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Chronic Intraventricular Administration of 1-Methyl-4-Phenylpyridinium as a Progressive Model of Parkinson's Disease

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Abstract

Animal models of Parkinson's disease (PD) that more closely exhibit the chronic neuropathology seen in the human condition are needed in order to reveal processes involved with progressive neurodegeneration and for testing potential interventions for retarding dopamine (DA) neuronal loss. Here we describe the recently developed chronic rat model of PD in which 1-methyl-4-phenylpyridinium ion (MPP⁺) is infused chronically into the lateral cerebral ventricle. We review features of this model that include loss of nigral DA neurons, swollen and abnormal mitochondria, striatal inclusion-like bodies and microgliosis. Advantages as well as limitations of the model are addressed.

Keywords

Chronic Parkinson's disease model; rat; MPP⁺; dopamine; neurodegeneration; stereology

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor-related symptoms due to the loss of nigrostriatal dopamine (DA) neurons. Although other neuronal populations are also affected in PD, it is the extensive loss of DA neurons that is characteristic of the disorder and it is these neurons that are primarily targeted for study in experimental models of PD.

Many PD models are based on chemical neurotoxins (e.g., MPTP [1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine], 6-hydroxydopamine, malonate) that produce an acute insult and a fairly rapid destruction of the DA neurons. The MPTP model is well known for its preferential targeting of DA neurons due to the selective uptake of the active metabolite MPP⁺ (1-methyl-4-phenylpyridinium ion) via the DA transporter. Although the acute MPTP model has provided significant insight into the pathology of PD, a model with chronic low-level perturbation of mitochondrial and cellular function may better reproduce the events

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicts of interest.

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relevant to the progressive degeneration of neurons. Chronic MPTP mouse models have been developed recently, e.g., the MPTP/probenecid model and the chronic MPTP model in which the neurotoxicant is administered over several weeks [1,2]. Our goal was to develop a chronic MPP⁺ rat model, as it would provide a second species for testing and also because the larger brain size in rats compared with mice makes it more amenable to various procedures such as microdialysis testing in small brain regions. We selected to use the intraventricular (icv) route of MPP⁺ delivery because 1) the rat is essentially insensitive to the neurotoxic effects of systemically administered MPTP, 2) MPP⁺ does not readily cross the blood-brain barrier, and 3) the icv route would eliminate the detrimental peripheral effects (e.g., cardiovascular) of systemic MPTP/MPP⁺. The characterization of this model and its use for evaluating neuroprotective strategies have been detailed previously [3,4]. In this article, we review the model and discuss some of its potential uses as well as its limitations.

2. Intraventricular delivery of MPP⁺

MPP⁺ is delivered via an Alzet osmotic minipump (DURECT Corp., Cupertino, CA, model 2ML4 which delivers 2 μ l/hour for 28 days) inserted under the skin in the back of the rat. The pump is attached via tubing to a cannula that is stereotactically placed for delivery of MPP⁺ into the anterior portion of the left lateral ventricle (the anterior portion of the lateral ventricle was targeted so as to minimize perfusion to the opposite hemisphere and therefore produce a unilateral lesion). No mortalities occurred even at high doses of MPP⁺. Rates of weight gain were similar in controls and treated rats after an initial lag period following surgery. Thus, we are able to achieve animals with good lesions of the nigrostriatal DA neurons without high mortality or morbidity.

3. Loss of DA neurons

3.1 Striatal neurochemistry

MPP⁺, delivered at 0.086 to 0.86 mg/kg/day for 28 days, produced a dose-dependent loss of DA ipsilateral to the icv infusion. No DA loss was observed in the contralateral striatum at any of the doses. At the lower MPP⁺ doses (0.086 and 0.142 mg/kg/day), DA content of the infused side was 63% and 47% of that of the respective contralateral side, whereas serotonin and GABA levels were unaffected. At the higher doses (0.432 and 0.860 mg/kg/day), DA loss was >90%, but significant reductions in serotonin also occurred. There was also a corresponding loss of tyrosine hydroxylase (TH) immunoreactivity in the dorsomedial striatum on the side of infusion. These findings indicate that selective damage to DA neurons can be achieved by using the lower doses of MPP⁺.

3.2 Nigral DA cell counts

A marked loss of TH-immunoreactive neurons was seen in the substantia nigra (SN). In rats treated with 0.142 mg/kg/day and euthanized at 28 days, TH-positive cells were reduced by 35%. In rats euthanized at 42 days, cell loss was significantly greater (65% reduction). Moreover, many of the surviving nigral DA neurons stained positive with silver staining, a sign of ongoing neurodegeneration. These findings indicate that chronic MPP⁺ administration leads to DA cell loss and that neurodegeneration progresses in the absence of continued MPP⁺ exposure. Although additional work needs to be performed, these initial findings are encouraging that the model will be extremely useful for studying the processes involved with progressive neurodegeneration.

4.0 Other pathological features

Other findings that make the model attractive are the presence of inclusion-like bodies, abnormal mitochondria and microgliosis. Inclusion bodies immunopositive for ubiquitin and

α -synuclein were found in the striatum near the infused ventricle, although none were found in the SN. However, with electron microscopy examination of SN tissue, abnormal mitochondria were found in SN DA neurons. These abnormalities included swollen mitochondria with broken cristae and electron-dense or electron-translucent material. Microglial activation (OX-42 immunoreactivity) was also found in the SN as well as the striatum and, interestingly, was observed both ipsilateral and contralateral to the MPP⁺-infused side.

5.0 Neuroprotection

The model has been used to test whether elevation of brain levels of the antioxidant glutathione could provide protection when administered concurrently with MPP⁺. Central delivery of MPP⁺ at 0.142 mg/kg/day for 28 days reduced striatal DA content by 70%. Administration of 10 mg/kg/day glutathione ethyl ester delivered icv via Alzet pump concurrently with MPP⁺ provided partial protection with DA levels being reduced by only 49% [4].

6.0 Advantages and limitations of the model

A chronic PD model that mimics all of the features seen in PD brains is highly desirable but unfortunately is not currently available. Chronic MPTP or MPTP/probenecid mouse models come closer to replicating PD pathology than do the acute MPTP mouse models [1,2]. A chronic progressive rat model of PD would be advantageous as a second species for testing. Although the chronic rat rotenone model [5] meets many PD criteria, it has high mortality and much variability, making it a difficult model with which to work, particularly for evaluating therapeutic interventions. The chronic MPP⁺ rat model described here is one in which neurotoxicant delivery is continuous, thus producing a state of persistent and steady-state inhibition of mitochondria. A unilateral lesion is produced that reduces adverse effects and eliminates the high mortality seen in many MPTP models. Inclusion bodies that stain for synuclein and ubiquitin (proteins that are abundant in Lewy bodies) are found in striatal neurons. Although inclusions were not detected in nigral DA neurons, swollen and abnormal mitochondria were found, reminiscent of abnormal mitochondria seen in other PD models [2, 6–8] and in cybrids from PD patients [9]. Whether longer MPP⁺ exposures or survival times will allow for the development of inclusions in nigral neurons remains to be determined. Although creation of the model is technically challenging, the reliability of the response and low variation in measurement outcomes makes it attractive for testing neuroprotective strategies, particularly during the early progressive stages, as this is precisely the stage in the disease course at which therapy is initiated.

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