MPTP-INDUCED PARKINSON-LIKE DISEASE IN SHEEP: CLINICAL AND PATHOLOGIC FINDINGS

A.M. Beale, a R.J. Higgins, b L.M. Work, c C.S. Bailey, c M.O. Smith, c T. Shinya, d and B.D. Hamrock e

aDepartment of Environmental Toxicology, Departments of Pathology, Surgery, and Anatomy, School of Veterinary Medicine, University of California, Davis, CA 95616, and bDepartment of Pharmaceutical Chemistry, University of California, San Francisco, CA 94143. (Address correspondence to Dr. Robert J. Higgins.)

Eight ewes, divided into two groups based on age, with group 1 7-8 and group 2 1-3 years old, respectively, were administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) intravenously (IV) at cumulative doses of 2.0 to 345 mg/kg body weight. Two group 1 sheep, given cumulative doses of 2 and 8.5 mg/kg, developed persistent severe neurologic signs of body stiffness and rigidity, paucity of movement, intention body tremors, and abnormal body posture and stance similar to those signs in MPTP-induced disease in people and primates. After their acute onset, these persistent signs were nonprogressive up to the observation period of 12 days post infusion. None of the younger ewes had persistent neurologic symptoms at equivalent cumulative doses (80 mg/kg). The only pathologic changes were microscopic lesions in the central nervous system, consisting of bilaterally symmetrical neuronal chromatolysis and necrosis limited to the substantia nigra and locus ceruleus. These lesions were found in two persistently affected and two younger sheep, suggesting age-based differences in dose response and the threshold of clinical expression of disease. Serum MPTP half-life was 17 days. Thus sheep exposed to MPTP could be an alternative model to the primate for a comparative study of clinical, pathologic, and biochemical mechanisms in MPTP neurotoxicity and Parkinson's disease in people.

INTRODUCTION

Interest in the etiology and underlying biochemical changes in Parkinson's Disease (PD) intensified following recognition that human exposure to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) resulted in a clinicopathologic syndrome similar to that of PD (Davis et al., 1979; Langston et al., 1983). In people, the behavioral and locomotor changes of resting tremor, bradykinesia, rigidity, the facial expression, floral posture, shuffling gait, loss of manual dexterity, and difficulty in swallowing are hallmarks of both the MPTP-induced disease and PD (Burns et al., 1985; Kohn, 1980). Likewise, in both diseases, similar lesions of degeneration
of dopaminergic neurons in the substantia nigra (SN) and locus ceruleus (LC) are seen (Burns et al., 1985). Major differences between MPTP neurotoxicity and PD are that the MPTP-induced symptoms occur much more rapidly, are nonprogressive, and occur independently of age (Kopin, 1988). The similarities, however, justified the need for an animal model system to study toxicokinetic, biochemical, and pathologic changes induced by MPTP and to screen possible environmental neurotoxins (Jellinger, 1987).

The most successful model in terms of sensitivity to cumulative dosage (5-15 mg/kg body weight), persistence of clinical symptoms, and similar neuropathologic lesions, has been the subhuman primate (Chiuhe et al., 1984a, b; Forno et al., 1986). However, major disadvantages include their prohibitive expense, nonavailability, and difficulty in handling. Other laboratory animals exposed to MPTP as potential models for PD include rodents (mice, rats, and guinea pigs) and cats. The major drawbacks to use of these animals are very high dose tolerance (from 30 to 150 mg/kg in rats and 300 mg/kg in mice) (Schenker et al., 1986; Sonsalla and Heikkila, 1986), induction of transient or atypical clinical signs with recovery over time (Schenker et al., 1986; Perry et al., 1985; Chiuhe et al., 1984b), and shared lack of typical neuropathologic lesions (Schenker et al., 1986; Perry et al., 1985; Chiuhe et al., 1984b, Heikkila et al., 1984).

Our initial studies in sheep demonstrated that, like primates, classical behavioral and neuropathologic changes could be induced and would persist following cumulative dosages of less than 10 mg/kg MPTP, with an age-dependent dose response. The primary objective of this study was the clinicopathologic characterization of the effects of parenteral MPTP in sheep. The results indicate that sheep satisfy criteria for a suitable model of MPTP-induced PD similar to that described for people.

MATERIALS AND METHODS

Animals

Twelve healthy, white, Merino x Columbia ewes, purchased from a local supplier, were separated into two different groups by age. Group 1 contained seven ewes older than seven years, and group 2 contained five ewes less than three years old. Two sheep were included in each group to serve as negative controls. The animals weighed from 43 to 65 kg. They were housed in enclosed runs and held for at least one week for clinical observation and routine clinical pathology screening prior to initiation of experiments. The animals were fed once daily, with either fresh or pelletized alfalfa; fresh water was available ad libitum.

Drug Administration Procedures

The MPTP, or saline alone in the four control ewes, was administered by controlled infusion through an indwelling catheter in the jugular vein. Each animal was restrained in a metabolism cage during the infusion process. The actual dosage of MPTP, varying from 1 to 3 mg/kg at each infusion, was calculated on the amount of free base in the administered MPTP/HCl and was diluted in one liter of sterile 0.9% NaCl for infusion.
The infusion rate was 1.6 ml/min for group 1 animals and 9 ml/min for group 2 animals, with each infusion time of about 2.5 and 1.75 hours, respectively.

### Schedule of Dosages

Our use of widely differing dosage schedules resulted from our initial experiments to determine a suitable dose-response relationship. All group 1 animals received 1 mg/kg MPTP every other day (see Table 1), except for ewe #900 for which the third dosage was 0.5 mg/kg MPTP and two controls that received normal saline alone on a similar schedule to #790 and #900. Ewes #800 and #950 received a total of 2 mg/kg and were held for seven and eight days, respectively; for clinical observation before euthanasia. Ewe #229 was given 6 mg/kg cumulatively, while #900 and #890 received 8.5 and 9 mg/kg, respectively. After the final dosage, ewe #229 was observed for nine days, #900 for 14 days, and #890 for 21 days before euthanasia.

In group 2 (see Table 1), ewe #767 was dosed with 3 mg/kg every other day to 9 mg/kg total, held for eight days of observation, and then terminated. Ewe #265 received 1 mg/kg on day 1 and 3 followed by 3 mg/kg on day 4 for a total of 5 mg/kg, and was killed on day 4. Ewe #689 received 3 mg/kg followed by 51 days of observation. A series of 1 mg/kg infusions were then administered on days 33, 35, and 37 with a two-day break before a series of 3 mg/kg infusions made:

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Total Dosage (mg/kg)</th>
<th>Day Number / Dosage (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>009</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>229</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>890</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>750</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>900</td>
<td>8.5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group 2</th>
<th>Total Dosage (mg/kg)</th>
<th>Day Number / Dosage (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>689</td>
<td>34.6</td>
<td></td>
</tr>
<tr>
<td>747</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>955</td>
<td>5.0</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.** Schedule of dosages.

<table>
<thead>
<tr>
<th></th>
<th>MPTP 1 mg/kg</th>
<th>MPTP 2 mg/kg</th>
<th>No treatment</th>
<th>Follow-up</th>
</tr>
</thead>
</table>

**Note:** Each box represents one day unless otherwise noted.
on alternate days through day 14 was begun. A total of 346 mg/kg was given to 389, Two control sheep were administered only normal saline, one on ewe 3689's schedule and the other on ewe 3285's schedule.

Sample Collection and Processing
Blood samples were taken from each animal during the observation period prior to exsanguination, immediately before the first preinfusion, and prior to euthanasia. The samples were submitted for routine blood chemistry and hematology analysis. Additional blood samples were taken from ewe 3689 at 30 minutes, 1 hour, 2 hours, 4 hours, and 8 hours; then at 24-hour intervals to day 14 and then every 48 hours to day 21. After collection, these samples were dispensed into separator tubes, allowed to clot at room temperature for one hour, centrifuged before a two-mL serum aliquot was removed, and then added to an equal volume of 5% trichloroacetic acid. After centrifugation, the supernatant was immediately stored at -20°C prior to quantitative analysis for 1-methyl-4-phenylpyridinium (MPP+)(microgram/mL) by the method of Shenk et al., 1987.

Clinical Examinations
The animals were monitored clinically during the infusion procedure for onset and development of neurologic symptoms, including gross, side-to-side head tremors, lip twitching, muscle fasciculations, convulsions, muscle rigidity, depression of pupillary reflexes, wild eyes (opisthotonic appearance), and dysphoria (see Table 2). Depending on the severity of symptoms, the animals were monitored and evaluated either hourly or daily. Body temperature, respiration, and heart rates were recorded both during infusion and at least daily thereafter in ewes 3690 and 3696. The consistency, color, and odor of bowel and bladder movements were noted in all animals during infusion.

Pathologic Examination
All the sheep were euthanized by 144 (Taylor Pharmaceuticals Co., IL) injected IV. The brain was immediately removed and sectioned sagittally. The brain was fixed by immersion in 10% buffered formalin, as were samples of liver, heart, lung, eye, kidney, skeletal muscle, and adrenal glands. Selected tissues from the brain and other organs were trimmed and treated routinely for paraffin embedding and 4-μm-thick sections were cut and stained with hematoxylin-eosin (HE) for routine light microscopic examination. Other selected sections were stained with Holmes silver, luxol fast blue (LFB), luxol fast blue-cresyl Echt violet (LFB-CVE), or Bodian stains. Glial fibrillary acidic protein (GFAP) was visualized immunocytochemically using a routine avidin-biotin-complex, horse radish peroxidase-labeled procedure (Andersson et al., 1987).
<table>
<thead>
<tr>
<th>ANIMAL NUMBER</th>
<th>EXPLOSIONS</th>
<th>HEAD</th>
<th>PAINING</th>
<th>TROUBLE</th>
<th>EDEMA</th>
<th>RIGIDITY</th>
<th>SYMPTOMS &amp; HYPOMNEA</th>
<th>POSTURAL ABNORMALITY</th>
<th>SN</th>
<th>LC</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>000</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>?</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>239</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>?</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>700</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>?</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>900</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>?</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>?</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GROUP 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>689</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>?</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>747</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>?</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>203</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>?</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Clinical Signs Key**
- Mild
- Moderate
- Severe
- ++ -- Presence of neurological signs lasting greater than 48 hours

**Pathology Key**
- Lesion present
- Lesion absent
- Not present
- Lesion combines

## RESULTS

### MPTP Administration

**General.** In both group 1 and group 2, all MPTP-treated animals showed varying degrees of acute transient neurological signs, which began within 15 minutes after the start and abated within one hour after the end of the infusion. These transient signs included 'hyperreactivity, ophthalmyso, kyphosis, and muscle fasciculations. Muscle fasciculations were pronounced 'n the lips and forequarters. Side-to-side head tremors, rigid facial expressions and rhythmic, jerky movements were frequently noted.

Persistent symptoms, lasting beyond the last infusion period, were seen only in group 1 animals and were characterized by body stiffness, paucity of movement, and abnormal body posture and stance (see Table 2). The cumulative amount of MPTP that would induce these persistent symptoms differed from animal to animal. However, initial signs generally were first noted during the last quarter of an infusion. The animals were then reluctant to move and would stand or lie very still, often in awkward or abnormal positions. Over the next 24 hours, it became increasingly difficult for the oes to prehend and eat feed.

Animals in group 1 also differed from those in group 2 in that they were less tolerant of both the rate and concentration of the infusion. In both groups, slowing the infusion rate to 2-4 ml/min alleviated the acute transient neurologic signs just described, apparently without affecting the onset or duration of the persistent symptoms.
During infusion, all the MPTP-treated group 1 and group 2 animals developed progressive softening of the feces, or frank diarrhea, and strong odor in their urine. Both changes resolved with 24 hours following cessation of infusion. None of the two animals in each group infused with normal saline developed any neurologic deficits.

**Group 1.** Two of the five sheep (#790 and #900) developed persistent, severe clinical symptoms, including paucity of movement, body stiffness, incoordination, intention tremors, and a decrease in conscious proprioception lasting 30 and 32 DPI respectively (see Table 2). All five animals developed persistent bradykinesia and hypokinesia, with ewes #790 and #900 severely, #900 strongly, #299 moderately, and #900 mildly affected. Only two developed severe postural abnormalities; the others were less affected. Although four of the five sheep would not do so voluntarily, they would eat if food was placed in their mouths. After cumulative dosages ranging from 1-5 mg/kg all five animals would not stand unassisted, and two of the five were never able to rise unassisted: #790 after 2 mg/kg and #900 after 6.1 mg/kg. However, in ewes #900, #229, and #900, this recurrence was a transient symptom that resolved within 48 hours after infusion ended. The affected animals exhibited concurrent muscle rigidity or fasciculations. In four of the five treated group 1 ewes, fasciculations could be induced by normal handling for periods of between 6 hours and 15 hours after each infusion.

After 2 mg/kg, ewe #790 developed persistent rigidity of the forelimbs, accompanied by muscle tremors (especially in the forequarters), incoordination, head panning, mydriasis, rigid facial expression, and rapid respiration rate (139-186/ minute). Twenty-four hours after the second infusion she remained in sternal recumbency with extended forelimbs. All her movements continued to be abrupt and jerky without remission until she was killed 10 DPI.

In two ewes, #900 and #900, bilateral patellar reflexes could no longer be elicited after 2 mg/kg and 5 mg/kg, respectively. Both sheep were uncoordinated, with general body stiffness but lacking spasticity, and were increasingly reluctant to move. Both, however, had normal menace and withdrawal reflexes. In addition, #900 showed increased jaw tone, digital separation, hyperextension of the fetlock joints, and difficulty rising by 4 DPI. By 9 DPI, conscious proprioception was severely depressed, the ewe had generalized muscle fasciculations, and she refused to walk unless forced. After 20 DPI, she appeared stuporous and took off balance with her hindfeet placed awkwardly under her body. This animal died suddenly 32 DPI.

**Group 2.** The neurologic changes seen in these animals were similar to the previously described acute symptoms. All neurologic signs were transient, characterized by a marked degree of hypersensitivity, head panning, and a wild appearance that developed during infusion and resolved within 20 minutes to one hour after infusion (see Table 2).

**Pharmacokinetic Results**

In ewe #69, the half-life for MPP+ was found to be greater than 11 days following a single infusion of 3 mg/kg MPTP, with serum levels of MPP+ slowly rising to a peak of 84 micrograms/ml after two days.
Pathologic Findings

In the group 1 sheep, lesions were restricted to microscopic changes in CNS in some of the treated sheep. No other lesions were seen on gross necropsy or microscopically in other organs. In the SN, the major lesions were bilaterally symmetrical and consisted of both neuronal chromatolysis and acute neuronal necrosis. The classical central chromatolytic changes were of swollen cytoplasm and cell processes, eccentric displacement of the nucleus, and peripheral margination or sometimes complete loss of Nissl substance, further demonstrated by LFB-CEV stains (see Figure 1). Necrotic neurons had swollen or shrunken eosinophilic granular cytoplasm and processes with faint nuclear staining (Figure 2), and eosinophilic granular axonal spheroids often appeared in the adjacent neuropil (Bodian, Holmes's stains). Other, less severe changes were sporadic vacuolation in the neuropil of the SN and a mild gemistocytic astrocytosis best visualized with the GFAP stain. Lesions in the LC, present only in one of 50 mice, were of acute neuronal necrosis without any cellular response or neuropil change.

Two of the sheep in group 2, #68 and #76, had lesions in the SN similar in nature and topography to those in group 1, although less severe. Finally, routine serum chemistry and hematologic values from blood samples of all ewes were unremarkable.

**FIGURE 1.** Photomicrograph of a transverse section through SN of ewe #90, showing widespread central chromatolysis (arrows) of most neurons (LFB-CEV × 280).
FIGURE 1. Acute neuronal necrosis (arrow) in the SN of one 4702 (16 x 9.8).

DISCUSSION

This study demonstrated that the systemic administration of MPTP to sheep selectively destroys neurons within the SN and sometimes the LC. This damage results in the development of a clinical picture of persistent behavioral and locomotor deficits, including bradykinesia and hypokinesia, postural changes, and intention tremors, which are very similar to the symptoms and pathology in people either affected with PD (Kopin, 1988) or exposed to toxic amounts of MPTP (Langston et al., 1985). The clinicopathologic features in some of these sheep were similar to those reported in primates experimentally administered MPTP (Chauvel et al., 1984a; b, Forno et al., 1984, 1986). Further, we have demonstrated that sheep are as dose-sensitive to intravenously administered MPTP as are primates (Forno et al., 1986). Together, these findings suggest that sheep may be a fact be a suitable alternative for studying the biochemistry of MPTP lesions with distinct advantages over primates in terms of ease of handling, greater availability, and greatly reduced cost.

As noted for primates (Forno et al., 1986) and mice (Langston et al., 1987; Ricarute et al., 1985) our sheep exhibited the phenonemon of age-dependent dose responsiveness, with older animals more neurologically sensitive that is, developing persistent signs at equivalent dosages than younger sheep. Although older sheep could tolerate a lower rate of infusion of MPTP, sheep in both groups were equally sensitive to MPTP with dramatically increased hyperactivity resulting from each successive dosage. This acute systemic effect, however, was reversed in all sheep within hours after the infusion without evidence of chronic neurologic signs. This age difference in dose sensitivity (Langston et al., 1987) may be the result of a more rapid conversion of MPTP to the toxic metabolite MPP⁺ in older
animals since monoamine oxidase-B activity, which catalyzes this conversion, increases with age in all species studied (Fowler et al., 1957; Wagner and Jarvis, 1966). Alternatively, it has been reported in other species. MPTP-seem to have a high-affinity binding to neuromelanin (D'Amato et al., 1986; D'Amato et al., 1987), and the neuromelanin content in the neurons of the SN in sheep and in other species (Marsden, 1986; Marsden, 1983) increases with age. This increase may explain some of the age-dependent response. This phenomenon may also reflect differences in MPTP availability owing to such factors as sequestration in a physiological sink (such as plasma protein binding; Baldessarini, 1983), or the rate of metabolism or excretion (Kogin, 1988).

The low cumulative dose responsiveness in sheep and primates, with persistence of induced neurological signs and lesions, contrasts with the extreme dose resistance in mice (Hekkila et al., 1984), guinea pigs (Perry et al., 1985; Chien et al., 1984), and rats (Chiew et al., 1984b) and their associated lack of permanent neurological signs or neuropathologic features. Although the mechanism underlying this major difference is unknown, species variations in the serum half-life of MPTP may be important. Our results indicate a half-life of 40-112 days in sheep, similar to that in primates and contrasting drastically with the half-life of two hours in mice (Carvey et al., 1986). This dramatic difference in serum half-life may reflect species differences in urinary excretion or resynaptic rates, because a renal organ, function carrier has been described for MPP⁺⁺ in the canine (Sokol et al., 1987). Alternatively, rodents may have a different metabolic pathway, or lack a physiological storage site, to provide a sustained release of MPTP. Obviously, comparative in vivo pharmacokinetic studies on MPTP and MPP⁺⁺ are needed to delineate the factors that may be responsible for these marked age, individual, and species variations.

One major concern with our proposed model is the marked disparity in the reproducibility of clinical and pathologic lesions. The wide variation in dosage schedules reflects our initial attempts to determine an effective dose. Only two of the five older sheep were clinically affected with persistent signs and had CNS lesions whereas the others appeared clinically refractory. This difference might be the result of a tolerance state (Kurlan and Stocel, 1982; Jenner and Marsden, 1987; Melamed et al., 1987) induced by differing dosage regimens (Scanzilla and Hekkila, 1986), especially since a Merino x Columbia breed was used. Breed and individual variations exist in primates (Forno et al., 1986), and although lesions in the SN, and possibly the IC, have been reported in cats (Schneider et al., 1984) and rodents (Hekkila et al., 1984), the lesion appears to be transient in these species.
Neuropsychiatric lesions in the SN or LC in the two sheep with persistent clinical signs were essentially similar in nature and topography to those described in primates (Forster et al., 1980) and humans (Langston et al., 1983). However, lesions in the LC, a hallmark of PD and reported once in MPTP-treated primates (Forno et al., 1984), were not seen. An unexpected finding was the presence of similar, though less severe, morphologic lesions in neurons of two younger sheep in which chronic neurolptic deficits were not elicited. This result only suggests some threshold of neuronal damage before expression of clinical disease, presumably a function of dopaminergic neurotransmitter activity (Goschke et al., 1985).

In summary, we have demonstrated that sheep may be a suitable alternative to the existing primate model of MPTP-induced PD. This claim is based not only on similarities to primates in clinical symptoms and neuropathologic lesions, but also in the dose–response relationships to age, sex, and cumulative dosages. Further, the sheep is a biologically well-described ruminant species that is relatively inexpensive and easy to obtain and handle. These results suggest that sheep may prove valuable for further biochemical and pathologic studies on the neurotoxic effects of MPTP in comparison to PD.

REFERENCES
Andressen CA, Kiguchi RL, Wahlberg AS, Osburn BR. Response of herder disease virus to oligodeoxynu-
D'Amato RJ, Lipman ZP, Snyder SH. Selectivity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) for monoaminergic neurons. Science 1984; 223: 859–861.


Heikkilä RI, Markovic L, Cabiati FL, Dosevicius BC. Protection against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by monoamine oxidase inhibitors. Nature 1984; 311:68-70.


Sennhan H, Massow ME, Ashburn AM, Lipton SB. Effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine on postmortem changes in dopamine turnover and transporter function in the mouse striatum. Eur J Pharmacol 1985; 113:151-166.


