The MPTP mouse model: Cues on DA release and neural stem cell restorative role

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Abstract

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to cause parkinsonism in humans and this fact is a major incentive for using this toxin as an animal model to study the pathogenesis of Parkinson’s disease (PD). Although the monkey MPTP model remains the best, most studies have been performed in mice. The so-called acute and sub-acute regimens are commonly used. Both induce tissue striatal dopamine (DA) depletion and nigral neuron death. Tissue striatal DA depletion does not necessarily correlate with impairment of striatal dopaminergic functioning. In freely moving mice, systemic acute or sub-acute MPTP directly induces prolonged release of striatal DA. Such DA release may be considered the first step in MPTP-induced striatal DA depletion. Reportedly, neural stem cells improve symptoms in the MPTP model of PD by interacting with the MPTP-induced pathological nigrostriatal milieu.

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1. The MPTP mouse model of Parkinson’s disease

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is known to cause parkinsonism in humans and this fact is a major incentive for using this toxin as an animal model to study the pathogenesis of Parkinson’s disease (PD) [1]. MPTP, a highly lipophilic compound, rapidly crosses the blood–brain barrier as well as cell membrane barriers, and it is metabolized to the final active toxin 1-methyl-4-phenylpyridinium ion (MPP+) by monoamine oxidase (MAO)-B in non-dopaminergic cells. As a polar molecule, MPP+ can only enter neurons by means of the plasma membrane monoamine transporter [1]. Although the monkey MPTP model remains the best, most studies have been performed in mice. In these studies, several MPTP dosing regimens have been used. The so-called acute regimen consists of multiple systemic administration of MPTP (usually 4 doses at 2-h intervals) per day, and the sub-acute regimen consists of a single systemic administration per day for several consecutive days (usually 5 days) or even weeks for the chronic case. According to Jackson-Lewis and Przedborski [1], tissue striatal dopamine (DA) depletion can range from 40% (when MPTP is given at 14 mg/kg per dose × 4) to approximately 90% (20 mg/kg per dose × 4) 7 days after the last MPTP dose. With this acute regimen, death of nigral neurons occurs in a non-apoptotic form with tissue striatal DA depleted by at least 40–50% in young adult C57Bl/6 mice by day 7 after MPTP administration. However, the extent of functional impairment of striatal fibres remains unknown [1,2]. In a study with Swiss mice subjected to systemic MPTP 30 mg/kg per day for 5 consecutive days [3], we demonstrated that death of nigral neurons occurred in
apoptotic form on day 6 after MPTP discontinuance, and tissue striatal DA was depleted by about 59% 1 day after MPTP discontinuance, with a stable trend to recovery (DA depletion of about 28%) from day 3 to day 14 after MPTP [4].

Immunostaining for tyrosine hydroxylase-positive (TH+) neurons and striatal fibers, as well as immunostaining for dopamine transporter (DAT), have been widely used to assess nigral dopaminergic neuron death and dopaminergic striatal denervation and recovery following MPTP intoxication. In primates, the rank order of MPTP-induced loss of these dopaminergic markers is TH+ > DAT [5]. Ashkan et al. [6], using single-photon emission computed tomography (SPECT) in a primate model of PD, found a good correlation of DAT SPECT with Nissl-stained cell counts as well as with behavioural scores. A discrepancy was found between a low TH+ level and the corresponding Nissl-stained cell number. Jackson-Lewis et al. [2], following an acute regimen (MPTP 20 mg/kg per dose × 4 at 2-h intervals) in C57/bl mice, found the same discrepancy (i.e. that the number of TH+ nigral neurons was lower than the corresponding Nissl-stained neurons) and concluded that MPTP can cause loss in TH without necessarily destroying neurons. Höglinger et al. [7], following an acute regimen (MPTP 10 mg/kg per dose × 4 at 2-h intervals) in C57BL/6 J mice, claimed an almost complete striatal denervation from day 1 after MPTP, on the basis of DAT and TH immunostaining. However, the number of nigral dopaminergic neurons did not differ from those in control samples.

2. Mechanisms of acute striatal DA depletion in acute and sub-acute MPTP intoxication

Striatal DA depletion in the animal model of PD depends mainly on nigral dopaminergic neuron death, with a consequent degeneration of striatal fibers. Reportedly, in the MPTP model, surviving striatal fibers acquire an increased DA synthetic capacity [8]. Several studies have shown that intrastriatal infusion of MPP+ causes the DA concentration to increase in dialysates from the striatum of freely moving mice. Rollema et al. [9] demonstrated that intrastratal administration of MPP+ in awake mice, rats and monkeys induced an immediate massive release of DA, accompanied by a pronounced decrease in the output of DA metabolites DOPAC and HVA. Uezono et al. [10] infused 10 and 100 µM of MPP+ for 60 min in the striatum of freely moving adult C57BL/6 mice and obtained a 28- and 93-fold increase, respectively, in DA baseline values. The mechanism of DA release by locally administered MPP+ has been related to energy impairment [11]. However, no information is available on the effects induced by systemic MPTP or intrastratal administration on striatal DA in vivo release. After its systemic administration, MPTP rapidly crosses the blood–brain barrier and all plasma membranes, including those of dopaminergic terminals. The question arises as to whether MPTP crosses dopaminergic terminals because they are void of any DA-releasing activity or because of the established MPP+ role in affecting DA release. As shown in Fig. 1, the acute systemic MPTP regimen (10 or 20 mg/kg per dose × 4 at 2-h intervals) induced a DA increase and its extracellular metabolite 3-methoxytyramine (3-MT) in dialysates from the striatum of freely moving C57BL/6 mice, along with a decrease in DOPAC and HVA concentrations, for each dose administered systemically. The increase in DA and 3-MT is a direct MPTP effect, since it is neither abolished nor reduced by systemic administration of the MAO-B inhibitor pargyline. Most likely, such prolonged DA release may be considered the
first step in MPTP-induced striatal DA depletion. The sub-acute regimen (25 mg/kg per day for 5 consecutive days) induced a progressive day-by-day decrease in dialysate DA and 3-MT, with concomitant behavioural impairment (swim test) (Fig. 2).

3. Attempt of restorative therapy with neural stem cells in the MPTP model of PD

Neural stem cells (NSCs) are presently employed by many researchers and clinicians as a restorative therapy for neurodegenerative diseases, including PD. The subventricular zone (SVZ) contains NSCs capable of giving rise to new neurons in adult mammalian brains. Dopaminergic neurons of the substantia nigra pars compacta (SNpc) project fibres to the SVZ that control proliferation and migration of NSCs. In sporadic PD, the generation of neural precursor cells in the subependymal zone is impaired by DA depletion, which is the hallmark of PD [7]. MPTP-induced degeneration of dopaminergic SNpc-SVZ fibres impairs NSC proliferation in primates [12] and in mice [7]. Proliferation is completely restored by a selective D2-like agonist. Fig. 3 shows the DA-induced increase in NSC viability in vitro. Human NSCs induced a behavioural improvement in an MPTP primate model of PD. Multiple modes of reciprocal interactions between exogenous NSCs and the pathological host milieu have been proposed as the underlying mechanism [13]. In addition, NSCs exerted neuroprotection in a murine model of focal ischaemia–reperfusion by changing the ischaemic microenvironment [14]. Thus, one of the targets of restorative therapy with NSCs might be the oxidative homeostasis in the extracellular nigrostriatal compartment.

At present, L-dopa remains the drug of choice in PD therapy. However, L-dopa and DA, being catechol-containing compounds, may undergo autoxidation in vivo in the striatal extracellular compartment [15], with the consequent generation of quinone derivatives and superoxide anion. L-dopa and DA autoxidation are increased by transition metals such as...
manganese (Mn) and iron [15]. In addition, DA and l-dopa are known to induce apoptosis in several cell lines [16], including NSCs [17]. These data may be of relevance to the oxidative homeostasis in the extracellular nigrostriatal compartment in PD, since dysregulation of iron metabolism and iron-induced oxidative stress are believed to be important pathogenetic mechanisms of neuronal death in PD [18]. Fig. 4 shows the inhibitory effects induced by Mn on NSC viability in vitro, on the associated l-dopa and the antagonism exerted by ascorbic acid. Fig. 5 shows the inhibitory effects of MPTP and MPP⁺ on NSC viability in vitro and the complete recovery of viability when DA + l-dopa + ascorbic acid were all added either to MPTP or to MPP⁺. DA, l-dopa and ascorbic acid are physiological components of the striatal extracellular compartment. Therefore, the striatal levels of DA and ascorbic acid, coupled with administration of exogenous l-dopa, might help NSCs in their restorative effort in the MPTP model of PD.

Conflict of interest

The authors have declared no conflicts of interest.

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References


