

The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): a technical review of its utility and safety

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Parkinson's disease (PD) is a common disabling neurodegenerative disorder the cardinal clinical features of which include tremor, rigidity and slowness of movement (Fahn and Przedborski 2000). These symptoms are attributed mainly to a profound reduction of dopamine in the striatum due to a dramatic loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) (Fahn and Przedborski 2000). Thus far, both the cause and the mechanisms of PD remain unknown. Over the years, investigators have used experimental models of PD produced by several compounds such as reserpine, 6-hydroxydopamine, methamphetamine, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to provide insights into the mechanisms responsible for the demise of dopaminergic neurons in PD. To this end, MPTP has emerged unquestionably as a popular tool for inducing a model of PD in a variety of animal species including monkeys, rodents, cats, and pigs (Kopin and Markey 1988). The sensitivity to MPTP and therefore its ability to induce parkinsonism closely follows the phylogenetic tree where the species most closely related to humans are the most vulnerable to this neurotoxin. Due to the significant neurotoxicity of MPTP, it is important that researchers appreciate the potential hazards of this toxin. Given this, the purpose of this review is to inform the researcher of the hazardous nature of MPTP and to provide guidance for its safe handling and use.

MPTP models of PD

MPTP is a by-product of the chemical synthesis of a meperidine analog with potent heroin-like effects. MPTP

can induce a parkinsonian syndrome in humans almost indistinguishable from PD (Langston and Irwin 1986). Recognition of MPTP as a neurotoxin occurred early in 1982, when several young drug addicts mysteriously developed a profound parkinsonian syndrome after the intravenous use of street preparations of meperidine analogs which, unknown to anyone, were contaminated with MPTP (Langston *et al.* 1983). In humans and non-human primates, depending on the regimen used, MPTP can produce an irreversible and severe parkinsonian syndrome that replicates almost all of the features of PD, including tremor, rigidity, slowness of movement, postural instability, and even freezing; in non-human primates, a resting tremor characteristic of PD has only been demonstrated convincingly in the African green monkey (Tetrad *et al.* 1986). The responses, as well as the complications, to traditional antiparkinsonian therapies are virtually identical to those seen in PD. It is believed that in PD the neurodegenerative process occurs over several years, while the most active phase of neurodegeneration is completed within a few days following MPTP administration (Langston 1987;

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Abbreviations used: MAO-B, monoamine oxidase B; MPDP⁺, 1-methyl-4-phenyl-2,3-dihydropyridinium; MPP⁺, 1-methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MSDS, material safety data sheet; PD, Parkinson's disease; PPE, personal protective equipment; SNpc, substantia nigra pars compacta.

Jackson-Lewis *et al.* 1995). However, recent data suggest that, following the main phase of neuronal death, MPTP-induced neurodegeneration may continue to progress 'silently' over several decades, at least in humans intoxicated with MPTP (Vingerhoets *et al.* 1994; Langston *et al.* 1999). Except for four cases (Davis *et al.* 1979; Langston *et al.* 1999), no human pathological material has been available for studies and thus, the comparison between PD and the MPTP model is largely limited to primates (Forno *et al.* 1993). Neuropathological data show that MPTP administration causes damage to the nigrostriatal dopaminergic pathway identical to that seen in PD (Agid *et al.* 1987), yet there is a resemblance that goes beyond the loss of SNpc dopaminergic neurons. Like PD, MPTP causes greater loss of dopaminergic neurons in SNpc than in ventral tegmental area (Seniuk *et al.* 1990; Muthane *et al.* 1994) and, at least in monkeys treated with low doses of MPTP but not in humans, greater degeneration of dopaminergic nerve terminals in the putamen than in the caudate nucleus (Moratalla *et al.* 1992; Snow *et al.* 2000). However, two typical neuropathologic features of PD have, until now, been lacking in the MPTP model. First, except for SNpc, pigmented nuclei such as locus coeruleus have been spared, according to most published reports. Second, the eosinophilic intraneuronal inclusions, called Lewy bodies, so characteristic of PD, thus far, have not been convincingly observed in MPTP-induced parkinsonism (Forno *et al.* 1993), although, in MPTP-injected monkeys, intraneuronal inclusions reminiscent of Lewy bodies have been described (Forno *et al.* 1986).

Modes of administration

To date, the most frequently used animals for MPTP studies are monkeys, mice and rats. The administration of MPTP through a number of different routes using different dosing regimens has led to the development of several distinct models, each characterized by some unique behavioral and/or biochemical features. The manner in which these models were developed is based on the concept of delivering MPTP in a fashion that creates the most severe and stable form of SNpc damage with the least number of undesirable consequences such as acute death, dehydration and malnutrition. Although MPTP can be given by a number of different routes, including gavage and stereotaxic injection into the brain, the most common, reliable, and reproducible lesion is provided by its systemic administration (i.e. subcutaneous, intravenous, intraperitoneal or intramuscular).

Monkeys

The most commonly used regimens in monkeys are the multiple intraperitoneal or intramuscular injections and the intracarotid infusion of MPTP (Petzinger and Langston 1998). The former is easy to perform and produces a

bilateral parkinsonian syndrome. However, often the monkey exhibits a generalized parkinsonian syndrome so severe that chronic administration of levodopa is required to enable the animal to eat and drink adequately (Petzinger and Langston 1998). On the other hand, the unilateral intracarotid infusion is technically more difficult, but causes symptoms mainly on one side (Bankiewicz *et al.* 1986; Przedborski *et al.* 1991), which enables the monkey to maintain normal nutrition and hydration without the use of levodopa.

For many years monkeys were mainly, if not exclusively, treated with harsh regimens of MPTP to produce an acute and severe dopaminergic neurodegeneration (Petzinger and Langston 1998). More recently, several investigators have treated monkeys with low doses of MPTP (e.g. 0.05 mg/kg 2–3-times per week) for a prolonged period of time (i.e. weeks to months) in an attempt to better model the slow neurodegenerative process of PD (Schneider and Roeltgen 1993; Bezard *et al.* 1997; Schneider *et al.* 1999). While both the acute and the chronic MPTP-monkey models are appropriate for the testing of experimental therapies aimed at alleviating PD symptoms, it is the chronic model that is, presumably, the most suitable for the testing of neuroprotective strategies.

Mice

In addition to monkeys, many other mammalian species are also susceptible to MPTP (Kopin and Markey 1988; Heikkila *et al.* 1989; Przedborski *et al.* 2000). Mice have become the most commonly used species for both technical and financial reasons. However, several problems need to be emphasized. First, mice are much less sensitive to MPTP than monkeys; thus, much higher doses are required to produce significant SNpc damage in this animal species, presenting a far greater hazardous situation. Second, in contrast to the situation in monkeys, mice treated with MPTP do not develop parkinsonism. Third, the magnitude of nigrostriatal damage depends on the dose and dosing schedule (Sonsalla and Heikkila 1986).

Rats

The use of MPTP in rats presents an interesting situation (Kopin and Markey 1988). For instance, rats injected with mg/kg doses of MPTP comparable to those used in mice do not exhibit any significant dopaminergic neurodegeneration (Giovanni *et al.* 1994a; Giovanni *et al.* 1994b). Conversely, rats injected with much higher doses of MPTP do exhibit significant dopaminergic neurodegeneration (Giovanni *et al.* 1994a; Giovanni *et al.* 1994b) although, at these high doses, rats have to be pretreated with guanethidine to prevent dramatic peripheral catecholamine release and extensive mortality (Giovanni *et al.* 1994a). These findings indicate that rats are relatively insensitive to MPTP, but regardless of this drawback, rats continue to be used often in MPTP studies (Storey *et al.* 1992; Giovanni *et al.* 1994a; Giovanni

et al. 1994b; Staal and Sonsalla 2000; Staal *et al.* 2000). In rats, the systemic administration of MPTP is rarely used and the vast majority of studies involve the stereotaxic infusion of MPTP's toxic metabolite, 1-methyl-4-phenylpyridinium (MPP⁺) (Storey *et al.* 1992; Giovanni *et al.* 1994a; Giovanni *et al.* 1994b; Staal and Sonsalla 2000; Staal *et al.* 2000).

Intervening factors

Several factors influence the reproducibility of the lesion in monkeys, rats, and mice. However, to our knowledge, the extensive and systematic assessment of these factors has only been done in mice, and can be found in the following references (Heikkila *et al.* 1989; Giovanni *et al.* 1991; Giovanni *et al.* 1994a; Giovanni *et al.* 1994b; Miller *et al.* 1998; Hamre *et al.* 1999; Staal and Sonsalla 2000), the highlights of which can be summarized as follows: different strains of mice (and even within a given strain obtained from different vendors) can exhibit strikingly distinct sensitivity to MPTP. This differential sensitivity acts in an autosomal dominant fashion (Hamre *et al.* 1999). Gender, age, and body weight are also factors that modulate MPTP sensitivity as well as reproducibility of the lesion, in that female mice are less sensitive and exhibit more variability in the extent of damage than males, as do mice younger than 8 weeks and lighter than 25 g. From our experience, optimal reproducibility in MPTP neurotoxicity is obtained using male C57 BL/6 mice 8–10 weeks of age and 25–30 g in weight. Also of importance is that, following MPTP administration, some mice will die within the first 48 h postinjection; note that C57 BL/6 mice from different vendors exhibit dramatically different magnitudes of acute lethality, ranging from 5% to 90%. This common issue is unlikely related to a toxic effect in the central nervous system but rather toxicity to the peripheral nervous and cardiovascular systems. Although, to our knowledge, this possibility has never been formally studied, we believe that, following acute MPTP administration, mice develop fatal alterations in heart rate and blood pressure. Moreover, MPTP intoxication causes a transient drop in body temperature, which not only can modulate the extent of dopaminergic damage (Moy *et al.* 1998), but can also contribute to acute lethality. Death rate can be reduced by maintaining the body temperature of the injected mice using a temperature-controlled warming pad (do not use a lamp, which can kill mice by overheating them as there is no control of the temperature).

Metabolism of MPTP

MPTP has a complex multistep metabolism (Tipton and Singer 1993; Przedborski *et al.* 2000). It is highly lipophilic, and freely and rapidly crosses the blood–brain barrier. Within a minute after MPTP injection, levels of the toxin are detectable in the brain (Markey *et al.* 1984). Once in the

brain, MPTP is metabolized to 1-methyl-4-phenyl-2,3-dihydropyridinium (MPDP⁺) by the enzyme monoamine oxidase B (MAO-B) in non-dopaminergic cells. Then MPDP⁺ is oxidized to the active MPTP metabolite, MPP⁺, which is then released into the extracellular space, where it is taken up by the dopamine transporter and is concentrated within dopaminergic neurons, where it exerts its toxic effects. The essential role of these different metabolic steps in MPTP-induced neurotoxicity and the fact that MPP⁺ is the actual culprit are demonstrated by the following observations: (1) pretreatment with MAO-B inhibitors such as deprenyl prevents MPTP biotransformation to MPP⁺ and blocks dopaminergic toxicity (Heikkila *et al.* 1984; Markey *et al.* 1984); (2) pretreatment with dopamine uptake inhibitors (e.g. mazindol) prevents MPP⁺ entry into dopaminergic neurons and also blocks dopaminergic toxicity (Javitch *et al.* 1985), at least in mice; and (3) striatal MPP⁺ content correlates linearly with dopaminergic toxicity in mice (Giovanni *et al.* 1991).

Body distribution and environmental contamination

Knowing where MPTP and its toxic metabolite, MPP⁺, accumulate both inside and outside of the body of the injected animal following MPTP administration is germane to the formulation of any set of standard practices for the safe use of MPTP.

Following MPTP administration to both mice and monkeys, only the interior surfaces of the cage, the surfaces that the animals and/or their excreta could physically touch, including food and drinking bottle, are contaminated with MPTP and its metabolites (Yang *et al.* 1988). Conversely, no evidence of contamination is found outside of the cage or on the outside surrounding surfaces (Yang *et al.* 1988). At two days postinjection, 70% of the total injected dose of MPTP and its metabolites is recovered from the inside cage-wash, urine and feces, of which about 15% in mice and 2% in monkeys is unmetabolized MPTP, while the rest is due to MPTP metabolites, such as MPP⁺. Moreover, it appears that the excretion of unmetabolized MPTP occurs mainly during the first day postinjection, while mainly MPTP metabolites are excreted up to 3 days postinjection (Yang *et al.* 1988). There is no evidence either in mice or in monkeys that MPTP and its metabolites are still being excreted after 3 days post MPTP administration. Although high concentrations of MPTP are found in the bile, the main route of MPTP excretion is the urine (Johannessen *et al.* 1986). MPTP in urine will likely be ionized and not volatile, and be well absorbed by the animal bedding. Also, less than 0.01% of the total injected dose of MPTP is detected as volatile MPTP, which probably originates from the animals exhaling MPTP or from vapors from contaminated urine (Yang *et al.* 1988).

One day after an injection of radiolabeled MPTP to mice, most of the radioactivity is localized in the brain and the adrenal gland, while all other organs contain 50–75% lower amounts of radioactivity (Johannessen *et al.* 1986). Analysis of the radioactive species recovered from different organs and body fluids such as bile, urine, blood, and CSF demonstrates variable amounts of unmetabolized MPTP soon after injection, but by 12–24 h postinjection, essentially all of the radioactivity corresponds to MPP⁺ (Markey *et al.* 1984; Johannessen *et al.* 1986).

From the above, it appears that the potential risks of exposure to MPTP are through direct contact with the animal, the animal cage inner surfaces, and its bedding material. There is minimal risk from exposure due to airborne or vapor-borne forms of MPTP. Although safety procedures, as outlined below, must always be followed, the period of maximal risk of MPTP contamination is from the moment of injection to the time that MPTP or its metabolites are no longer found in the excreta of treated animals; as a precautionary measure, we recommend extending the period of high-risk from 3 days to 5 days post-MPTP injection.

Personal protection

Prior to discussing MPTP preparation, injection and animal experimentation, it is necessary to discuss the issues of the recommended facility and personal protective equipment (PPE). As a rule, only investigators and/or staff members who are trained in handling hazardous agents and who are familiar with MPTP safety procedures and practices should prepare and administer MPTP, and monitor the animals during the high-risk period (i.e. 5 days post-MPTP injection). Of note, any staff member who undertakes these tasks should give informed consent and not be coerced into taking on MPTP-related duties. Moreover, it is strongly recommended that all aspects of the MPTP experiment, including storage and solution preparation, take place in a dedicated procedure room (for small animals) or area within the animal room (for large animals), and not in a regular laboratory. For personal safety, when using MPTP, researchers are required to wear the PPE described below, during the preparation of the MPTP solution, the injection period, and 5 days postinjection. Thereafter, regular laboratory attire as required to handle animals is sufficient.

It is important to emphasize that in laboratories committed to MPTP research, one cannot exclude that exposure to even trace amounts of MPTP over many years of the same investigator and/or staff member may have negative consequences. This is one more reason why a heightened standard of protection must be implemented for any individual involved in MPTP experiments.

Dedicated procedure room and area within animal room

All MPTP experiments including preparation of solutions must be performed in a procedure or animal room under negative-pressure because aerosols from MPTP and its metabolites can be generated from bedding, excreta and animal fur. All animals should be acclimated to the room for 4–7 days prior to any MPTP experiment to allow for monoamine stabilization before MPTP injection since monoamine level alterations may affect intragroup lesion reproducibility. The procedure or animal room should have a 12-h light-dark cycle, a bench with a working area, a sink, and be temperature-controlled. For small animals like mice, it should also be equipped with an animal rack to hold all of the cages and a fume hood. All furniture should be of stainless steel or of any material, except wood, that is acid-resistant and washable. All working surfaces including the fume hood and animal racks should be covered with materials that are absorbent on the face-up side and non-absorbent on the face-down side. The entire floor of the procedure room or working area in the animal room for large animals should be covered with plastic-backed absorbent sheets. A warning sign clearly stating ‘Danger! MPTP Neurotoxin Use Area – Entry Restricted’ must be posted on the outside of the procedure or animal room door. The room must be locked at all times and the animal care staff informed of the ongoing use of MPTP and its dangers. They must also be informed that this room is off limits unless allowed to enter by the responsible investigator.

This procedure room or designated procedure area should be completely equipped with all of the necessary supplies for the MPTP experiments. It should also contain a sharps disposal container clearly labeled as hazardous waste, a container lined with a hazardous waste disposal bag for solid waste (diapers, gloves, animal shavings, etc.), gloves, absorbent pads, paper towels, markers, weighing scales for animals and MPTP, sterile saline, syringes with needles, 1% bleach (sodium hypochlorite) solution in water, a strong biodegradable detergent, personal protective equipment (see below), and deprenyl (selegiline), an MAO-B inhibitor, for accidental exposure to MPTP. It is imperative that the material safety data sheet (MSDS) for MPTP, which is supplied by the manufacturer, be kept in the room. Thus, once in the room or area, there should be no need to exit during the injection period.

Personal protection equipment

PPE must be worn during all procedures involving MPTP, including during the 5 days post-MPTP injection. The PPE is far more important when injecting mice than monkeys as mice require significantly higher doses of MPTP. The PPE consists of a one-piece garment with an

attached hood, elasticized wrists and attached boots made of a lightweight, chemically and biologically inert, non-absorbent material that is tear-resistant and provides protection from airborne particles. This garment should be easy enough to get into and economical enough to throw away after one wearing. For example, coveralls made of Tyvek fabric with elasticized wrists and boots and an attached hood (Kaplar, Guntersville, AL, USA) can be used. A full-face respirator with removable HEPA filter cartridges that is fit-tested to the individual is preferred for facial and respiratory protection. Alternatively, a half-face air-purifying respirator with removable HEPA cartridges that is approved by the National Institute of Occupational Safety and Health (NIOSH)/Mine Safety Health Administration (MSHA) for respiratory protection against dusts that is fit-tested to the individual using the respirator can also be used. The respirator is re-usable and should be thoroughly wiped with 1% bleach solution then washed with detergent after each use; wipes must be disposed of in the hazardous waste container. Splash-proof goggles and double-layered nitrile under latex gloves complete the PPE attire. All items comprising the PPE attire can be obtained from a large general laboratory supply company. The office of environmental health and safety in any Institution where MPTP is to be used must be consulted for guidance in obtaining PPE attire for use with MPTP.

Housing

For small animals such as mice, disposable cages and accessories are strongly recommended as they permit incineration of waste without bedding changes. Covering cages with filter bonnets is recommended to significantly reduce both room contamination and cross contamination of other animals. Small animal cages should be placed on the animal rack in the procedure room prior to and during the five-day period post-MPTP injection. All injections must be performed in the fume hood in the procedure room.

For large animals such as monkeys, enclosed cages should be used. The base of the cage and the drop pan must be lined with plastic-backed absorbent pads.

MPTP storage and handling

MPTP can be purchased from several commercial sources. Unless specifically required, do not use MPTP as the free base, but only as the hydrochloride or other non-volatile salt conjugate. MPTP storage and handling must be restricted to the procedure room or designated area within the animal room. Minimize the use of large volumes, concentrated solutions, and handling of MPTP powder and never transport MPTP solutions or opened vials of MPTP outside of the dedicated room. MPTP may be purchased in small quantities of 10 mg or 100 mg in glass septum bottles. Vials

of MPTP must be kept closed until used and stored at room temperature in a container within a vacuum-sealed desiccated container. This second container should be kept in a locked cabinet with a permanently affixed 'MPTP – Neurotoxin' label. This cabinet must be secured to a non-removable surface in the procedure room or area.

Only investigators appropriately trained in the handling of MPTP should perform manipulations involving the powder. Use of glass containers will reduce handling problems that result from the electrostatic properties of plastic. It is strongly recommended that a balance dedicated to weigh MPTP powder be kept in the procedure room. Prior to weighing MPTP powder, cover the weighing area with pads dampened with 1% bleach solution to reduce the risk of airborne MPTP powder particles. To minimize the risk of MPTP powder spills, the weighing procedure described by Pitts *et al.* (1986) is a safe method: tare a small container (e.g. small scintillation glass vial with a screw cap); take the tared container and place an approximated amount of MPTP in it, close and wipe container with 1% bleach solution; weigh container; then add solvent to give desired concentration; again wipe container and all other items with 1% bleach solution; dispose of all wastes in a hazardous waste container. Alternatively, if a given experiment requires a total daily dose of less than 10 mg or 100 mg of MPTP, then it is safer not to open the vial and weigh the powder but to add the desired volume of solvent/vehicle directly to the sealed 10 mg or 100 mg vial. It must be understood that this MPTP solution has to be used in one day and the remainder discarded since MPTP in solution oxidizes at room temperature; prior to discarding the used MPTP sealed vial, inject a volume of 1% bleach solution equivalent to the volume of MPTP solution remaining into the vial, then discard the vial as biohazardous liquid waste. We previously found that storing MPTP solution at -80°C retards its oxidation as MPTP solution appears stable up to 2 months (personal observation). However, unless one has a dedicated -80°C freezer for MPTP storage, other issues such as laboratory safety will arise and that even without mentioning the negative impact of thawing and freezing of MPTP solution on its neurotoxic potency.

Animals should be injected only with sterile solutions of MPTP prepared by either filtration through a disposable $0.22\ \mu\text{m}$ filter unit or by dissolving the compound in sterile saline or water. Do not autoclave MPTP solutions, as this will vaporize the compound and may lead to exposure from inhalation.

Injection of MPTP

As mentioned above, a number of different injection regimens have been used to produce the desired MPTP lesions. These are based on a number of factors, including experimental design, degree of desired lesion, and species

used. As indicated, mice, which typically require greater amounts of MPTP to produce lesions, can be injected either subcutaneously or intraperitoneally, single or multiple injection, and with a wide range of concentrations. Whatever the regimen used, it is recommended that all MPTP injections to mice be performed in a fume hood. Vials from which MPTP is drawn should have a septum or be covered with parfilm to eliminate potential aerosols and spills and to avoid drops on the needle end. Change gloves frequently during the course of and at the end of the injection schedule. This will prevent any contamination of the PPE and decrease the possibility of overt contamination of equipment.

On the day of or on the evening before the experiment, all animals are weighed and coded. About a half-hour before starting the injection schedule, sterile MPTP solution should be prepared to the desired working concentration. During animal injection, care must be taken to avoid self-inoculation; special attention to animal restraint will significantly reduce this risk. For injection, place the mouse cage in the fume hood and when injecting, hold the animal so that any urine spray will fall into the cage and not on the surrounding areas, since mice, when held, tend to expel urine which can contain significant amounts of MPTP (Yang *et al.* 1988). Make sure the mouse is not held so tightly as to cause backflow of the injected MPTP from the peritoneum. Larger animals such as squirrel monkeys must be placed in restrainers for injection. It is not practical to inject large animals in a fume hood. Inspect injection site for leakage or spilled solution and wipe with a small pad dampened with 1% bleach solution. When discarding syringes, do not clip, recap or remove needles from syringes; fill the syringe with 1% bleach solution and then place the syringe with attached needle in a sharps container to be disposed of as biohazardous waste. At the end of the injection schedule, the remaining MPTP solution must be destroyed with an equivalent volume of 1% bleach solution as described above.

Cage changing

The greatest potential for exposure to MPTP and its metabolites is from contaminated bedding and caging immediately following MPTP injection and during the period that MPTP or its metabolites are likely to be in the excreta of treated animals. Therefore, when handling cages and their contents, it is important that the PPE be worn.

Used disposable mouse cages containing contaminated bedding should be dampened with 1% bleach solution and then be carefully placed into a plastic biohazard bag, tied off, and sent for incineration. When using re-usable cages, bedding should also be dampened with 1% bleach solution, then carefully placed in the biohazard bag, packaged and disposed of as biohazardous waste. Immediately after

emptying re-usable cages, soak cages and accessories with 1% bleach solution for 10 min, rinse, then wash with detergent and rinse thoroughly with water. Mouse cages may then be sent to central cage washing facilities. The absorbent material that covered the rack surfaces should be sprayed with 1% bleach solution, allow to soak for 10 min and then disposed of as hazardous waste. For large animal cages, spray plastic-backed absorbent pads that line the cage bottoms and drop pans with 1% bleach solution, allow to soak for 10 min, then remove pads and place them in the biohazardous waste container; replace used linings with fresh pads. This needs to be done on a daily basis. Wash cages and accessories thoroughly with 1% bleach solution, rinse, then wash with detergent and rinse thoroughly with water. The procedure described above assumes that MPTP-injected animals remain in the same cage for 5 days postinjection and change out should occur only after the 5 days postinjection period. In the case of prolonged MPTP exposure protocols (i.e. weeks to months), while the procedure room or area will remain off-limits throughout the treatment period (plus the five days postinjection period), for mice, change only cage bottoms once a week following the procedure described above and, for monkeys, it is advisable to move monkeys to clean cages every other week and to handle the dirty cages as described above.

Counter tops in the procedure room or area should be cleaned with 1% bleach solution. Floor coverings should be carefully removed and disposed of as hazardous waste. Routine animal care can be re-instituted five days post last MPTP injection and once the procedure room or area has been cleaned by the responsible investigator and/or staff member.

Animal tissues

Potential risk of exposure to MPTP or MPP⁺ may occur when animals are killed for tissue collection up to 5 days following MPTP administration. During this period, mice should be killed in the fume hood and the appropriate PPE worn by the researcher during blood and tissue harvesting procedures. All working surfaces are lined with plastic-backed absorbent pads, which should be changed if stained with body fluids. Since decapitation is the primary method of killing for small animals in MPTP studies, care should be taken to prevent blood spatters, and urine and feces should be contained. Brain tissues are best dissected on an inverted glass Petri dish covered with water-dampened filter paper and placed on regular ice. All instruments, including the Petri dish used for dissection, should be soaked in 1% bleach solution for 10 min, rinsed, then washed with detergent and rinsed with water. Collected tissues should always be handled with double gloves, and brain remnants and the remaining carcass, which may contain MPTP and

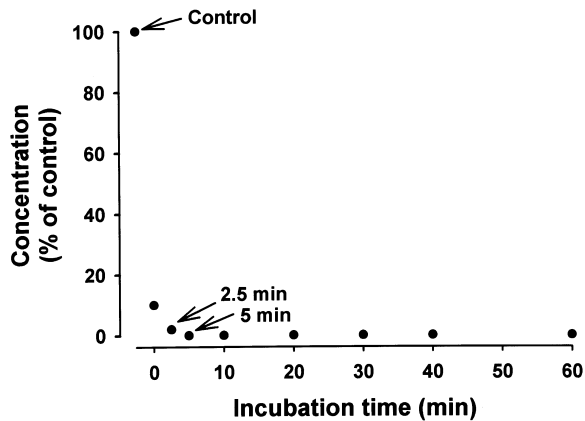


Fig. 1 Time-dependent effect of 1% bleach solution on MPTP. A 5-mg/mL of MPTP-HCl solution in saline was incubated at room temperature for different lengths of time with 1 volume (v/v) of 1% bleach (sodium hypochlorite) solution in water. After the indicated time of incubation, an aliquot of the mixture was injected into an HPLC-UV system and MPTP levels were quantified as described (Przedborski *et al.* 1996).

metabolites (Yang *et al.* 1988), must be discarded following biohazardous waste practices for animal waste.

For the perfusion of small animals, a grid overlaying a collection pan works best. Thus, blood and perfusion solution will be collected in the pan and can then be poured into a bottle or can be discarded as biohazardous waste. As per proper biohazardous waste disposal, the outside of the waste container must be wiped with 1% bleach solution.

For the perfusion of large animals, plastic tubing should be attached to the drain of the dissection table and a liquid biohazard waste container. This will catch any perfusion solution and prevent contamination of the water system. The collected perfusate will be discarded as biohazardous waste. After the perfusion procedure, the table must be washed with 1% bleach solution, rinsed, then washed with detergent and rinsed with water.

Decontamination, cleaning, and disposal

Often, one may see that 0.1 M HCl is used for cleaning up following MPTP experiments. However, we have HPLC evidence showing that HCl, up to 2 M and after incubation for more than 1 h at room temperature, does not destroy MPTP at all. Conversely, a 5% potassium permanganate solution in water completely destroys MPTP almost immediately. However, since potassium permanganate is such a powerful oxidant, it can produce hazardous exothermic reactions with several compounds like detergents and must be neutralized with ascorbic acid prior to being discarded as non-toxic waste. We have also found that bleach is as efficient as potassium permanganate in destroying MPTP, yet more friendly to use as it does not

cause dangerous reactions with detergents and does not require specific treatment prior to discarding. Bleach is commercially available as a 5–10% stock solution. It can be readily diluted to the desired concentration with water and kept at room temperature indefinitely. Using a 1% bleach solution in water, which corresponds to twice the Environmental Protection Agency (EPA) recommended concentration for disinfection, we found that the action of bleach on 5 mg/mL of MPTP-HCl in saline is rapid in that after 5 min, at room temperature, there is no longer any detectable MPTP (Fig. 1). The ‘almost’ instantaneous destruction of MPTP by the bleach solution, as illustrated in Fig. 1, is not a surprising finding since the bleach-mediated reaction corresponds not to an enzymatic reaction but to a straight biochemical oxidation. In addition, we found that 10 min incubation of 5 mg/mL MPTP-HCl with different concentration of bleach solutions, ranging from 0.5 to 2.5%, had similar effects on MPTP. Therefore, our recommendation for MPTP decontamination is 10 min of soaking in 1% bleach solution. In contrast to their effects on MPTP, neither 2.5% bleach solution nor 5% potassium-permanganate destroyed MPP^+ , even after an overnight incubation. This is not surprising, as MPP^+ is notoriously stable and resists destruction even after exposure to extremely harsh chemical and physical treatments. High doses of MPP^+ administered systemically (i.e. 25 mg/kg intraperitoneal) to mice produce oxidative damage to the lung, but fail to affect the nervous system (Johannessen *et al.* 1985). This is consistent with our observation that the intraperitoneal or subcutaneous injection of different doses of radiolabeled and non-radiolabeled MPP^+ to mice failed to show any accumulation of radioactivity in the striatum or to produce any damage to the dopaminergic systems of the brain (unpublished observation). Nevertheless, the direct injection of MPP^+ into the striatum does produce dopaminergic neurotoxicity (Giovanni *et al.* 1994b). These data indicate that the work-related hazards of MPP^+ involve peripheral organs such as the lungs and then only if high amounts reach the blood stream or the respiratory tract. Therefore, MPP^+ is far less hazardous than its parent compound and thus the real safety goal is the destruction of MPTP.

Only investigators appropriately trained in the handling of MPTP should clean up spills. Prior to any decontamination procedure, determine the maximum quantity of MPTP involved in the spill and the location of the spill.

If the room is properly maintained as stated above, linings and underpads will catch any spills. In case a liquid spill does occur, wearing the PPE, the researcher should immediately spray the linings and underpads with 1% bleach solution, allow to soak for 10 min, then remove, and place these in hazardous waste disposal bags. In the event that pads and linings have not caught all of the spill, absorb MPTP spill with absorbent plastic-backed pads to prevent

MPTP solution from contaminating gloves and discard as hazardous waste. The dry area is then soaked with 1% bleach solution, rinsed with water, then washed several times with detergent, rinsed with water, and dried with pads. Discard these materials in hazardous waste bags as well. Recover work area and inform the environmental health and safety office that an MPTP spill has occurred and what measures were used to remove that spill.

To clean up MPTP powder spills, cover with a disposable towel dampened with 1% bleach solution, then pick up all materials and put into a hazardous waste container. Then, soak the area with 1% bleach solution, rinse with water, then wash several times with detergent, rinse with water, and dry with pads. Discard these materials in hazardous waste bags. Recover area, then inform the environmental health and safety office that a MPTP powder spill has occurred and what measures were taken to contain and clean up the powder spill.

If clothes become contaminated with MPTP, immediately remove clothing and shower. After obtaining fresh clothing, report directly to a medical service. A very careful evaluation of any potential MPTP exposure is critical (see medical emergency and surveillance). Persons assisting exposed individuals should wear the PPE attire.

Plan experiments to avoid generating large quantities of contaminated glass or metal; these materials are difficult to incinerate, and large quantities can create waste disposal problems. Contaminated glass and metal can be decontaminated using 1% bleach solution followed by detergent washes and rinses. Decontaminate all equipment with wipes dampened with 1% bleach solution before repair work is performed, before transferring equipment to other operations, and before discarding. Pay special attention to internal parts of equipment that may have become contaminated.

Prevention, medical emergency and surveillance

To date, there has been no report in the literature of the inadvertent exposure of a researcher to MPTP while conducting MPTP experiments. A single report of a research chemist who suffered a fatal exposure to large amounts of MPTP during its synthesis has been documented and represents the only inadvertent human exposure to MPTP (Langston and Ballard 1983). However, despite the safe track record of MPTP use, precautionary emergency procedures must be employed to avoid potential injury from acute exposure to the toxin (such as a needle prick).

As indicated above, MAO-B inhibitors prevent the conversion of MPTP to its toxic metabolite, MPP⁺ thereby preventing neurotoxicity. For example, pretreatment of animals with deprenyl, a potent irreversible MAO-B inhibitor prevents MPTP-induced neurotoxicity (Cohen *et al.* 1984; Mytilineou and Cohen 1985; Fuller *et al.* 1988). On the other hand, except for a single report (Tatton

1993), there is no evidence that MAO-B inhibition by deprenyl or by other compounds, following exposure to MPTP provides any neuroprotection. However, in case of accidental exposure to MPTP, in an attempt to block the conversion of any remaining MPTP to MPP⁺ it is recommended that deprenyl be administered immediately. As far as we know, there is no established deprenyl regimen for accidental exposure to MPTP. Since the goal here is to prevent the conversion of MPTP by inhibiting MAO-B, as rapidly and profoundly as possible, we suggest an initial large dose of deprenyl (e.g. four 5 mg tablets) be taken orally at once. Although it may be prudent to continue deprenyl medication (e.g. 5 mg twice a day) for some time, it is unknown whether this is justified. Short-term surveillance is necessary for the appearance of hypotension from the deprenyl or the development of acute parkinsonian symptoms from the MPTP exposure. In addition, following the administration of a large dose of deprenyl, individuals must be cautious in consuming tyramine-containing foods (i.e. cheese) and in taking medications containing pharmacologically active amines. Prior to beginning any MPTP investigation, deprenyl must be available for emergency use and must be kept in a closed container at all times in the procedure room or area for immediate use, if necessary. Furthermore, it is advisable that individuals who are planning to embark upon a series of MPTP experiments consider a treatment of 5 mg twice a day of deprenyl prior to (e.g. 3–5 days before) and during the experiments. This may be especially indicated for a person first learning the protocol or if there is an increased risk of contact with MPTP. This should be done only after consulting one's personal physician.

Conclusion

To date, MPTP remains the best experimental model of PD. To this end, it is extensively used in various animal species and especially in mice. However, even as a research tool, MPTP is an extremely hazardous compound, which can be injected, ingested, inhaled, and/or absorbed. Because of its demonstrated toxicity to humans, the use of MPTP among researchers is a serious concern. Over the years, a better understanding of the physicochemical properties of this toxin, its metabolism, and its body distribution has enabled investigators to develop practices and procedures for the safe use of this compound. These include improved procedures for preparing MPTP solutions and for its injection into animals, proper protective equipment, reducing potential exposure from animal excreta, proper decontamination and disposal procedures, and medical treatment and surveillance in case of accidental exposure. Despite the fact that we have tried to cover the most common situations and topics related to MPTP use, this review cannot cover all possible aspects of the safe use of

this hazardous compound. Accordingly, there can be no substitute for common sense and proper laboratory practices in the use of dangerous compounds such as MPTP. It is hoped, however, that this review has built upon the guidelines presented by others in the past and, in conjunction with our recent knowledge of MPTP, will lead to the effective and safe use of the MPTP animal model of PD.

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