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Standard Operating Procedure (SOP) for systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in non-human primates

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ABSTRACT

This protocol details standard operating procedure (SOP) for systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in non-human primates.

ATTACHMENTS

673-1419.docx

GUIDELINES

Preface

Because of its relative selectivity for dopamine neurons, the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is frequently used to induce parkinsonism in primates. MPTP can be used by systemic (s.c., i.m., or i.v.) injections, or through intracarotid injections. The procedures described here apply to the use of systemic injections of the compound.

MPTP use poses health risks for the personnel administering the agent. Accidental MPTP exposure can produce parkinsonism in humans. MPTP may enter the body via absorption through the skin or eyes, injection, inhalation of vapors or powders, or by ingestion. Many of the points made below are in place to address these safety concerns.

An essential part of the biocontainment effort is focused on quarantine the animals during the MPTP exposure. To this end, they are placed into a dedicated room prior to the injections and remain in this room for a 72 hour period following the injections. During this time all of their excreta are collected and disposed of in dedicated biohazardous waste containers for later incineration. The relevant animal housing and husbandry details are mentioned in sections 3.4-3.6

Personnel education

Keywords: Preparation of MPTP solutions, MPTP administration procedures, MPTP Animal Room Arrangements , Animal Housing and Husbandry

- Personnel working with MPTP need to be fully informed of the potential hazards before beginning to work with the chemical. Personnel who administer MPTP may have to undergo specialized training provided by occupational health personnel.
- The use of full-body personal protective equipment (see below) is essential. This includes the use of a well-maintained half-mask or full-face respirator with appropriate filter cartridges (at Emory University, a 3M™ Multi Gas/Vapor Cartridge/Filter 60926, P100 is used for this purpose). Personnel using respirators undergo annual health assessments to assess their fitness for using the respirator.
- Most institutions will have a Chemical Safety Board which needs to approve the use of this agent.
- Personnel has access to doses of selegiline (5 mg tablets) to use in cases in which accidental exposure to MPTP has occurred. Personnel receives training on when and how to use this agent.

References

- National Institutes of Health, Office of Research Services. “Procedures for Working with MPTP or MPTP-treated Animals.” Retrieved from: https://www.ors.od.nih.gov/sr/dohs/Documents/Procedures_for_Working_with_MPTP_or_MPTP_Treated_Animals.pdf.
- Przedborski S, Jackson-Lewis V, Naini AB, Jakowec M, Petzinger G, Miller R, Akram M. The parkinsonian toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP): a technical review of its utility and safety. J Neurochem. 2001 Mar;76(5):1265-74.

MATERIALS

Materials needed

Personal protective equipment (PPE)

- Disposable water-repellent coverall (i.e., Tyvek suit)
- Two pairs of chemical-resistant (nitrile) gloves that cover the wrists of the coveralls
- Headcover
- Shoe covers
- Safety goggles or face shield (if using a half-face respirator)
- Half- or full-face mask respirator (requires annual medical clearance and fit-test)
- Cut-resistant gloves and sleeves

Facility requirements

- Given the extent of preparation and effort required for prevention of biohazard exposure after the injections, it is best to carry out the MPTP injections in a dedicated room
- The MPTP solutions should be prepared in a chemical fume hood or Type II B2 Biological Safety Cabinet

Other materials

- MPTP (e.g., Sigma-Aldrich, catalog number M0896-10MG)
- Sterile saline for injection
- Blunt needles
- Syringes
- Absorbent pads
- 1% bleach solution in water
- Biohazard disposal bags and boxes/containers
- Selegiline, 5 mg tablets (optional)

Note

The Containment Manager routinely inspects the selegiline lock box in the MPTP anteroom to ensure that the initial unexpired prophylaxis dose is available, in a box labeled “For prophylaxis after MPTP exposure - Only for personnel cleared to take selegiline.”

Receipt of MPTP

1 Place the package delivered to the research lab into a chemical fume hood dedicated to MPTP solution preparation.

2 Don a lab coat and two pairs of nitrile gloves and inspect the packaging to ensure no damage.

3 Open the box and visually inspect the bottle.

3.1 If all packaging and bottles are intact, the MPTP bottle is removed from the packaging and then stored in a locked cabinet.

3.2 If the package or its contents is damaged, or the lid is not secure:

- Close the box and chemical fume hood sash and don all PPE listed in Materials section.
- Add 1% bleach solution to each vial to inactivate the MPTP. Discard the MPTP in a dedicated biohazard container.
- Notify the manufacturer that the product arrived damaged and was discarded.
- Contact the Environmental Health and Safety Office to dispose of the biohazard container.

Preparation of MPTP solutions

4 MPTP solutions are considered to be stable for 24 hours at 4 °C, 1-3 months at -20 °C, and 2-6 months at -80 °C. However, given the toxicity of the agent, storage should involve dedicated MPTP storage freezers. The general consensus is that it is preferable to use freshly prepared MPTP solutions.

5 Only investigators and/or staff members trained in and familiar with the use of MPTP should prepare and administer MPTP.

6 MPTP should be prepared under fume hood in the designated MPTP preparation suite.

7 Anybody working with MPTP needs to wear the PPE mentioned in Materials section.

8 A warning sign is posted on the outside of the suite door to alert others that work with MPTP is underway, and that PPE is required to enter.

9 All surfaces that may come in contact with MPTP have to be covered with absorbent plastic-backed disposable bench paper.

Note

The plastic-backed paper is disposed of into biohazard bins after each procedure or whenever a spill occurs.

10 Prepare MPTP solution under the fume hood, by adding the appropriate amount of diluent (saline) into the MPTP bottle.

Note

MPTP-HCl dissolves rapidly in aqueous solution. The appropriate amount of MPTP can be retrieved from the MPTP bottle with a needle/syringe.

11 Before removal from the hood, wipe down the outside of the container and all other items used during preparation with 1% bleach solution.

12 Rinse contaminated equipment with 1% bleach solution before disposal.

13 If the MPTP solution needs to be transported out of the MPTP preparation suite, store the solution in a closed, unbreakable, secondary container. Label the containers to indicate their contents

("Danger: MPTP; dangerous chemical; Neurotoxin"). Place an absorbent material in the secondary container to absorb the MPTP solution in the event of a spill.

MPTP administration procedures

14 Prior to MPTP injections, move the animals into the approved MPTP housing area.

15 Use needle-safe systems, needle-locking or single-unit syringes.

16 Fill the Syringes for dosing in the chemical fume hood using a blunt needle to draw MPTP from the vial into the syringe.

17 Institution Animal Care and Use Committees, or veterinary SOPs may limit the maximal dose of MPTP that can be administered to animals (per day or per week). As an example, an IACUC policy at Emory University limits the total daily dose given by systemic injections to 0.8 mg/kg.

18 After withdrawal of MPTP for injection, inactivate the remaining MPTP solution in the vial with the bleach solution and discard into a biohazard material (sharps) container.

19 Dispose the needle and syringe post-injection immediately into a sharps container.

20 Inspect the injection site for leaking or spilled solution. Absorb Leaking or spilled solution with an alcohol-dampened pledget or sterile gauze.

21 Order of PPE removal (after completion of injections).

21.1 First remove shoe covers and disposable coveralls (Tyvek suit).

21.2 Remove outer layer of gloves.

21.3 Remove respirator.

21.4 Clean respirator after each use.

- Remove cartridges and wipe down using a 1% bleach solution.
- Spray respirator (without cartridges attached) with 1% bleach solution or wash with soap and water.
- Allow respirator to air dry in an appropriate area (e.g., locker).
- Place dry respirator and cartridges in a sealed plastic bag for storage.

21.5 Remove head cover.

21.6 Remove the inner layer of the gloves.

21.7 Place disposable garments in a biohazard bag, and mark the bag as “pathological waste. “

21.8 Place bags in the biohazard container pending incineration.

21.9 Thoroughly wash exposed areas of skin.

MPTP Animal Room Arrangements

22 For the time of the injection and a 72-hour post-injection containment period, place animals into the dedicated MPTP treatment suite.

23 The MPTP treatment room may be shared by several animals receiving MPTP treatment concomitantly. These can either be in a single rack of cages, or separate racks. If animal(s) have to enter an isolation room during the quarantine period of (an)other animal(s), house the animals that receive MPTP injections later in a different cage rack.

24 All animals housed in the same rack must leave the MPTP room simultaneously.

Animal Housing and Husbandry

25 House the animals injected with MPTP in areas isolated from the general animal colony.

26 Access to the MPTP animal room is limited to trained personnel for a minimum of 72:00:00 3d following the injection of MPTP.

27 Special arrangements for animal housing are made with the Veterinary and Animal Care Departments (see above).

28 Given safety concerns, house the animals in a single-tier rack whenever possible or in the lower cages of double-tier racks.

29 During the 72-hour quarantine period, label animal cages to indicate clearly that the cages contain animals treated with MPTP.

30 PPE required for entry into the NHP animal area during periods that MPTP or its metabolites are likely to be in the excreta of treated animals are listed in Materials section.

31 Remove PPE immediately after exiting the MPTP isolation room, following the procedures mentioned Step 21.

32 As aerosols of MPTP and/or its metabolites may be generated from bedding, excreta, or the animal's hair/coat, take special precautions during cage cleaning activities to reduce the creation of aerosols.

32.1 Line cage pans with disposable plastic-backed absorbent pads. Use dry bedding as an alternative if necessary.

32.2 Prior to removal of pads or bedding, spray excreta with 1% bleach solution.

32.3 If dry bedding is used, dampen the bedding with 1% bleach and allow 10-15 minutes of contact before disposal to reduce airborne particles.

32.4 Carefully remove absorbent pads, bedding, and excreta from the drop pan and place in a

biohazard bag for incineration.

32.5 Transfer the animal to a clean cage after 72:00:00 following the MPTP injection. 3d

32.6 Spray cages and pans with 1% bleach solution before cage washing.

33 Once the animal(s) have been transferred to clean cages, and the room has been decontaminated, the use of special MPTP PPE is not required, and the room may be cleaned according to standard husbandry procedures.

Transport of MPTP-treated animals

34 Animal transport during the quarantine period should be avoided if at all possible.

35 If animal transport is unavoidable, anesthetize the animal. Then, cover all surfaces the animal touches with absorbent, plastic-backed disposable paper, and decontaminate the surfaces later with a 1% bleach solution.

36 Place a diaper on the animal.

37 Transport the animal wrapped in a plastic bag or similar (avoid wrapping the head of the animal).

Procedures in case of a spill

38 Evacuate people from the immediate area.

39 Don the appropriate PPE (if not already wearing it).

40 If possible to do so without contaminating yourself, spray the spill with 1% bleach solution.

41 Cover the spill with an absorbent, plastic-backed pad.

42 If available, contact the Environmental Health and Safety Office Spill Team if assistance is needed. Provide information on the agent, the size and location of the spill, and contact information.

43 When they arrive, inform the spill response team of any actions and specifics related to the spill.

44 Remove all contaminated clothing and place it in a sealed plastic bag, making sure not to contaminate yourself or the environment.

45 Dispose all contaminated materials used to clean spill of in the MPTP suite.

46 Follow the rules mentioned in Step 21 for PPE removal after decontamination of the area.

Exposure

30m

47 Prompt medical attention is essential should an MPTP exposures occur.

48 In the event of a recognized exposure to MPTP, personnel who have completed a evaluation and have been trained in the use of selegiline can take 4 5-mg tablets (total of 20 mg) of selegiline HCl immediately.

49 Immediately wash the exposure site with soap and water for 00:15:00. If eyes or mucous membranes are involved, flush these for 00:15:00 with water.

30m

50 Follow all reporting requirements at your institution.