Published in final edited form as:
Parkinsonism Relat Disord. 2008 ; 14(Suppl 2): S112–S115. doi:10.1016/j.parkreldis.2008.04.012.

Modeling PD pathogenesis in mice: advantages of a chronic MPTP protocol

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Abstract

Formidable challenges for Parkinson's disease (PD) research are to understand the processes underlying nigrostriatal degeneration and how to protect the dopamine neurons. Fundamental research relies on good animal models that demonstrate the pathological hallmarks and motor deficits of PD. Using a chronic regimen of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and probenecid (MPTP/p) in mice, dopamine cell loss exceeds 60%, extracellular glutamate is elevated, cytoplasmic inclusions are formed and inflammation is chronic. Nevertheless, isradipine, an L-type calcium-channel blocker, attenuates the degeneration. These data support the validity of the MPTP/p model for unravelling the degenerative processes in PD and testing therapies that slow their progress.

Keywords

probenecid; L-type calcium channel; grid test; alpha-synuclein; substantia nigra; dopamine; isradipine

1. Introduction

Parkinson's disease (PD) is characterized by progressive loss of dopamine neurons and terminals from the nigrostriatal pathway and by a slow onset of motor symptoms. To provide insight into the pathophysiological processes of this disease, animal models should mimic as many of the clinical features as possible. The loss of the dopaminergic pathway can be replicated in rodents using various surgical, toxic or genetic approaches. Over the past couple of decades, one neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), has become a widely used method for modeling PD. However, for most MPTP models, the loss of

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Conflict of interest statement
The authors have declared no conflicts of interest.

Role of the funding source
Funding was provided by the United States Army Medical Research and Material Command (grant number W81XWH-05-1-0580 to G.E.M.) and the NIH (grants NS41799 to G.E.M., and NS047085 to D.J.S.).

dopamine is rapid and not progressive, and the motor disability is often difficult to demonstrate, especially when tested some time after toxin application [1]. A model that shows great promise, particularly in its progressive nature, involves the administration of MPTP and an adjuvant, probenecid (MPTP/p), that blocks the rapid clearance of the toxin and its metabolites [2]. Chronic MPTP/p treatment produces many of the pathological hallmarks and motor deficits of PD, making it an excellent choice for studies of pathogenesis, for testing neuroprotective therapies and developing biomarkers to detect the disease presymptomatically [3,4]. This review covers the key features of this model and discusses its applicability to neuroprotective strategies.

2. Preparation of the chronic MPTP/p model

Male C57/bl mice, initially weighing 20−24g, are injected with 10 doses of MPTP hydrochloride (25mg/kg in saline, s.c.) and probenecid (250mg/kg in tris-HCl buffer) over 5 weeks at 3.5-day intervals. Control mice are injected with vehicle (saline or probenecid) in the same volume and on the same schedule. Three days before treatment, and each week thereafter, mice are tested for coordination and rigidity using the grid test [1,3,5]. Briefly, mice are placed in the center of a wire mesh grid, which is then rotated 180 degrees to suspend them upside down. Mice are allowed to move freely on the grid and their movements are filmed for 60 s. Forepaw foot-faults and total forepaw steps are recorded for each of three trials and a ratio (foot-faults/total steps) is established per mouse. Data are pooled for each group and paired Student's *t*-tests compare within- groups' grid activity pre- and post-treatments, and unpaired *t*-tests compare between-groups' grid performance.

After the final behavioral test, mice are perfused transcardially with fixative and their brains prepared for light (LM) or electron (EM) microscopy. For LM, adjacent sections are immunoreacted for tyrosine hydroxylase (TH), Mac-1 and alpha-synuclein. The total number of TH-immunoreactive, Nissl-stained neurons and Mac-1-immunoreactive cells (microglia) are estimated with optical disectors (optical fractionator approach) using dedicated software (StereoInvestigator, Microbrightfield, Williston, VT). Inclusions are identified using LM, confocal microscopy and EM. Sections for EM are immunoreacted for TH and prepared for examination in a Philips 400 electron microscope. Mice were killed by cervical dislocation and decapitation and then striatal dopamine concentration is determined electrochemically (Coulochem II, ESA, Chelmsford, MA).

3. Dopamine loss, motor dysfunction, inflammation and inclusion formation

The chronic MPTP/p model shows a significant reduction in the number of neurons in the substantia nigra pars compacta (SNpc). Shortly after MPTP/p treatment, approximately 50% of dopamine neurons are lost, increasing to nearly 70% 3 weeks after toxin treatment (Table 1). Striatal dopamine levels are reduced by 90−93% within a week, and by 70−80% of the total at 3 to 24 weeks after MPTP/p treatment [4]. The low level of striatal dopamine is matched by a significant loss of TH-immunopositive fibers throughout the caudate putamen, especially in central and medial parts [2]. Dopamine loss correlates well with motor deficits. As early as 3 days post-MPTP/p treatment, mice show a significant disability on the grid and the impaired performance persists up to 6 months post-MPTP/p treatment. Typically, vehicle-treated mice perform significantly better (ratio of foot-faults/total steps: 0.036 ± 0.01) than MPTP/p-treated animals (0.167 ± 0.04; $p < 0.001$, Student's *t*-test).

There is a strong inflammatory response in the SN 3 weeks after MPTP/p treatment (density of microglia for the MPTP/p group: $2.76 \pm 0.04 \times 10^4/mm^2$ versus vehicle [probenecid] group: $2.39 \pm 0.09 \times 10^4/mm^2$; $p < 0.05$, Student's *t*-test). Reactive microglia with large cell bodies and short processes are also found after MPTP/p treatment and persist for months [6].

The formation of inclusion bodies has been demonstrated for several chronic MPTP models, but not for acute or subchronic models [7-11]. Presumably, the slow administration of MPTP/p can induce prolonged damage to mitochondria and precipitate alpha-synuclein toxicity, resulting in cytoplasmic accumulation of alpha-synuclein and ubiquitin proteins. For the MPTP/p model, inclusions have been identified in cell bodies and dendrites of TH-immunoreactive neurons as early as 2 to 3 weeks after toxin administration. These inclusions immunostain for alpha-synuclein, DJ-1 and ubiquitin (Fig. 1 [7,8]) and, at the EM level, are granular, contain lipid droplets, proteinaceous deposits and parallel membranes (Fig. 2 [7]). Ultrastructurally, the granules have the appearance of lipofuscins or secondary lysosomes (Fig. 2), cellular organelles that accumulate with age but at a significantly faster rate in neurodegenerative disease [12]. In PD, lipofuscins are closely associated with lipid droplets and neuromelanin, and may be important for the development of Lewy bodies [12].

4. Cell death in the MPTP/p model

MPTP intoxication rapidly and persistently depletes ATP and increases reactive oxygen and nitrogen species molecules that induce cell death pathways. In acute or subchronic MPTP models, less than half of the SNpc dopaminergic neurons are destroyed, whereas nigrostriatal degeneration with the chronic MPTP/p regimen is more extensive (Table 1 [2]). This is because striatal dopamine depletion peaks within 24 hours after a single dose of MPTP, but that loss is extended with MPTP/p, presumably due to the probenecid's competitive block of active transport of the toxin at the kidney and blood-brain-barrier [2]. This means that more dopamine neurons die over time. The chronic MPTP/p model also reveals numerous pathological features, such as persistent inflammation, alpha-synuclein-positive inclusions, and aberrant elevations in extracellular glutamate (Meredith and Meshul, unpublished results), all of which would increase vulnerability to calcium (Ca^{2+}) influx and excitotoxicity. Prolonged intervention with compounds that reduce Ca^{2+}-dependent cellular stress could, therefore, be tested with this model.

5. Neuroprotection

Adult SNpc dopamine neurons are Ca^{2+}-dependent autonomous pacemakers, the basal activity of which is driven by the relatively rare, voltage-dependent, L-type Ca^{2+} channel $Ca_v1.3$ [13]. Pacemaking elevates cytosolic Ca^{2+}, and would therefore harm neurons that are energy-compromised through mitochondrial stress (as in PD [12,14]). If the Ca^{2+} dependence of pacemaking could be changed, perhaps through the blockade of $Ca_v1.3$, some protection may be afforded to dopamine neurons. We conducted *in vivo* experiments using mice administered isradipine, a potent L-type Ca^{2+} channel blocker, during treatment with MPTP/p. Mice were implanted with extended release pellets with biodegradable-carrier bound isradipine (60 days, 3μg/g/day) or inert placebo pellets (Tocris, Ellisville, MO) 1 week before toxin or vehicle treatment. One week after treatment, mice were tested on the grid and, 24 hours later, euthanized and TH-immunoreacted so that Nissl-counterstained neurons in the SNpc could be counted. Isradipine significantly improved performance compared to mice implanted with placebo and, although MPTP/p-treated mice with isradipine lost significantly more SNpc neurons than those treated with vehicle, isradipine attenuated the loss compared to MPTP/p-treated, placebo-implanted mice (Table 1 [3]). Protection was not due to isradipine affecting MPTP metabolism, because brain 1-methyl-4-phenylpyridinium ion (MPP+) levels did not differ between toxin-treated groups [3].

In conclusion, mice treated with MPTP/p exhibit many features of PD, including dopamine cell loss, motor deficits, inclusion formation and inflammation. The model is thus an attractive choice for testing neuroprotective strategies or for developing biomarkers for early detection of disease.

Acknowledgements

This article is based on a presentation given at the LIMPE Seminars 2007 'Experimental Models in Parkinson's Disease' held in September 2007 at the "Porto Conte Ricerche" Congress Center in Alghero, Sardinia, Italy. Technical assistance was provided by J. Jackolin, F. Jodelka, S. Blume, and Drs. M. Mo and P. Loomis, and the work was supported financially by a grant from the United States Army Medical Research and Materiel Command (USAMRMC) NETRP Program and USPHS grants from the National Institutes of Health (NIH), USA.

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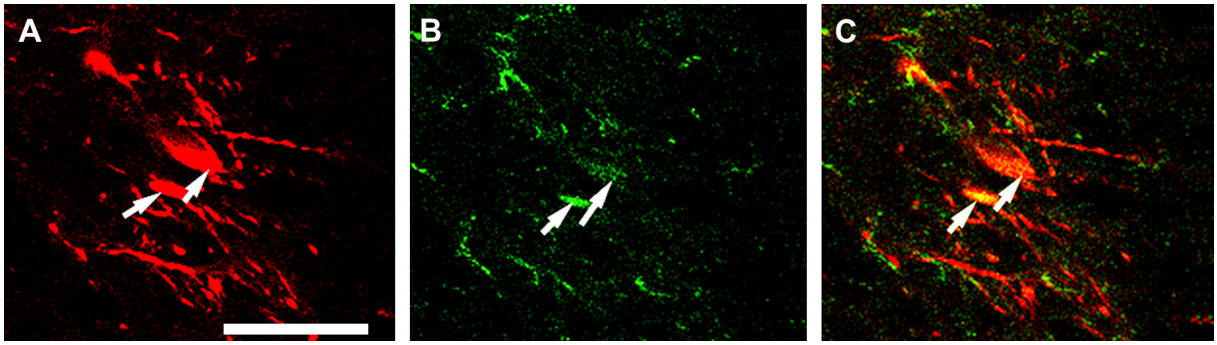

Fig. 1.

Images of TH and alpha-synuclein immunoreactivity in the SNpc. (A) TH-immunoreactive neurons, (B) alpha-synuclein-immunoreactive puncta (note the varicosities of alpha-synuclein terminals), and (C) merged image showing the alpha-synuclein-immunoreactive granular inclusions in TH-immunoreactive neurons. Scale bar in A is valid for A, B and C, and equals 25μm.

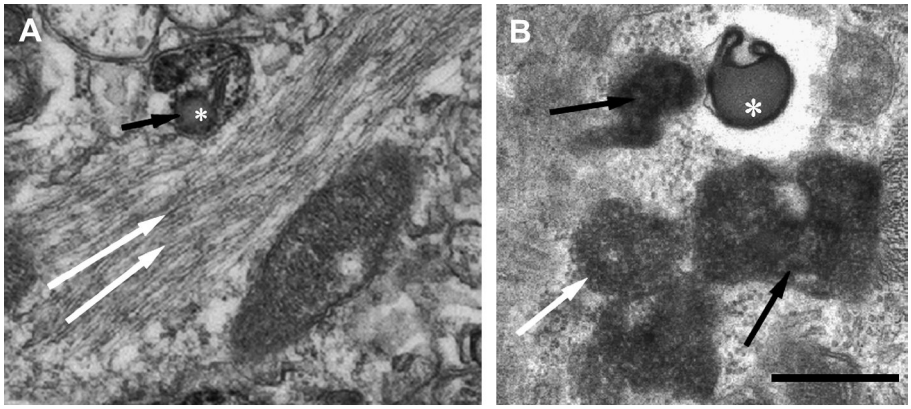

Fig. 2.

Ultrastructural appearance of inclusions in the SNpc of MPTP/p-treated mice. (A) A TH-immunoreactive membrane-bound structure is filled with a proteinaceous deposit (black arrows) and an electron lucent lipid deposit (asterisk). Note extracellular parallel fibers (white arrows). (B) Proteinaceous (black arrows) cytoplasmic deposits and lipid (asterisk) in the SNpc. Scale bar in B is valid for A and B, and equals 0.5μm.

Table 1

Stereological results for TH-immunoreactive, Nissl-stained neurons in the SNpc, including reference volume (Vr) and estimated total number of neurons. Data are derived from two experiments, separated below by double lines.

Treatment	N	Vr ± SEM (mm^3)	Total number of neurons ± SEM ($\times 10^3$)	Coefficient of error
MPTP/p	8	0.164±0.003[†]	3.139 ± 0.12[a]	0.11
Probenecid	6	0.170±0.004	9.440 ± 0.11	0.09
Saline	5	0.178±0.008	9.672 ± 0.10	0.10
MPTP/p + placebo	4	0.186 ± 0.02[†]	3.669± 0.27[a]	0.10
MPTP/p + isradipine	7	0.185 ± 0.019	6.789± 0.56,[bc]	0.08
Saline + isradipine	5	0.193 ± 0.023	9.607 ± 0.3	0.07

[a] $p < 0.01$ (Student's *t*-test), significantly less than in vehicle-treated group(s).

[b] $p < 0.01$ (Student's *t*-test), significantly less than in saline/isradipine-treated group.

[c] $p < 0.001$ (Student's *t*-test), significantly greater than in MPTP + placebo group.

[†] No significant difference between groups.