound

Merck Serono S.A., Geneva, Switzerland, provided safinamide. A fresh safinamide or amantadine (Sigma–Aldrich, Canada) aqueous solution was prepared on every experimental day. A volume between 10 and 20 ml of the safinamide or amantadine solution was administered by nasogastric gavage under normal restraining condition. The nasogastric tube was removed after administration prior to the behavioral assessment. L-DOPA methyl ester and benserazide (Sigma–Aldrich, Canada) were dissolved in sterile 0.9% saline solution and pH adjusted to 6.

2.3. Acute dose–response behavioral effect of safinamide (Experiment 1)

Three weeks before beginning the acute study, seven animals were primed by repeated administration p.o. (thrice per week) with L-DOPA/benserazide 100/25 or 50/12.5 adjusted according to the motor response of the animals. Monkeys were first evaluated following vehicle (water) administration alone (baseline) and with vehicle + L-DOPA/benserazide administered subcutaneous. L-DOPA doses were adjusted for each monkey and varied from 15 to 35 mg/kg and were always given with a fixed dose of benserazide (50 mg total). Ascending doses of safinamide (3, 10 and 30 mg/kg) as add-on to L-DOPA were tested. Safinamide was given 1 h before L-DOPA administration.

2.4. Acute behavioral effect of safinamide, crossover design (Experiment 2)

A crossover design was used in Experiment 2 with six of the MPTP monkeys used in Experiment 1. On the first observation day, half of the MPTP-treated monkeys were administered vehicle and the others safinamide 20 mg/kg, followed 1 h later by L-DOPA/benserazide. After a one-week washout period, a crossover of the treatments was performed. All other experimental conditions were as in Experiment 1.

2.5. Semi-chronic behavioral effect of safinamide, crossover design (Experiment 3)

Eight MPTP monkeys (5 were different from those used in Experiments 1 and 2) were used in a semi-chronic experiment. For the first seven days, half of them received each day at 7 h 30 min safinamide 10 mg/kg p.o. and the others vehicle p.o., and at 8 h 30 min all received L-DOPA/benserazide subcutaneous; then, at 15 h 30 min, the dosing of safinamide or vehicle was repeated. Hence, half the monkeys received two administrations of safinamide at 10 mg/kg for a total of 20 mg/kg/day of safinamide. After a one-week washout period, a crossover of the treatments was performed. All other experimental conditions were as in Experiment 1. Motor behavior was assessed after the morning drug administrations. Experimental days were separated by two-day holiday following the last oral administration of L-DOPA (50 or 100 mg) to maintain priming. One monkey was removed from the analyses because its motor behavior induced by L-DOPA was not stable.

2.6. Acute dose–response behavioral effect of amantadine and safinamide (Experiment 4)

Seven MPTP monkeys, (six were the same as in Experiment 3), were used in an acute dose–response. Ascending doses of amantadine (0.3, 1, 5 and 20 mg/kg) were given orally 1 h before L-DOPA administration. L-DOPA was tested 3, 9, 15 and 18 days after the last amantadine test as a washout phase. Five days after the last L-DOPA test, safinamide (3 and 20 mg/kg p.o.) was given as add-on to L-DOPA. In the second washout phase, L-DOPA was tested 6 days after the last administration of safinamide. Then, three combinations of both medications (1 and 3, 5 and 3, 5 and 20 mg/kg of amantadine and safinamide respectively) as add-on to L-DOPA were tested separated by a seven days washout period. All other experimental conditions were as in Experiment 1. Between test days, oral L-DOPA (50 or 100 mg) was administered to maintain priming.

2.7. Safinamide assay

In Experiments 1 and 2, a blood sample was taken 5 h after administration of L-DOPA (6 h after safinamide administration) after the behavioral measures. In Experiment 3, one blood sample was taken in the morning before safinamide administration, another sample 6 h after, and five blood samples on Day 8, one at the time of safinamide administration and the others 1, 2, 4, and 6 h afterwards. Blood samples (1 ml) were collected in EDTA tubes and centrifuged (10 min, 1000 × g) at 4 °C. Plasma samples were then stored at −80 °C to be later analyzed. Plasma concentrations of safinamide were assayed by liquid chromatography–mass spectrometry/mass spectrometry (method in Study Number 0070/456, on file at Covance Laboratories Limited, Otley Road, Harrogate, HG3 1PY, United Kingdom).

2.8. Behavioral assessment

The animals were scored every 15 min for antiparkinsonian and dyskinetic responses during the whole duration of response to L-DOPA. The observer was unblind to the treatments in Experiment 1 in order to monitor the acute response of the animals to safinamide. The observation was made "live" through a one-way screen in Experiments 1 through 3, whereas in Experiment 4 animals were filmed and scored blind *a posteriori*. The observer was blind in Experiments 2–4. Disability and dyskinesia scales developed in our laboratory were used to evaluate the parkinsonian syndrome and dyskinesias in MPTP monkeys [16,17]. Parkinsonian symptoms were rated on a scale with a maximal (more severe) score of 16. Dyskinesias were rated for the face, neck, trunk, arms and legs and summed (maximal score: 21). The duration of response was calculated from the beginning of the antiparkinsonian response to L-DOPA until its effect subsided.

2.9. Data analysis

For each treatment day and for each monkey, a mean parkinsonian score and a mean dyskinetic score (total period) were obtained by averaging all 15-min scores obtained during the whole duration of the L-DOPA response. Parkinsonian and dyskinesia scores were analyzed with a Friedman nonparametric test followed by a Dunn's multiple comparison test. A Wilcoxon test was used to compare two groups. Values for duration and delay of responses were analyzed by analysis of variance for repeated measures followed by a Dunnet's multiple comparison test. Correlations between plasma safinamide concentrations and dyskinesia scores were made using the Pearson Correlation test. A p value < 0.05 was considered significant. All analyses were done using Prism 4.0 for Macintosh (GraphPad, La Jolla, California, USA).

3. Results

3.1. Acute dose–response behavioral effect of safinamide

Compared with baseline, L-DOPA reduced parkinsonian symptoms; this response was maintained in animals treated with all three doses of safinamide added to L-DOPA (Fig. 1A). Moreover, the duration of the L-DOPA response was prolonged by each dose of safinamide (Fig. 1B). Safinamide did not affect the elapsed time after L-DOPA administration for behavioral response ($F_{4,24} = 2.83$, $p = 0.82$, data not shown). Safinamide was also associated with dose-dependent improvements in LID. There were reductions in the mean dyskinesia score over the whole assessment period for each dose of safinamide (Fig. 1C). The time course of dyskinesia scores clearly showed a reduction in intensity and duration of dyskinesias during the L-DOPA behavioral response (Fig. 1D).

The results obtained in the crossover experiment were very similar to those obtained in the dose–response experiment. The antiparkinsonian effect of L-DOPA was maintained with 20 mg/kg safinamide (Fig. 2A), and the duration of the L-DOPA response was prolonged (Fig. 2B). Safinamide did not affect the elapsed time after L-DOPA administration for behavioral response ($F_{2,10} = 6.13$, $p = 0.67$, data not shown). Safinamide reduced mean LID (Fig. 2C). The time course of dyskinesia scores with 20 mg/kg add-on safinamide is similar to results of Experiment 1 with a reduction in intensity and duration of dyskinesias (Fig. 2D).