

## Neuroprotective effects of rotigotine in the acute MPTP-lesioned mouse model of Parkinson's disease

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Received 19 July 2007; received in revised form 21 November 2007; accepted 1 December 2007

### Abstract

Dopamine agonists used to manage Parkinsonian motor symptoms have been suggested to be neuroprotective. The study was designed to assess the neuroprotective potential of the D<sub>3</sub>/D<sub>2</sub>/D<sub>1</sub> dopamine receptor agonist rotigotine in the acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned (MPTP) mouse model of Parkinson's disease by measuring mesencephalic degenerating neurons using FluoroJade staining and the remaining dopaminergic nerve endings in the striatum using dopamine transporter binding. Continuous administration of rotigotine at a dose of 3 mg/kg significantly attenuated MPTP-induced acute cell degeneration in the FluoroJade-staining paradigm. Rotigotine (0.3–3 mg/kg) partially protected dopamine nerve endings from MPTP-induced degeneration in a dose-dependent manner. These data suggest that rotigotine, at the doses employed, significantly protected dopamine neurons from degeneration in an acute mouse model of MPTP intoxication.

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**Keywords:** Rotigotine; Dopamine agonist; Continuous administration; Striatum; Substantia nigra pars compacta; Transdermal patch; Dopamine transporter

In contrast to other neurodegenerative conditions, symptomatic therapy exists for Parkinson's disease (PD). Unfortunately, there is no proven therapy to prevent or slow the progressive neuronal cell death, or restore abnormally behaving neurons to a normal state [7]. There has been increasing interest in the development of drugs to modify the biochemical abnormalities that cause the neurodegeneration and thus alter the course of PD, either by retarding the rate of cell death or by restoring function to neurons that are likely to undergo degeneration [6]. In this context, dopamine (DA) receptor agonists have shown promise. Not only do these drugs provide symptomatic relief of PD but they also appear to be associated with a significant decrease in the rate of motor complications and to be capable of protecting against some of the adverse consequences of levodopa use (for

review, see Ref. [23]). Evidence based largely on experimental *in vitro* and *in vivo* rodent studies is emerging suggesting that some DA agonists may have neuroprotective properties in addition to their symptomatic effects (for review, see Refs. [19,24]). In this context, the present study was designed to investigate the potential of the D<sub>3</sub>/D<sub>2</sub>/D<sub>1</sub> DA receptor agonist rotigotine to slow down PD progression in an acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mouse model of Parkinson's disease. As neurodegeneration is occurring stochastically over some period of time, rotigotine was administered continuously using a slow release formulation mimicking the release from a transdermal patch formulation as used clinically.

Experiments were carried out in accordance with European Communities Council Directive of 24 November 1986 (86/609/EEC) for care of laboratory animals. All efforts were made to minimise animal suffering and to use the minimum number of animals necessary to perform statistically valid analysis. C57BL/6J@RJ male mice (Breeder: Elevage Janvier, 53940 Le Genest, France) weighing 26–29 g were housed in a temperature-controlled room under a 12 h light/dark cycle with free access

**Abbreviations:** DA, dopamine; DAT, dopamine transporter; FJ, FluoroJade; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; SD, standard deviation; SN, substantia nigra.

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Table 1  
Experiment no. 1 design

Groups	Day 1		Days 2–8		Brain
	Rot/Veh	MPTP/sal	Rot/Veh	<i>n</i>	
A1	–	–	–	10	FF
A2	Veh	Sal	Veh	11	FF
A3	Veh	MPTP	Veh	10	FF
A4	0.3	MPTP	0.3	6	FF
A5	1.0	MPTP	1.0	6	FF
A6	3.0	MPTP	3.0	7	FF
A7	3.0	Sal	3.0	12	FF

Rot: rotigotine (mg/kg); Veh: vehicle; Sal: Saline; FF: fresh frozen. On day 1, animals received rotigotine or its vehicle followed by four injections of MPTP or saline (i.p.) at 2 h intervals. They then received rotigotine or its vehicle once daily on days 2–8. Brains were collected fresh frozen on day 8.

to food and water. To induce neurodegeneration of half of the dopaminergic neurons in the substantia nigra, mice were treated with MPTP hydrochloride on day 1 (four injections of MPTP; 20 mg/kg i.p. at 2 h intervals; saline as vehicle) as previously described [2,4,18,20]. Experimental groups received an oily suspension of rotigotine (s.c., SPM 962, batch no. 20004007, Schwarz BioSciences GmbH) or rotigotine vehicle (s.c., batch no. 20104016, Schwarz BioSciences GmbH). Two experimental designs were used. In experiment 1 (Table 1), mice received rotigotine/vehicle slow release formulation shortly before 1st MPTP/saline administration (day 1) and subsequently once daily from days 2 to 8. They were euthanized 4 h after the last rotigotine or vehicle administration on day 8, i.e. 7 days after the MPTP injections [12], by cervical dislocation followed by decapitation. In experiment 2 (Table 2), mice received rotigotine/vehicle slow release formulation on day 0, i.e. 18 h prior to MPTP/saline (day 1). They were then anesthetized and euthanized by paraformaldehyde perfusion 24 h later.

DAT binding using [<sup>125</sup>I]-(*E*)-*N*-(3-iodoprop-2-enyl)-2β-carboxymethyl-3β-(4'-methylphenyl)-nortropine (PE2I; Chelatec, France) was measured on fresh frozen cryostat-cut 20 μm thick slide-mounted coronal sections as previously described [2,3,9]. Briefly, sections were incubated for 90 min at 25 °C with 100 pM [<sup>125</sup>I]PE2I in pH 7.4 phosphate buffer (NaH<sub>2</sub>PO<sub>4</sub> 10.14 mM, NaCl 137 mM, KCl 2.7 mM, KH<sub>2</sub>PO<sub>4</sub> 1.76 mM). After thorough rinsing and drying, sections were

Table 2  
Experiment no. 2 design

Groups	Day 1		Day 2		Brain
	Rot/Veh	MPTP/sal	<i>n</i>		
B1	Veh	Sal	5	PFA	
B2	Veh	MPTP	5	PFA	
B3	0.3	MPTP	5	PFA	
B4	1.0	MPTP	5	PFA	
B5	3.0	MPTP	5	PFA	
B6	3.0	Sal	3	PFA	

Rot: rotigotine (mg/kg); Veh: vehicle; Sal: Saline; PFA: perfused with paraformaldehyde. On day 0, animals received rotigotine or its vehicle 18 h prior four injections of MPTP or saline (i.p.) at 2 h intervals. They were then anesthetized and killed by perfusion 24 h later.

exposed to β radiation-sensitive film (Hyperfilm βmax, Amersham, UK) in X-ray cassettes, for 2 days. Densitometric analysis of autoradiographs was performed using an image analysis system (Densitag V. D2.99, Biocom) as previously described [2,3,9]. The optical density was assessed in both the dorsal and ventral part of the rostral caudate-putamen defined according to a mouse brain atlas [8]. Four sections per animal were analysed by an examiner blinded to the experimental conditions. Optical densities were averaged in each animal and converted to amount of radioactivity bound by comparison to the previously measured sections from control animals [9]. Mean radioactivity bound and S.D. were then calculated for each group. Data are expressed in fmol/mg of tissue equivalent.

FluoroJade-staining was performed as in Schmued and Hopkins [27]. Deparaffinized and rehydrated 18 μm-thick sections were incubated in a solution of 0.01% FluoroJade (Histo-Chem, Jefferson; AR, USA) in 0.1% acetic acid for 30 min and were visualized under epifluorescence (with a fluorescein/FITC filter). Images were captured with an imaging system (Sony MC3255 camera, KS100 Rel.3.0). For final output, images were processed using PhotoImpact 4 software. For each animal, FluoroJade-positive (FJ+) cells were counted by two independent examiners, one being blind to the treatment schedule. FJ+-cells were counted in two representative mesencephalic planes. All fluorescent profiles irrespective of the presence of a nucleus were counted. Mean number of FJ+-cells ±S.D. were calculated for each group.

Rotigotine vehicle/MPTP treatment (A3) elicited a significant reduction in DAT binding in both the dorsal (84% decrease) and ventral (84% decrease) parts of the striatum, compared to animals receiving rotigotine vehicle/MPTP vehicle (A2) (both  $P < 0.001$ ; Figs. 1 and 2). In the dorsal part of the striatum, rotigotine (0.3, 1 and 3 mg/kg)/MPTP-treated groups (A4, A5 and A6, respectively) had significantly higher DAT binding than animals treated with rotigotine vehicle/MPTP (A3) (100%, 340% and 365% higher, respectively,  $P < 0.05$ ,  $P < 0.001$  and  $P < 0.001$ ,

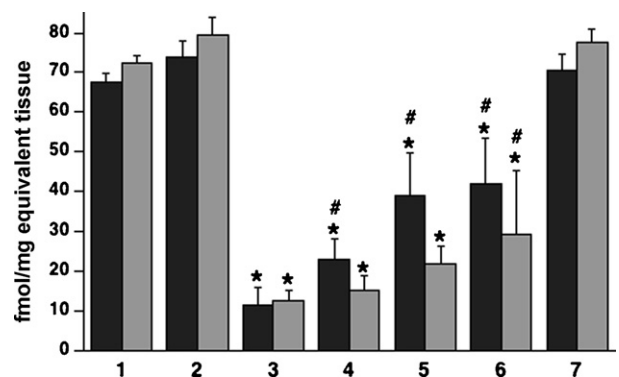


Fig. 1. Effect of rotigotine on DAT binding. DAT binding (fmol/mg equivalent tissue) in dorsal (dark columns,  $F_{(6,61)} = 155.23$ ,  $P < 0.0001$ ) and ventral part (grey columns,  $F_{(6,61)} = 222.27$ ,  $P < 0.0001$ ) of the striatum in the seven groups of experiment 1 animals. (\*) Significant decrease in dopamine transporter binding compared to Group 2; (#) significant preservation of binding in comparison with Group 3. Data were analysed with a one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison's test (Intercooled Stata 6.0, Stata Corporation, College Station, TX).

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