REVIEW ARTICLE

MPTP-Induced Neurotoxicity and the Quest for a Preventative Therapy for Parkinson's Disease

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ABSTRACT: Less than 10 years have passed since the discovery that 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is capable of producing parkinsonism in both humans and non-human primates. In that time, there has been considerable interest in the possibility that the pathogenesis of idiopathic Parkinson's disease (PD) might involve a process analogous to that of MPTP toxicity. One hypothesis holds that PD might arise, at least in part, from exposure to an MPTP-like environmental toxin. Rapid progress has been made towards elucidating the precise mechanism by which MPTP exerts toxicity, and clarifying the relationship of MPTP toxicity to idiopathic PD. The goal of these efforts is to develop a therapy that inhibits the underlying disease process in PD.

RÉSUMÉ: Neurotoxicité induite par le MPTP et recherche d'une thérapie préventive pour la maladie de Parkinson. Moins de 10 ans se sont écoulés depuis la découverte que la 1-méthyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) peut produire le parkinsonisme chez les primates humains et non-humains. Depuis ce temps, la possibilité que la pathogenèse de la maladie de Parkinson idiopathique (MP) pouvait impliquer un processus analogue à celui de la toxicité du MPTP a suscité un intérêt considérable. Selon une hypothèse, la MP pourrait résulter, du moins en partie, d'une exposition à une toxine environnementale similaire au MPTP. Des progrès rapides ont été accomplis pour élucider le mécanisme précis de la toxicité du MPTP et clarifier la relation entre la toxicité du MPTP et la MP idiopathique. Le but de cex efforts est de développer une thérapie qui inhibe le processus sous-jacent à la MP.

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Recent reports have indicated that deprenyl, an inhibitor of the catecholamine catabolic enzyme monoamine oxidase, type B (MAO-B), may delay the onset of disability associated with early, otherwise untreated, cases of idiopathic Parkinson's disease (PD).[1,2] The efficacy of deprenyl in the treatment of PD has long been recognized in Europe[3-6] where its beneficial effects have generally been ascribed to an increase in the dopamine content of the presynaptic nerve terminals.[3,7] One interpretation of the recent North American results is that deprenyl might not simply provide symptomatic therapy, but might actually interfere with the underlying pathogenetic mechanisms of PD and thereby slow the progression of the disease itself.[1,2] This notion had originally been proposed by Birkmayer and colleagues in the mid-1980s[3,8] but, until recently, lacked compelling supportive evidence from a prospective trial.

The MPTP Model of Parkinsonism

Much of the impetus for proceeding with randomized clinical trials of deprenyl originated with the observation that the meperidine derivative 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) could produce a clinical syndrome strikingly similar to idiopathic PD.[9,10] Subsequent animal experiments showed that primates treated with MPTP develop bradykinesia, rigidity and tremor (although the tremor tends to resemble a cerebellar action tremor rather than the classic resting tremor of PD: cf. CD Marsden, as quoted in reference 11). Neuropathological examination reveals damage to the dopaminergic neurons of the substantia nigra and, to a lesser extent, the noradrenergic cells of the locus coeruleus; this damage is accompanied by eosinophilic intraneuronal inclusion bodies, which resemble Lewy bodies.[12]

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Neurochemical analysis demonstrates depletion of dopaminergic markers from the nigrostriatal tract,[13-17] and, to a lesser extent, from the mesolimbic system, the mesocortical system and the retina.[17-19] Noradrenergic indices are also decreased in MPTP-treated primates[20,21] while the neuropeptide profile following MPTP treatment less consistently mimics that observed in PD.[13]

The remarkable similarities between idiopathic PD and the syndrome induced by MPTP have focused attention on the pathophysiology involved in MPTP-induced toxicity. The postulated steps are outlined in Figure 1, and are described below in detail.

Step 1: Oxidation of MPTP. MPTP can readily cross the blood-brain barrier, but the substance itself is not toxic.[22] Instead, toxicity is thought to be produced by 1-methyl-phenyl-pyridinium (MPP+), a metabolite of MPTP. The first step in this process is the oxidation of MPTP to 1-methyl-phenyl-dihydro-pyridinium (MPDP), via MAO-B; MPDP then spontaneously oxidizes to MPP+.[23,24] The administration of nonspecific (e.g. pargyline) or specific (e.g. deprenyl, MD240928) blockers of MAO-B activity prior to MPTP treatment prevents the conversion of MPTP to MPP+ and results in attenuation of toxicity, both *in vivo* and *in vitro*.[25-28] Specific blockers of MAO-A activity, on the other hand, are unable to inhibit MPTP-induced toxicity.[25,26] Interestingly, the catecholaminergic neurons of the substantia nigra and locus coeruleus do not appear to contain appreciable quantities of MAO-B and therefore may not be capable of oxidizing MPTP.[29-32] Instead, MPTP is thought to be metabolized to MPP+ within astrocytes and, to a much lesser extent, within serotonergic neurons,[28,32-35] the two central nervous system cell classes in which MAO-B is predominantly located.[30,31]

Step 2: MPP+ in the Extracellular Fluid. Following the oxidation of MPTP, MPP+ is found within the extracellular fluid,[28,35] although the precise mechanism by which it gets there has not been clarified. One possibility is that MPDP is released from astrocytes/serotonergic neurons and oxidized to MPP+ in the extracellular space. Another is that the conversion of MPDP to MPP+ takes place within astrocytes/serotonergic neurons, with subsequent secretion of MPP+ into the extracellular fluid.

Step 3: Uptake of MPP+. Once MPP+ is present within the extracellular fluid, it is taken up into catecholaminergic neurons via the appropriate high-affinity uptake systems.[36,37] Pretreatment with specific (GBR 13098, maprotiline) or nonspecific (mazindol, nomifensine) dopamine and noradrenaline uptake inhibitors results in attenuation of MPTP-induced dopamine and noradrenaline depletions.[25,27,38-41] Conversely, prior inhibition of catecholamine vesicular transport with reserpine or tetrabenazine potentiates MPTP-induced dopamine depletions, presumably because MPP+ is not sequestered into storage vesicles and is therefore free to exert its toxic effects within the cytosol.[42]

Step 4: Interaction with Neuromelanin. Neuromelanin (NM), a granular pigment found in selected areas of the mammalian brainstem and dorsal root ganglia,[43] plays an important role in the cytotoxic effects of MPP+. It has been suggested that NM binds to MPP+, thus preventing its clearance from the brain, and that the gradual release of MPP+ from this intracellular depot is crucial in the production of cytotoxicity.[44] Pretreatment of primates with the antimalarial drug chloroquine results in attention of MPTP-induced toxicity,[44] presumably because chloroquine binds to NM, and thereby interferes with the binding of MPP+ to NM.[44-47]

The presence of high concentrations of NM in the primate and human, but not in the rodent,[43] may help account for the extraordinary variability in species vulnerability to MPTP: 0.33-

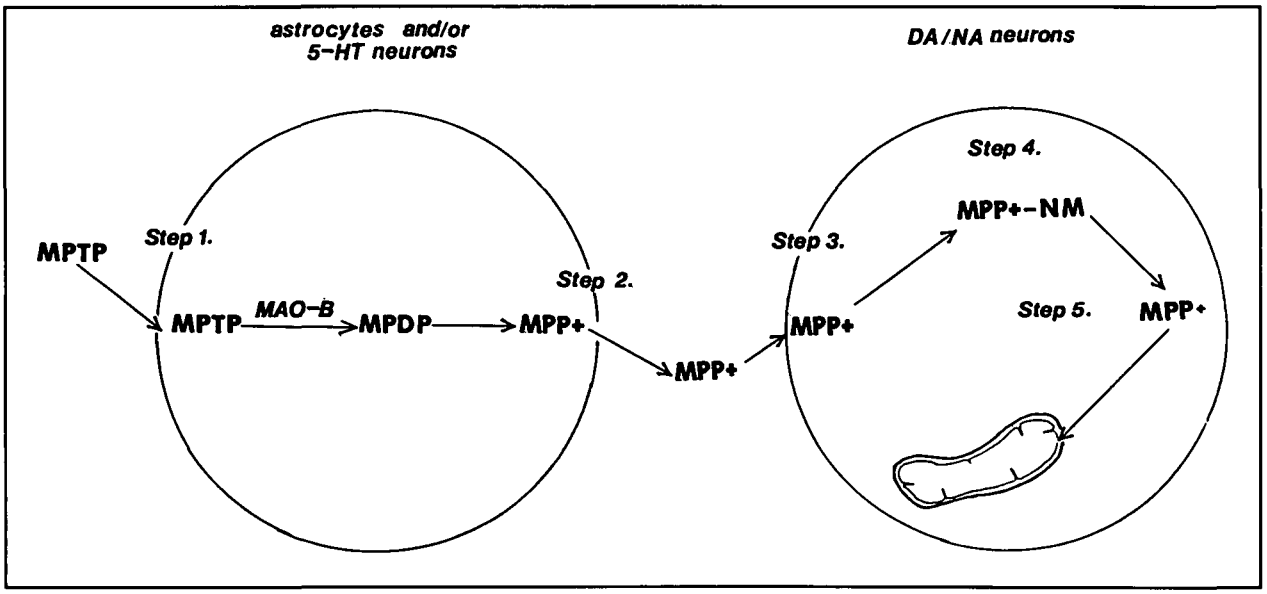

Figure 1 — Postulated Steps of MPTP-Induced Cytotoxicity. Step 1. MPTP crosses the blood-brain barrier and is taken up into astrocytes and/or serotonergic neurons where it is metabolized into MPDP via MAO-B. MPDP then spontaneously oxidizes to MPP+. Step 2. MPP+ is released from astrocytes/serotonergic neurons and enters the extracellular space. Alternatively, MPDP may be released from astrocytes/serotonergic neurons, with conversion to MPP+ occurring in the extracellular space. Step 3. MPP+ is taken up into dopaminergic/noradrenergic neurons. Step 4. MPP+ binds to neuromelanin (NM). Step 5. MPP+ is released from NM, and taken up into mitochondria, where it interferes with the synthesis of ATP.

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3.0 mg/kg of MPTP administered once per day for a few days produces permanent depletion of striatal dopaminergic markers in primates, [20,48-50] while 10-50 mg/kg of MPTP administered several times per day over a few days results in only transient suppression of dopaminergic indices in rodents.[14,15] The tendency of NM to accumulate with age may also partially explain why older animals are more susceptible than younger animals to the toxic effects of MPTP.[43,44]

Step 5: Induction of Cell Death. The final step in MPTP-induced toxicity is thought to involve interference with mitochondrial respiration. MPP+ is taken up into the mitochondria via an energy-dependent uptake system (normally used for the uptake of pyruvate), resulting in the concentration of MPP+ within the mitochondrial matrix. MPP+ inhibits Complex I, a complicated membrane-bound system which catalyzes the transfer of electrons from NADH to ubiquinone (coenzyme Q). This may result in a bioenergetic deficiency if the electron transport chain is sufficiently blocked to inhibit oxidative phosphorylation and decrease the synthesis of ATP.[51-53] Alternatively, relatively high-energy electrons diverted from the electron transport chain may go on to generate cytotoxic free radicals (see below). Either of these processes could lead to cell death. It was recently reported that Complex I is defective in patients with idiopathic PD,[54,55] reinforcing the similarities between PD and MPTP-induced parkinsonism.

Another potential mechanism of cell death in both MPTP-induced toxicity and idiopathic PD is through the generation of cytotoxic free radicals. It is known that the amount of NM in human brain increases with age, perhaps as a result of oxidation of catecholamines.[43,56] The oxidation of dopamine or MPTP by MAO results in the formation of hydrogen peroxide (H_2O_2). Transition metals such as iron (II) interact with hydrogen peroxide, leading to the production of free radicals such as the highly reactive cytotoxic hydroxyl radical (-OH) or superoxide radicals (O_2^-). These in turn induce lipid peroxidation, resulting in increased cell membrane fluidity and cell degeneration.[57-59] Under normal circumstances, hydrogen peroxide in the brain is inactivated by glutathione peroxidase in the presence of its rate-limiting substrate, reduced glutathione. Both PD and MPTP-induced parkinsonism are associated with decreased levels of glutathione,[60-62] potentially rendering the brain more vulnerable to damage from free radicals. At the same time, levels of iron are elevated more than two-fold in PD substantia nigra,[61,63,64] potentially increasing the generation of free radicals. More specifically, it is iron (III) that is significantly increased in PD, resulting in a shift of the iron (II)/iron (III) ratio in favour of iron (III). It was recently demonstrated that neuromelanin, which is traditionally regarded as a scavenger of free radicals, may instead increase the rate of -OH production if the predominant form of iron is iron (III).[65] This selective increase of iron (III) in PD substantia nigra shifts the ratio of iron (II)/iron (III) from the 3:1 ratio seen in normals towards 1:1,[61] which is the optimal ratio for initiation of -OH formation and membrane lipid peroxidation.[66,67] The result is that in both MPTP-induced toxicity and PD there may be increased production of free radicals and a decrease in scavenging enzymes. One of the objectives of the multicentre DATATOP study is to examine whether alpha-tocopherol, a component of vitamin E which is known to trap free radicals,[59,68] might retard the progression of idiopathic PD. The data are not yet sufficiently conclusive to permit a statement concerning its potential efficacy.[2]

Environmental Neurotoxins and PD

The knowledge that PD is probably not genetically transmitted[69] and the ability of MPTP to mimic the features of idiopathic PD have given rise to the idea that PD may be caused by an environmental substance similar in structure to MPTP.[70] Support for this notion has come from clinical studies, animal experimentation and epidemiological investigations. An early case report was that of a chemist who developed PD at age 37, eight years after beginning to work with MPTP.[71] A PET scan study of asymptomatic subjects who had self-administered MPTP in low doses showed decreased striatal 6-fluorodopa activity.[72] More recently, 22 individuals with a mean age of 34.7 years and a history of exposure to MPTP have been found to exhibit signs of mild parkinsonism.[73]

Additional evidence in support of the "environmental hypothesis" has come from animal experimentation. While none of the commonly occurring environmental contaminants that are structurally similar to MPTP has thus far been shown to produce a parkinsonian syndrome,[74-76] several laboratory-synthesized analogues of MPTP have demonstrated a capacity for killing dopamine neurons. The expression of cytotoxicity by some of these compounds is dependent upon their oxidation by MAO-A instead of MAO-B.[77]

Epidemiological studies have found that idiopathic PD is more prevalent in industrialized than in nonindustrialized countries and, within the former, more prevalent in rural than in urban areas.[70] One possible interpretation of these findings is that environmental factors such as drinking well water or exposure to pesticides and herbicides may contribute to the pathogenesis of PD.[70,77,78]

Towards a Preventative Therapy for PD

The hypothesis behind the recent trials of deprenyl in early PD was that the pathogenesis of PD might involve a neurotoxic process analogous to that involved in MPTP-induced cytotoxicity. While the initial reports give hope that deprenyl may indeed be able to delay the clinical progression of PD[1,2] this issue remains controversial. An alternative explanation for the apparent efficacy of deprenyl in early PD might be that deprenyl simply provides symptomatic relief to PD patients, by inhibiting the breakdown of dopamine in presynaptic terminals. This possibility was carefully considered by the authors of the DATATOP study, but was rejected. A double-blind trial of deprenyl therapy in patients with PD who were not taking levodopa found it to be of little if any symptomatic benefit.[79] Furthermore, among the patients studied in double-blind fashion by Tetrud and Langston and in the DATATOP study, there was no change in parkinsonian or depression scores among deprenyl-treated patients either when drug treatment was started ("wash-in") or when it was stopped ("wash-out").[1,2] It has been pointed out, however, that the one-month wash-out period used by Tetrud/Langston and the DATATOP investigators may not have been adequate, as the pharmacological effects of deprenyl may persist for more than one month after its withdrawal.[80-82] The recent decision to extend the DATATOP wash-out period should help clarify

whether the effect of deprenyl is primarily symptomatic, or whether it indeed slows the course of PD.

The precise relationship between MPTP-induced parkinsonism and idiopathic PD remains, at this point, unclear. One possibility is that idiopathic PD develops from a process exactly analogous to that of MPTP-induced toxicity. In that case, one would expect to be able to inhibit the progression of PD by interfering with any of the 5 steps outlined in Figure 1. Among the drugs which block MPTP toxicity, only deprenyl has been systematically studied in PD patients. Whether chloroquine or a dopamine uptake inhibitor might be effective in delaying the progression of PD symptoms is unknown. Another possibility is that the putative effect of deprenyl may be related to its ability to inhibit the oxidative stress associated with increased dopamine turnover in the early stages of PD.[83] The oxidation of dopamine by MAO-B, however, would appear to take place outside of dopaminergic neurons, since MAO-B in brain seems to be present only in glia and in serotonergic neurons.[30,31] One would therefore expect that the selective MAO-B inhibitor, deprenyl, would selectively inhibit oxidative free radical formation in non-dopaminergic cells, as opposed to the melanized dopaminergic neurons which are targeted in PD. Given that dopamine appears to be deaminated by MAO-A as well as by MAO-B,[31,32] one wonders whether a nonspecific MAO inhibitor might not be even more effective than deprenyl in treating patients with early PD.

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