Experimental and Clinical Neurotoxicology

SECOND EDITION

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MPTP and Analogs

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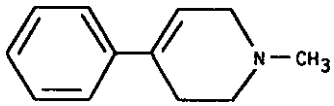

MPTP
C12H15N

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine

NEUROTOXICITY RATING
Clinical
A Extrapyramidal syndrome (parkinsonism)
Experimental
A Extrapyramidal dysfunction (parkinsonism; substantia nigra degeneration)

The neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was identified in 1983 as the contaminant of synthetic illicit drugs that caused the abrupt onset of parkinsonian signs in young drug addicts (27).

MPTP is commercially available as a hydrochloride salt. In this form, MPTP is a white, odorless powder (MP, 241°-243°C) that is soluble in water. Because of its toxicity to humans, special precautions must be taken for its use and disposal. MPTP solutions should be treated with a 50% excess of commercial bleach before disposal (60).

MPTP has been extensively used for research purposes. The clinical, neuropathological, and neurochemical features of MPTP-induced toxicity are *very* similar to those observed in idiopathic parkinsonism, (a) raising the possibility that MPTP-like toxins may play a role in the etiology of Parkinson's disease, and (b) providing a valuable experimental tool for the development of *in-vitro* and *in-vivo* models of nigrostriatal degeneration.

General Toxicology

Because of its lipophilic structure, MPTP is not only rapidly distributed throughout body tissues but also crosses the blood-brain barrier. Once in the CNS, however, MPTP does not cause neurotoxicity unless it is activated to its fully oxidized metabolite, 1-methyl-4-phenylpyridinium (MPP'). This metabolism occurs in two steps: first, MPTP is oxidized to the intermediate 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP') and then MPDP' is converted to MPP'. The first step is catalyzed by the enzyme monoamine oxidase (MAO) type B (9), while the conversion of MPDP' to MPP' is likely to occur *via* auto-oxidation (59). The critical role played by MPP' in MPTP toxicity is demonstrated by findings showing that (a) MAO B inhibitors completely protect against MPTP toxicity both in *vivo* and *in vitro* (16,22,29), and (b) MPP' itself causes toxic effects similar to those seen after systemic administration of MPTP (3). On the other hand, systemically administered MPP' does not cross the blood-brain barrier; therefore, MPP'-induced neurotoxicity only occurs when this pyridinium compound is directly injected into the brain. Thus, conversion of MPTP to MPP' in peripheral tissue can be considered a detoxification pathway, since peripherally generated MPP' is relatively "inert" from the point of view of inducing CNS damage.

Nigrostriatal dopaminergic neurons represent the primary target of MPP' toxicity. However, they are unlikely to be the site for MPTP bioactivation to MPP'. Immunocytochemical studies show that dopamine-containing neuronal groups do not express MAO B, whereas this enzyme is located within serotonergic neurons and astrocytes (30). MPTP neurotoxicity is not significantly affected when serotonergic neurons are lesioned (33), thus leaving astrocytes as the most likely cells that generate MPP' in the CNS. Once MPP' is produced within glial cells, it can reach the extracellular space by at least two mechanisms: (a) it is released from damaged astrocytes as a consequence of cell membrane disruption (18,59); and (b) despite its charged chemical structure, it possesses enough lipid solubility to

cross undamaged astrocyte membranes (18). A third mechanism could also account for the presence of MPP' in the extracellular space: MAO-generated MPDP' could cross astrocyte membranes in the form of the lipophilic free base 1,2-MPDP and then undergo auto-oxidation to MPP' (18).

The fate of MPP' in the extracellular space represents a critical step in the mechanism of action of MPTP that explains at least in part the selectivity of this neurotoxicant. It seems likely that MPP' would be cleared from the CNS with only minor neurotoxic effects if it were not recognized as a substrate by the catecholamine uptake system (24). MPP' can therefore be accumulated into dopaminergic neurons where it reaches high enough concentrations and persists long enough to cause irreversible damage. The importance of the active uptake of MPP' in relation to MPTP neurotoxicity is demonstrated by studies showing that dopamine-uptake blockers are capable of preventing the neurotoxic effects of MPTP in rodents (34,50). The effectiveness of uptake blockers against MPTP neurotoxicity in the monkey model is less clear (26,54), possibly due to the different regimens of administration of these agents by different groups of investigators.

Active uptake of MPP' into dopaminergic neurons does not completely explain MPTP selectivity, however. For example, it cannot explain why dopaminergic neurons are particularly vulnerable to MPTP toxicity as compared to other neurons that also express catecholaminergic-uptake sites. It has been suggested that the presence of neuromelanin may be another mechanism contributing to MPTP selectivity toward the nigrostriatal dopaminergic system. MPP' binds with high affinity to neuromelanin and could therefore be selectively sequestered within neuromelanin-containing neurons of the substantia nigra (13). At least three lines of evidence support a relevant role for neuromelanin in MPTP neurotoxicity. First, chloroquine, a compound able to displace MPP' from neuromelanin-binding sites, has been shown to attenuate neurotoxic effects of MPTP in primates (12). Second, injection of synthetic neuromelanin enhanced MPTP-induced dopaminergic neurotoxicity in rodents (35). On the other hand, animal species that do not have a melanized substantia nigra (e.g., aged mice) appear to be susceptible to the neurotoxic effects of MPTP.

Animal Studies

Research on the effects and mechanism of action of MPTP has involved a remarkable variety of animal models, ranging from the medicinal leech to monkeys. A 1984 report first documented the observation that certain strains of mice, particularly C57BL/6 mice, were more sensitive than other rodents to the neurotoxic effects of MPTP (21). These animals have since become the most widely used rodent model for MPTP studies, providing a great body of valuable information. There is little doubt, however, that when one compares the behavioral, neuropathological and neurochemical features of MPTP toxicity with those of idiopathic parkinsonism, the most reliable animal model is the nonhuman primate. For example, parkinsonian signs such as bradykinesia, postural abnormalities, and tendency to freeze are consistent effects of MPTP neurotoxicity in monkeys (14), while MPTP causes relatively nonspecific behavioral changes in rodents. Thus, the comparison of neuropathological and neurochemical features between MPTP-induced and idiopathic parkinsonism in the following paragraphs relates primarily to findings in the primate model.

The main neuropathological feature of idiopathic parkinsonism is a degeneration of neuromelanin-containing neurons in the pars compacta of the substantia nigra, with the lateral and ventral cell groups being most severely affected. Degeneration of pigmented noradrenergic neurons of the locus ceruleus is also typically observed. Another neuropathological hallmark is the presence of eosinophilic inclusions first described in 1912 by F.H. Lewy and since called Lewy bodies. These cytoplasmic inclusions are observed not only in the substantia nigra and the locus ceruleus but also in sites such as the dorsal motor nucleus of the vagus, the nucleus basalis of Meynert, and the sympathetic ganglia. They are not specific for Parkinson's disease and are thought to be indicative of an ongoing degenerative process.

MPTP-induced parkinsonism is characterized by a marked destruction of neurons in the zona compacta of the substantia nigra (4,28). The distribution of lesions within this area appears to depend upon the severity of injury and the duration of survival of exposed animals (19). In animals surviving for more than 6 weeks, the middle and lateral cell groups seem to be more severely affected, thus resembling the pattern of neuronal degeneration in idiopathic parkinsonism. Another similarity between the MPTP model and Parkinson's disease concerns the involvement of the locus ceruleus; nerve cell loss has been reported to occur in this area of the brain in older squirrel monkeys after a protracted MPTP regimen (20). Although eosinophilic intraneuronal inclusions have been observed in monkeys exposed to MPTP, they cannot be considered classic Lewy bodies because of significant histopathological differences (14,19). It has been hypothesized that MPTP-induced inclusions may represent Lewy bodies at an early stage (so-called "pre-Lewy bodies") and that differences between inclusions in MPTP and idiopathic parkinsonism may result from the acute vs. more chronic type of neuronal degeneration. This hypothesis has yet to be proved, and therefore

the absence of typical Lewy bodies remains the most evident neuropathological distinction between MPTP neurotoxicity and Parkinson's disease.

Dopamine is the neurotransmitter used by pigmented neurons which project their axons from the zona compacta of the substantia nigra into the striatum. It is not surprising, therefore, that degeneration of these neurons in Parkinson's disease as well as in MPTP neurotoxicity leads to depletion of dopamine in the nigrostriatal pathway. The pattern and time course of MPTP-induced dopamine depletion have been elucidated in the monkey model and appear to differ significantly in the substantia nigra and the striatum. In the substantia nigra of squirrel monkeys, dopamine levels fall to <50% of the initial values just 24 h after systemic MPTP exposure and remain decreased for at least 10 days (23). This dopamine depletion is accompanied by reduced levels of the dopamine metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). In the striatum, no decrease in dopamine levels is measured at 1 and 3 days after MPTP injection to squirrel monkeys, and actually dopamine values in the putamen are increased in MPTP-treated vs. untreated animals (23). Then, after 5 days of MPTP exposure, a dramatic dopamine depletion occurs in both the caudate and the putamen and persists for at least several days (23). Striatal levels of DOPAC and HVA are consistently reduced from 1-10 days after MPTP injection.

The reason(s) for the different time course of dopamine depletion in the substantia nigra and striatum remain to be elucidated and may include different responses of cell bodies and nerve terminals to MPTP injury. It is possible, for example, that a "paralysis" of dopamine release from nerve terminals in the striatum may result from an initial damage to cell bodies in the substantia nigra, leading to unchanged or increased striatal dopamine levels. The pattern of MPTP-induced dopamine depletion is also likely to reflect tissue features characteristic of the nigrostriatal pathway of primates. Indeed, striatal dopamine depletion in the rodent model (i.e., in mice) occurs within hours of systemic administration of MPTP, and dopamine levels remain below initial values during the following days and weeks (7,26). Understanding the different mechanisms of MPTP-induced neurochemical changes in the primate and the rodent models may help to explain the greater sensitivity of monkeys to MPTP neurotoxicity (26).

It has been reported that, in idiopathic Parkinson's disease, the loss of dopamine is significantly greater in the putamen than in the caudate (25). Whether a similar regional pattern occurs in MPTP neurotoxicity has often been debated. It seems likely, however, that the controversial results reported by different research groups (23,44) are due to variations in MPTP dose, route, and regimen of administration in the different monkey models. However, the finding that dopamine levels in the putamen are more severely depleted than in the caudate of squirrel monkeys injected with MPTP (23) bears significant implications in bringing the MPTP model even closer to idiopathic parkinsonism. This regional pattern of MPTP neurotoxicity is further indicated by data showing a more extensive loss of ^{3}H-mazindol binding sites in the putamen as compared to the caudate of squirrel monkeys (36).

Although dopamine depletion represents the most dramatic and consistent neurochemical feature of MPTP toxicity, several reports suggest the involvement of other neurotransmitter systems, such as the noradrenergic system (45). This is not surprising in view of the pathological findings of extranigral MPTP damage (e.g., to the locus ceruleus) and the possible alterations of nondopaminergic systems in Parkinson's disease.

In Vitro Studies

Several *in vitro* models have been used to evaluate and clarify molecular aspects of MPTP toxicity. The role of the mitochondria) enzyme MAO B in MPTP bioactivation has been determined in mitochondrial preparations (9). Mitochondria have also been used to discover and characterize the ability of MPP' to block mitochondrial respiration, a property that is likely to underlie its cytotoxic effects *(see* Toxic Mechanisms *vide infra) (40).* Synaptosomal preparations have provided valuable information concerning the uptake of MPP' into nerve terminals and the metabolic changes following this active accumulation (55). Mesencephalic neuronal cultures have helped characterize the selective cytotoxic effects of MPP' toward dopaminergic neurons (37), while glial cultures have allowed detailed study of the metabolic pathways of MPTP biotransformation (17). Studies with adrenal chromaffin cells have suggested that sequestration of MPP' by chromaffin vesicles may protect against its cytotoxicity (48), and findings with preparations of brain microvessels support the concept that peripheral metabolism of MPTP prevents its access into the CNS and the consequent neurotoxic effects (49).

This brief review emphasizes the wide range of information obtained using *in vitro* models. By elucidating the mechanism of action of MPTP, these studies have shed light on molecular processes that are likely to be of more general neurotoxicological relevance, such as the role of MAO B in the bioactivation of neurotoxins. Furthermore, *in vitro* studies with MPTP/MPP' have provided valuable models of dopaminergic degeneration. For example, the efficacy of potential therapeutic agents (e.g., neurotrophic factors) against dopaminergic cell death can now be tested in MPP'-treated mesencephalic cultures (42).

Human Studies

Although it is estimated that approximately 400 intravenous narcotics users were exposed to MPTP, a frank parkinsonian syndrome was observed in only seven subjects. A number of reasons can explain this numerical discrepancy, including degree of exposure (i.e., number of doses) and, perhaps, greater vulnerability of the dopaminergic system of the affected population. Affected individuals rapidly developed a clinical syndrome virtually indistinguishable from idiopathic Parkinson's disease (27). This syndrome included not only all the major parkinsonian features (i.e., tremor, rigidity, bradykinesia, and postural instability), but also more subtle signs of the disease such as hypomimia, micrographia, and seborrhea. MPTP patients responded to L-dopa treatment and actually developed the same side effects as those seen in patients with idiopathic parkinsonism (e.g., end-of-dose "wearing off" and peak-dose dyskinesia). A significant reduction in levels of HVA, a major dopamine metabolite, was measured in the cerebrospinal fluid of severely affected MPTP patients (5). Furthermore, positron emission tomography (PET) scanning using ^{1}'F-fluorodopa as the tracer revealed a similar reduction in nigrostriatal radioactivity in MPTP-exposed subjects as compared to parkinsonian patients (6).

Three patients affected with MPTP-induced parkinsonism have undergone bilateral fetal mesencephalic grafting. Transplantation of fetal dopamine-rich neuronal tissue has been reported to exert functional effects in experimental animal models as well as in patients with Parkinson's disease (2,31). An initial report evaluating the effects of mesencephalic grafting in two of the three MPTP patients revealed significant motor function improvement and increased striatal uptake of fluorodopa at 1 and 2 years postoperatively in both patients (58). This positive response to implantation of dopamine-rich mesencephalic tissue further supports the view that the clinical effects of MPTP result from the selective damage of the nigrostriatal dopaminergic system.

Toxic Mechanisms

MPTP-induced neurotoxicity is the consequence of a seemingly concerted sequence of pharmacological and biochemical events, some of which have previously been discussed. Briefly, (a) MPTP crosses the blood-brain barrier by virtue of its lipophilic structure; (b) in the brain, MPTP is converted to its toxic metabolite MPP' *via* MAO B localized within glial cells; (c) MPP' is released from astrocytes into the extracellular space; and (d) dopaminergic neurons actively accumulate MPP' through their catecholamine-uptake system. The final step of this sequence is neuronal cell death, but the ultimate mechanism by which MPP' induces cytotoxicity is still a matter of debate.

It was initially suggested that MPP' could induce the formation of oxygen radicals leading to cytotoxicity *via* a condition of oxidative stress. Experimental evidence supporting this hypothesis has been controversial, however, and most likely the contribution of oxidative stress to MPP'-induced toxicity is *only* secondary (15). The search for a toxic property of MPP' that could directly link its intracellular presence to cytotoxicity led to the finding that MPP' is able to interfere with mitochondrial function. MPP' has been shown to be accumulated into mitochondria *via* the mitochondrial transmembrane potential (46,52). High concentrations of intramitochondrial MPP' are able to inhibit the flow of electrons through the respiratory chain; the site of MPP' inhibition appears to be the same as the classic complex I inhibitor rotenone (40). Blockade of electron flow may lead to oxygen radical formation that could then contribute to the cytotoxic effects of MPP' (10). However, the most serious consequence of complex I inhibition by MPP' is likely to be an impairment of oxidative phosphorylation with depletion of cellular energy supplies in the form of adenosine triphosphate (ATP). Indeed, cytotoxicity caused by both MPTP and MPP' *in vitro* has been clearly correlated with ATP depletion (16,59). Furthermore, it has been shown that the addition of substrates for glycolysis-dependent ATP production can delay MPTP/MPP'-induced cell death (16). A decrease in ATP has also been documented in mice after exposure to MPTP; this decrease selectively occurs in the striatum and ventral mesencephalon, and its time course seems to parallel the accumulation and clearance of MPP' in these areas of the mouse brain (8).

Impairment of mitochondrial function by MPP' could also account for the involvement of excitotoxicity in the cascade of MPTP-induced neurotoxic events. Although studies using N-methyl-D-aspartate (NMDA) receptor antagonists as protective agents against MPTP have generated conflicting results *(7,56,57),* there are reasons to suspect that they play a role in neurotoxicity. For example, increased extracellular levels of excitatory amino acids (EAAs) and/or increased sensitivity of FAA receptors seem probable in view of the following: depletion of energy supplies is likely to affect the uptake and inactivation of EAAs, leading to their accumulation in the synaptic cleft; and, membrane depolarization due to ATP depletion could relieve the voltage-dependent Mg^{r}' block of NMDA channels, resulting in increased receptor sensitivity. Thus, impaired mitochondrial function and excitotoxicity are likely to be linked, and the effects of EAAs may play a role in MPTP neurotoxicity as a consequence of MPP'-induced ATP depletion.

MPTP Analogs

Many MPTP analogs have been synthesized and tested both in *vitro* and *in vivo* in order to identify the molecular properties that would predictively underlie neurotoxicity. These structure-activity studies first focused on the relationship between neurotoxicity of MPTP analogs and their ability to be oxidized by MAO. Several compounds were found to be good substrates for either MAO A or MAO B or both (61). This metabolic activation appeared to be necessary, but not sufficient, however, to predict the neurotoxic effects of MPTP analogs. For example, 1-methyl-4-(2',6'-dimethylphenyl)-1,2,3,6-tetrahydropyridine was found to be metabolized by MAO at significantly higher rates than MPTP, but did not cause significant reduction in striatal dopamine levels after systemic administration to mice (61). Thus, other properties besides being a MAO substrate are necessary for MPTP analogs to become neurotoxic; knowledge of the events underlying the action of MPTP has helped to identify the critical factors. Such factors include the ability of MPP' analogs to (a) function as substrates for the dopamine-uptake system, and (b) interfere with mitochondrial respiration. Cultured mesencephalic neurons were used to show a correlation between selective neurotoxicity of MPP' analogs and uptake into dopaminergic neurons (51); also, compounds with high affinity for the dopamine-uptake system did not exert neurotoxic effects unless they were also potent inhibitors of mitochondrial respiration. This correlation between respiratory chain inhibition and neurotoxicity of MPTP/MPP' analogs has been emphasized in a number of reports (47,53). It is noteworthy, however, that extremely potent mitochondrial inhibitors (i.e., 4'-alkylated analogs of MPP'), which lack affinity for the uptake system, did not target dopaminergic neurons but caused a nonselective damage of neurons in culture (51). Thus, structure-activity studies with MPTP/MPP' analogs have emphasized the need for at least three critical molecular properties that would make these compounds selectively neurotoxic: (a) MAO activation of the pyridine derivative to the corresponding pyridinium metabolite, (b) active uptake by dopaminergic neurons, and (c) inhibition of oxidative phosphorylation after accumulation into mitochondria.

The search for compounds structurally similar to MPTP that could be formed endogenously within the CNS has pointed to tetrahydroisoquinolines (TIQs) and tetrahydro-0-carbolines (TBCs) as possible endotoxins. These compounds could be formed in the brain via spontaneous Pictet-Spengler cyclization of catecholamines and tryptamine. TIQs have been detected in the human brain and, in particular, in the brain of patients with Parkinson's disease (41). They have been reported to decrease dopamine and tyrosine hydroxylase levels in the nigrostriatal pathway of monkeys after prolonged administration (38), although these findings have not been confirmed by other investigators (43). TBCs have also been detected in mammalian brain (32). N-Methylated derivatives of TBCs have been shown to possess biochemical properties similar to MPP' (e.g., inhibition of mitochondrial respiration) as well as dopaminergic neurotoxicity (1,32,52). Thus, N-methylation has been suggested to be a toxification route for potential endogenous toxins (11).

Both TIQs and TBCs seem to be similar to MPTP in their requirement for activation to the corresponding quinolinium and carbolinium metabolites to become neurotoxic. Although controversial data have been reported concerning the ability of TIQs to be metabolized by MAO (39,52), it appears that TIQs and TBCs are not substrates or are very poor substrates for MAO. It is possible, therefore, that lack of dramatic neurotoxicity following systemic administration of TIQs or TBCs is the consequence of a limited production of the corresponding toxic metabolites. Hence, detection of quinolinium and carbolinium compounds in mammalian brain, as well as evaluation of possible metabolic pathways for the production of these MPP'-like agents, is a critical area for investigation (11,32). Findings from these studies may provide evidence in favor of an etiological role of TIQs and/or TBCs in Parkinson's disease.

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