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*Review*

# The Neurotoxin 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP): A Key to Parkinson's Disease?

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Since the recognition that the compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent inducer of chronic and irreversible parkinsonism in humans, it has been the object of intense and extensive research activity. The fact that MPTP selectively kills cells in the pars compacta of the substantia nigra and produces a syndrome in human and nonhuman primates that is virtually indistinguishable from the idiopathic disease has led to hope that understanding the mechanism of action of this toxin may shed light on the underlying biochemical pathology that is responsible for cell death in Parkinson's disease. A brief overview of Parkinson's disease is presented, and current data regarding the effects, biotransformation, and mechanism of action of MPTP are discussed.

KEY WORDS: MPTP; dopamine; Parkinson's disease; neurotoxins; MAO.

## IDIOPATHIC PARKINSON'S DISEASE: A BRIEF OVERVIEW

Parkinson's disease, first described in 1817 by James Parkinson in "An Essay on the Shaking Palsy," is a progressive neurodegenerative disease, the hallmark of which is the triad of symptoms tremor, bradykinesia (slowness of movement), and rigidity. Although irreversible "parkinsonism" occasionally develops as a sequellae to viral disease, stroke, or other brain insult, the vast majority of the more than 400,000 cases of Parkinson's disease in the United States is idiopathic, with little or nothing known regarding the etiology. Affecting 1 in 100 individuals over the age of 50 years, idiopathic Parkinson's disease (IPD) is characterized by a progressive loss of motor function resulting in mask-like facies, stooped posture, shuffling gait, drooling, and increasingly slow and difficult initiation of movement. In the advanced stages patients become virtually frozen.

Gross examination of parkinsonian brain autopsy material shows a loss of pigmentation in the area of the midbrain known as the substantia nigra (SN). Microscopic study of the SN reveals marked cell-body loss, the presence of extraneuronal pigment, gliosis, and eosinophilic inclusion bodies known as Lewy bodies. Biochemical analysis reveals a severe depletion of dopamine in the striatum (the region where nigral neurons terminate). The depletion of dopamine correlates with the severity of the disease, and in cases of hemiparkinsonism, the dopamine deficit is contralateral to the parkinsonism. Normal functioning of the nigrostriatal pathway can be viewed as a process whereby dopamine, produced in the cell bodies of the substantia nigra, is transported via the long connecting axons to the terminals in the striatum. There, dopamine acts as a neurotransmitter, playing a crucial role in the initiation of voluntary movement. In Parkinson's disease the cell bodies in the nigra die, resulting in a severe reduction in dopamine and a consequent poverty of voluntary movement.

Current therapy represents a major advance for modern pharmacology and rational drug development. On the basis of biochemical evidence, and seeking to replenish the deficient dopamine, clinicians administered the amino acid dopamine precursor, L-dihydroxyphenylacetic acid (L-dopa), to parkinsonian patients (dopamine itself does not cross the blood–brain barrier), and the success of L-dopa has made it the mainstay of therapy. Unfortunately, although L-dopa provides striking relief for many of the symptoms of IPD, it possesses numerous and serious side effects and does nothing to arrest the inexorable deterioration. Inevitably, advanced patients become "frozen" and unable to care for themselves.

Research in the etiology of IPD and the development of new drugs has been hindered by the lack of both an animal model and knowledge regarding the cause of nigral cell death.

## EFFECTS OF MPTP

Although first described by Ziering *et al.* (1) almost 40 years ago, the potent and selective neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Fig. 1a) was recently recognized when a group of young heroin addicts unknowingly injected this substance as a contaminant in "synthetic heroin" (2). The systemic administration of MPTP to human and nonhuman primates produces a syndrome characterized by profound rigidity, akinesia, and tremor, which is reversed by dopamine agonist and precursor therapy. MPTP has also been shown to reduce drasti-

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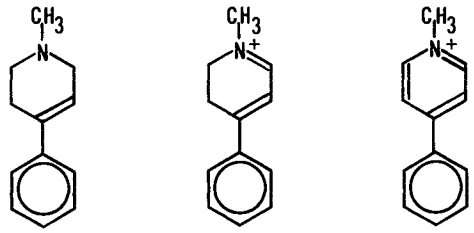


Fig. 1. (a) MPTP; (b) $MPDP^+$; (c) $MPP^+$.

cally nigrostriatal dopamine and to be selectively toxic to the cell bodies of the substantia nigra (SN) in human (3) and nonhuman primates (4,5), producing severe cell loss and glial scarring there while leaving other brain areas undamaged (Fig. 2). Hence MPTP is probably one of the most selective central nervous system (CNS) toxins described to date. Its selectivity is for the same cell group that bears the brunt of the damage in idiopathic Parkinson's disease (IPD).

The fact the MPTP produces a behavioral syndrome and neuropathological damage so strikingly similar to idiopathic Parkinson's disease has not escaped the attention of numerous investigators. Work on the mechanism of action of this toxin has been intense as a result (6,7). MPTP has already been successfully used to produce the first animal model for Parkinson's disease; thus, it is now possible to develop and evaluate new pharmacological and surgical approaches to therapy in nonhumans. Since MPTP is selectively toxic to the same cells that die in IPD, it has raised hopes that elucidating the mechanism of action of MPTP may shed light on the manner of nigral cell death in idiopathic Parkinson's disease. Furthermore, recent reports that chemists working with MPTP have developed early Parkinson's disease (8) have led to the suggestion that MPTP or a similar chemical present in the environment could actually be responsible for the disease itself (9).

## MODELS OF MPTP TOXICITY AND BIOACTIVATION

The biotransformation and toxicity of MPTP have been studied *in vivo* and *in vitro*. Since the effects of MPTP administration to different animal species produce a tremendous variation in response (10–12) and there is still some dispute whether or not "true" toxicity (i.e., neuronal cell loss) results in any nonprimate species, the major animal models are briefly discussed.

The only animal that, to date, has been shown to develop unequivocal cell loss and a permanent parkinsonian behavioral syndrome is the primate. Extremely variable results have been reported in rodent species, and conflicting reports regarding cell damage in rodents have been published (13,14). The lower cost and ease of handling small

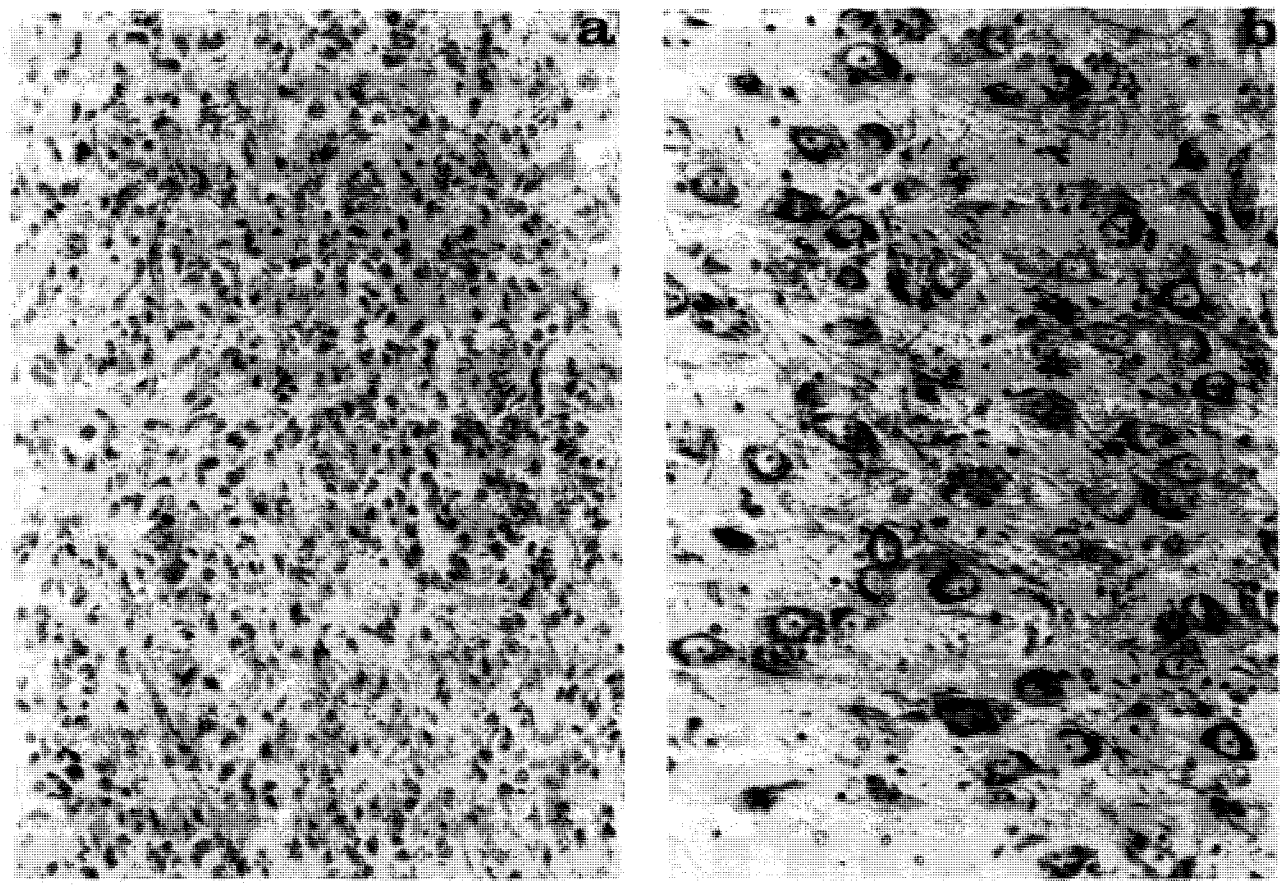


Fig. 2. (a) MPTP-treated squirrel monkey. Substantia nigra showing severe nerve cell loss in the zona compacta. Luxol fast blue–cresyl violet stain. (b) Untreated squirrel monkey with the normal number of cells in the compact zone of the substantia nigra. Luxol fast blue–cresyl violet stain. × 220; reduced 10% for reproduction.

rodents for laboratory studies, however, have led many investigators to persist, and the MPTP-treated mouse has been widely studied. Mice treated with MPTP develop a long-lasting depletion of striatal dopamine, similar depletions in striatal dopamine uptake (13–15) and binding of mazindol to striatal membranes (16), and histopathological evidence of striatal terminal damage (15).

The significant recovery of these parameters with time, however, as well as conflicting reports as to whether actual cell death occurs, makes it difficult to distinguish long-term pharmacological effects from acute toxicity in the mouse. The recent observation that aged mice treated with MPTP develop cell loss, while young mature mice do not (17), suggests that the effects of MPTP in rodents are age dependent and that this observation may account for these conflicting reports. Thus the MPTP-treated mouse has emerged as a useful model, with the caveat that major findings need to be confirmed in the primate.

MPTP has also been administered to numerous other species including amphibians, guinea pigs, cats, dogs, and the medicinal leach and has been reported to produce a variety of neurotransmitter, behavioral, pigmentation, and cytotoxic effects. For a summary of these the reader is referred to a recent symposium on this topic (18).

*In vitro* models that have been used to study MPTP biotransformation and toxicity have focused primarily on tissue culture and brain homogenates. The addition of MPTP to explant cultures of nigrostriatal origin is reported to cause damage to dopaminergic cells as judged by reduced dopamine, dopamine metabolite (DOPAC and HVA), and tyrosine hydroxylase levels and reduced catecholamine fluorescence on microscopic examination (19,20).

The biotransformation of MPTP has also been studied in liver microsomal preparations (21), brain homogenates, and mitochondrion-enriched fractions of brain homogenates (22), and although these models do not directly link biotransformation to toxicity, they have revealed some valuable information regarding MPTP metabolism.

## BIOTRANSFORMATION

Early studies in primates showed that MPTP rapidly disappears from all tissues except the eye (23). No basic, acidic, or neutral metabolites were detected in organic extracts of biological tissues; however, large amounts of the highly water-soluble pyridinium compound 1-methyl-4-phenylpyridinium ion ($MPP^+$; Fig. 1c) were identified in virtually all tissues. $MPP^+$ has now been positively identified by means of TLC, HPLC, GC/MS, and NMR (21,23,24). Although biological precedents for the oxidation of partially reduced pyridines to pyridinium ion can be found and this biotransformation pathway has been exploited as a novel system for drug delivery to the brain (25), the conversion of a tetrahydropyridine to a pyridinium congener is not a commonly described metabolic pathway. Once formed, $MPP^+$ has an extremely long half-life in primate brain (between 48 and 100 hr) (26), and intraneuronal trapping of this charged species was suggested as the mechanism for its toxicity (24).

In contrast, although $MPP^+$ is also formed in the rodent brain (24,27), it does not persist in the CNS to the same extent as in the primate, as the half-life of $MPP^+$ in the mouse is approximately 4 hr (27). Whether this is the result of further biotransformation or faster excretion is not known; however, the pattern of $MPP^+$ persistence in the CNS parallels the relative sensitivity of rodents and primates to MPTP toxicity.

We have studied the biotransformation of MPTP in tissue culture and have found that $MPP^+$ is produced in glial and mesencephalonic cultures that have been incubated with MPTP (28). *In vitro* studies (21,22) showed that while rat liver microsomal preparations convert MPTP to its demethylated analogue, the conversion of MPTP to $MPP^+$ occurs in homogenates of rat brain. Further, most of the capacity to convert MPTP to $MPP^+$ was found in the mitochondrial fraction. These *in vitro* studies also established two important new aspects of the biotransformation of MPTP. First, monoamine oxidase (MAO) is involved in the oxidation of MPTP to $MPP^+$, and second, the reaction proceeds via the dihydropyridinium intermediate ($MPDP^+$; Fig. 1b). In this *in vitro* system, deprenyl and pargyline were found to inhibit selectively the conversion of MPTP to $MPP^+$, while clorgyline was much less active. MPTP has since been shown to be a substrate for both forms of the enzyme (29,30); however, the sensitivity of the *in vitro* process to inhibition by deprenyl indicated that MAO B is the relevant form of the enzyme *in vivo*. These results suggested that the suicide inhibitors pargyline and deprenyl might protect animals against MPTP toxicity. This, in fact, proved to be the case. Pretreatment of monkeys with pargyline (31) or deprenyl (32) completely prevented nigral cell destruction and the development of parkinsonian syndrome that results from a standardized dosage schedule of MPTP. In addition, the conversion of MPTP to $MPP^+$ *in vivo* was virtually blocked after pretreatment with pargyline (31). Similarly, in the mouse pretreatment with pargyline inhibits the biotransformation of MPTP to $MPP^+$ (24) and prevents the dopamine-depleting action of MPTP (33). Pargyline is also effective in protecting nigral explant cell cultures from the effects of MPTP (19).

The finding that MAO B inhibitors prevent the effects of MPTP is somewhat surprising, since most of the intraneuronal MAO in dopaminergic neurons is believed to be MAO A (34) (MAO B is the predominant form in glial and serotonergic cells). This implies that the biotransformation of MPTP to $MPP^+$ occurs outside of the target cells.

It is also of note that deprenyl (a selective MAO B inhibitor) has been used, together with L-dopa, in the treatment of Parkinson's disease in Europe. The rationale for the use of this MAO B inhibitor has been to prolong the action of L-dopa by protecting the dopamine formed from degradation by MAO. Preliminary reports (35,36) that in patients treated with deprenyl, the degenerative, progressive nature of Parkinson's disease appeared slowed and the life expectancy and therapeutic response are prolonged have stimulated interest in MAO inhibitor therapy as a strategy to alter the course of the disease. It has been suggested that MAO acting upon some endogenous (dopamine) or exogenous substrate may produce free radicals, toxic intermediates, or metabolites responsible for neurodegenerative changes seen in certain diseases (37); moreover, oxidized dopamine is toxic to neuroblastoma cells (38).

## MECHANISM OF ACTION

While the protection of animals by MAO inhibitors focuses on the conversion of MPTP to $MPP^+$ as a key step that is required for neurotoxicity, it does not explain the selectivity of MPTP for dopaminergic cells of the nigrostriatal pathway, nor does it distinguish between the two candidates for the actual toxic species $MPDP^+$ and $MPP^+$.

Although only the last provides mechanisms for both toxicity and selectivity, current thinking regarding the actual toxic species focuses on four hypotheses (Fig. 3). First, the production of peroxide, superoxide, or hydroxyl radicals, as by-products of the MAO-mediated oxidation of MPTP represents the cytotoxic process. Although the role of oxygen radicals in cell damage is well established, future investigations into the qualitative and quantitative distribution of MAO are required and must explain the selectivity of MPTP toxicity in spite of the ubiquity of MAO in the brain. The second hypothesis is based on the assumption that $MPDP^+$ should be a highly reactive intermediate, capable of covalent binding to cellular macromolecules. $MPDP^+$ is an electrophilic species that readily forms adducts with cyanide (39). Recent studies (40) suggest that a hydrophobic microenvironment would be required to maintain stable amino or sulfhydryl adducts *in vivo,* since these are unstable in aqueous solution. Redox cycling, between either $MPDP+$/MPTP or $MPP+$/$MPDP^+$, leading to the production of free radicals and subsequent cell death, represents the third hypothesis to explain MPTP toxicity. The measurement of oxidation and reduction potentials under physiological conditions will clarify the role of redox cycling mechanisms.

The final hypothesis regards $MPP^+$ as the toxic species and suggests that its selectivity for the dopaminergic system arises from its accumulation there in greater amounts. Thus it provides an explanation for both cell death and selectivity. $MPP^+$ is a known toxin and was developed and utilized as an herbicide (41). In primates, distribution data suggest that $MPP^+$ accumulates with time in the substantia nigra (26). This hypothesis is further supported by studies showing that $MPP^+$, but not $MPDP^+$ or MPTP, is a highly active substrate for the dopamine uptake system (16). In rodent striatal synaptosomal preparations, $MPP^+$ is taken up with approximately the same affinity ($K_M$ = 0.18 $\mu M$; $V_{max}$ = 2 nmol/g tissue/min) as dopamine. This uptake is reversible, competitive with dopamine, and the rank order of several blockers is the same for both dopamine and $MPP^+$, suggesting a common site. The affinity of $MPP^+$ for uptake by noradrenergic systems is approximately 1/10 that for dopaminergic systems and, hence, might explain the selectivity of MPTP for the nigrostriatal neurons. Although it will need to be confirmed in the primate, the fact that dopamine uptake blockers protect against the dopamine-depleting effects of MPTP in rodents (42) further supports this contention.

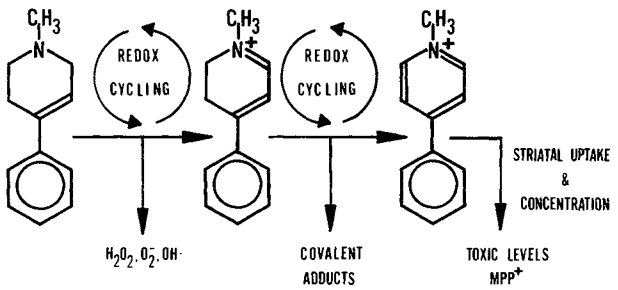


Fig. 3. Possible mechanisms of MPTP neurotoxicity: MPTP is oxidized by monoamine oxidase to $MPP^+$, via a dihydropyridinium intermediate. Possible toxic species include (1) peroxide, superoxide, or hydroxyl radical produced as a result of MAO activity; (2) the reaction of the dihydropyridinium intermediate with cellular macromolecules; (3) redox cycling of $MPDP^+$/MPTP or $MPP^+$/$MPDP^+$ and consequent production of free radicals; and (4) uptake and concentration of the toxin $MPP^+$ via the dopamine uptake system, leading to toxic concentrations of $MPP^+$ in nigral cells.

## CONCLUSION

MPTP is a recently discovered neurotoxin that has already provided some fascinating and important insights into the functioning of the central nervous system. It has yielded the first animal model of Parkinson's disease and its cytotoxic neurochemical and age-related effects closely parallel the disease. Understanding the biotransformation and metabolism of this compound has been crucial to understanding its mechanism of action. At this early stage in our knowledge, it is clear that, at the very least, we stand to learn a great deal about the neurons of the substantia nigra. It is even possible that MPTP may be a tool that allows us to understand one of the major neurodegenerative diseases of aging.

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