# Preclinical Toxicity Study of Intrathecal Administration of the Pain Relievers Dextrorphan, Dextromethorphan, and Memantine in the Sheep Model

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■ **ABSTRACT**

Objectives To determine the toxicity window for the continuous intrathecal administration of dextrorphan, dextromethorphan, and memantine via an implanted delivery pump.

Materials and Methods Using 48 sheep with programmable continuous intrathecal infusion systems we determined the behavioral, motor, neurological, and histopathological changes produced by a 43-day continuous infusion study of dextrorphan, dextromethorphan, and memantine dissolved in 0.9% NaCl. Daily doses of each N-methyl-D-aspartate (NMDA) antagonist were 0.013, 0.051, 0.203, 0.510, 0.811, and 2.533 mg/kg/day, flow rates ranged from 13.25 ml/day to 0.051 ml/day at a concentration of 10 mg/ml. Control animals received saline in the range of 7.9985 ml/day to 1 ml/day.

Conclusions Infusion of saline in the control animals produced no behavioral or motor changes. However, infusion of dextrorphan, dextromethorphan, and memantine at the higher doses (> 0.051 mg/kg/day) produced dose-dependent negative behavioral, motor, and histopathologic changes as indicated by a series of nonparametric statistical analyses. The minimal toxic doses were dextrorphan dose 3, dextromethorphan dose 1 and memantine dose 1. This study suggests that continuous intrathecal infusion of dextrorphan, dextromethorphan, and memantine via an implantable pump system can cause significant toxicities at the higher doses studied. ■

**KEY WORDS:** continuous, dextromethorphan, dextrorphan, infusion, intrathecal, memantine, sheep.

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N-methyl-D-aspartate (NMDA) receptors in the dorsal horn of the spinal cord are involved in the mediation of nociceptive information (1–3) and their involvement in maintaining pathologic pain states is well documented in the literature (4). Behavioral studies demonstrated that the injection of NMDA receptor antagonist DL-2-amino-5-phosphonopentanoic acid (AP5) into the subarachnoid space suppressed the response to mechanical and thermal nociceptive stimuli (5). Recently, additional studies that focused on phasic pain or hyperalgesia showed that selective NMDA antagonists suppressed periph-

eral inflammation (6), formalin-induced pain behavior (7), tail ischemia (8), hyperalgesia induced by painful mononeuropathy (9,10), allodynia (11), and mechanical hyperalgesia (12). Dubner suggested that the dorsal horn nociceptive neurons mediated hyperalgesia following nerve injury or tissue inflammation which involved the release of excitatory amino acids and their action at the NMDA receptor sites (12). This means that NMDA antagonists can benefit clinical applications as analgesics in the future. The NMDA antagonists dextrorphan and memantine produce analgesia in neuropathic pain animal models (4,13). Tal et al. found the analgesic activity of dextrorphan to be temporary and suggested frequent dosing or continuous infusion. However, there are no data on the safety, side-effect profile, or toxicity of long-term administration in animals or man (14). To establish toxicity levels of continuous intrathecal administration of the NMDA antagonists dextrorphan, dextromethorphan, and memantine, sheep implanted with intrathecal delivery systems were studied prior to preclinical studies.

Epidural and intrathecal administration of opioids like morphine are well studied methods for controlling postoperative and cancer-induced pain (15,16). Because of the concern over the side effects of intrathecal opioids, especially delayed respiratory depression, other classes of drugs are under examination for spinal analgesia (17). With few exceptions, these drugs lack animal neurotoxicity data (18–21). The behavioral and histopathologic effects of long-term continuous intrathecal infusion of dextrorphan, dextromethorphan, and memantine need examination (14). We therefore undertook the study of the behavioral, neurologic, and histologic effects of chronic (up to 43 days) intrathecal infusion of these drugs in the sheep model.

Activation of the NMDA type of excitatory amino acid receptors increases the Ca2 + conductance of the cation channel due to extracellular accumulation of glutamate (22–24). The excessive levels of glutamate or related neurotransmitters are associated with neuroneal cell death in hypoxic-ischemic brain injury (1,25), epilepsy, trauma (26), and several neurodegenerative diseases such as Huntington's disease, Parkinson's disease (27), and acquired immunodeficiency syndrome dementia (28).

The open-channel (use-dependent) NMDA subclass glutamate receptor antagonist dextromethorphan [(+)-3-methoxy-N-methylmorphinan] and its active metabolite dextrorphan [(+)-3-hydroxy-N-methylmorphinan-D-tartrate] are dextrorotary morphinans. Dextromethorphan and dextrorphan are widely used non-narcotic antitussives (29) and more recently found to possess anticonvulsant properties in many seizure models (30,31). Memantine [1-amino-3, 5-dimethyladamantane], an aminoadainantane derivative of the same subclass of NMDA receptors as dextromethorphan and dextrorphan, is among the few NMDA receptor antagonists used clinically for many years for the treatment of Parkinson's disease (32–35). These ligands are well tolerated (oral and intravenous administration), and their safety, compared with that of MK-801 or phencyclidine, may be due to their rapid-response kinetics (2,22,32,36,37). Memantine also shows promise in the treatment of dementia (38–40), spasticity (41) and seizures (31).

## MATERIALS AND METHODS

After approval by the Institutional Animal Care and Use Committee at The University of Texas M.D. Anderson Cancer Center; this study was conducted at M.D. Anderson Cancer Center-Science Park's Department of Veterinary Sciences (Bastrop, TX) in strict accordance to the Guide for the Care and Use of Laboratory Animals published by National Research Council (42). The 48, one- and two-year-old Rambouillet/Suffolk-cross sheep used in this study weighed between 22 and 58 kg. The male sheep ($N = 39$) weighed an average of 43.65 kg and the female ($N = 2$) weighed an average of 36.0 kg. The sizes of the groups were originally two sheep per dose for each of the three agents. However, some sheep at the same dose were equivocal and we added a third sheep to add finer detail to the gradation of changes in some of the groups. Also, dose level 5 of memantine was not administered to any sheep due to the toxic effects already evident at dose level 4.

### Drugs

Our lab obtained dextromethorphan from Sigma Chemical Co. (St. Louis, MO) and purchased dextrorphan and memantine from Research Biochemicals International (Natick, MA). The agents were prepared as the following salts: dextrorphan tartrate (10 mg/ml, pH 3.3), dextromethorphan hydrobromide (10 mg/ml, pH 5.96), and memantine HCl (10 mg/ml, pH 5.90). The vehicle utilized was 0.9% NaCl

(pH 5.7). The agents were individually prepared and sterilized by passing solutions through 0.22 μm Millipore filters (Bedford, MA) into sterile vials in a sterile hood (Nuaire Class II Type A/B3 cabinet, Plymouth, MN) and stored at room temperature. Our lab did not independently analyze the agents. The manufacturer lists the purities to be: dextrorphan > 99%, dextromethorphan > 99%, memantine > 95%. The absence of other manufacturer products was determined using high-performance liquid chromatography.

## Animal Preparation

Each animal received cefazolin as a perioperative antibiotic. Administration of 0.2 mg/kg diazepam and 6.0 mg/kg ketamine allowed for anesthetic induction, intubation, and surgical anesthesia. Two-percent inspired halothane maintained this state of anesthesia.

A midline incision over L-6 to S-1 exposed the muscle fascia. A 16-G Tuohy needle inserted 2.5 cm caudad into the intravertebral space at L-7/S-1 allowed for a bloodless procedure. Slow advancement of the needle until the dura was punctured allowed freely flowing cerebrospinal fluid (CSF) to emerge from the hub of the needle. Threading an intraspinal catheter (Medtronic catheter, model 8703, Minneapolis, MN; outer diameter 1.2 mm) into the Tuohy needle and advancing the catheter cephalad into the subarachnoid space provides drug delivery to the approximate level of T-13/L-1 (catheter is threaded approximately 17 cm cephalad). A 2–0 silk suture secures the metal connector and the pump connector assembly which link the intrathecal catheter to the pump (Medtronic Synchromed model 8615 and 8617). After making a pocket in the left paralumbar fossa the pump connector was tunneled to that area and connected to the pump (Fig. 1). A 2–0 silk suture secured the pump to the muscle fascia. At the time of surgery, and for the week following surgery, the pumps delivered 1 ml of saline per day. We collected bacterial cultures of the saline perfusate prior to implantation of the pumps. A solution made of saline and gentamicin irrigated the wounds followed by a local anesthetic (lidocaine). 3–0 vicryl closed the wounds in layers. A technician administered analgesics (butorphanol, 5 mg, IV) prior to the sheep emerging from anesthesia.

After 1-week postoperative recovery, studies began. Analysis of results included daily body temperature, behavior, and motor changes (Table 1 and Table 2) as well as micturition and defecation habits (no volumetric measurements were taken); weekly blood pressure recordings (Colin Medical Instruments Corp. Press-Mate blood pressure monitor, San Antonio, TX); and complete blood profiles taken on days 1, 15, and 43. Pumps were aseptically refilled as needed (Medtronic refill kit model 8551).

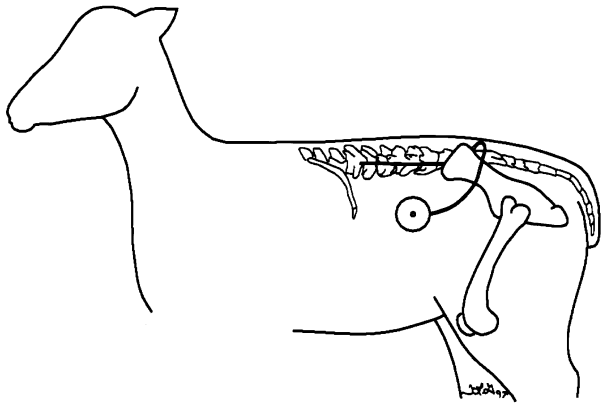

**Figure 1.** Diagram of the Medtronic Programmable continuous intrathecal drug delivery system in the sheep model.

Sheep randomly received either dextrorphan, dextromethorphan, or memantine; no animal received a combination of any of these drugs (Table 2). A dose concentration of 10 mg/ml was chosen because it would be a clinically used concentration. This concentration is high enough to be useful for an implanted pump in humans and easy to use for calculations, and is a concentration where the drug

**Table 1.** Four-grade Scale for the Evaluation of Behavioral and Motor Changes

| Grade | Behavioral and motor change |
|---|---|
| 0 | Animal standing, eating, drinking, and ruminating, with normal respiratory and heart rates. Sheep able to rise and ambulate without any difficulty. |
| 1 | Shuffling of either rear leg or slight limp. Animal biting at site of catheter tip location. Slight distortion of normal spinal axis. |
| 2 | Loss of righting reflex in one of the rear legs; sheep able to stand without assistance but with some difficulty. Decreased interest in eating, ruminating, and environment. |
| 3 | Inability to maintain standing posture. Attempts by technician to help animal stand are unsuccessful. Cutaneous noxious stimuli of hind legs does not elicit any response. |

**Table 2.** Pathoanatomic/Behavioral Results

| Drug | Dose Level | N = | Behavior and Motor Grade | Day of First Symptom | Intrathecal Fibrous Reaction[a] | Inflamatory Cells Present[a] | Necrosis[a] | Days on Drug |
|---|---|---|---|---|---|---|---|---|
| Dextrorphan | | | | | | | | |
| | 1–0.013 mg/kg/day | 2 | 0, 0 | na, na | +, + | +, rare | 0, 0 | 43, 43 |
| | 2–0.051 mg/kg/day | 2 | 0, 0 | na, na | 0, + | +, + | 0, 0 | 43, 43 |
| | 3–0.203 mg/kg/day | 3 | 2, 0, 0 | 6[b], na, na | + + +, +, + | + +, +, + | + +, 0, 0 | 43, 43, 43 |
| | 3.5–0.507 mg/kg/day | 1 | 3 | 20 | 0 | + + | + + | 41 |
| | 4–0.811 mg/kg/day | 2 | 3, 3 | 4, 3 | + +, + + + | + + +, + + + | + + +, + + + | 6, 5 |
| | 5–2.533 mg/kg/day | 2 | 3, 3 | 1, 1 | + + +, + + + | + + +, + + + | + + +, + + + | 7, 8 |
| Dextromethorphan | | | | | | | | |
| | 1–0.013 mg/kg/day | 2 | 1, 0 | 16, na | +, 0 | +, 0 | 0, 0 | 43, 43 |
| | 2–0.051 mg/kg/day | 2 | 2, 0 | 28, na | 0,0 | + +, 0 | + +, 0 | 43, 43 |
| | 3–0.203 mg/kg/day | 2 | 3, 2, 2 | 8, 14, 36 | 0, +, + + | + + +, + + +, + + + | + + +, + + +, + + + | 14, 43, 43 |
| | 3.5–0.507 mg/kg/day | 3 | 3, 3, 3 | 2, 2, 7 | +, + + +, + + + | + + +, + + +, + + + | + + +, + + +, + + + | 35, 17, 36 |
| | 4–0.811 mg/kg/day | 2 | 3, 3 | 1, 3 | 0, + + + | + + +, + + + | + + +, + + + | 4, 14 |
| | 5–2.533 mg/kg/day | 1 | 3 | 2 | 0 | + + + | + + + | 5 |
| Memantine | | | | | | | | |
| | 1–0.013 mg/kg/day | 3 | 0, 0 | na, na | +, 0 | +, 0 | 0, 0 | 43, 43 |
| | 2–0.051 mg/kg/day | 2 | 0, 1 | na, 7 | + + +, 0 | + + +, 0 | + +, 0 | 43, 43 |
| | 3–0.203 mg/kg/day | 2 | 0, 3 | na, 3 | 0, + + | 0, + + + | 0, + + + | 43, 10 |
| | 3.5–0.507 mg/kg/day | 2 | 3, 3 | 4, 5 | + + +, + + | + + +, + + + | + + +, + + + | 7,17 |
| | 4–0.811 mg/kg/day | 2 | 3, 3 | 1, 13 | + +, + + | + + +, + + + | + + +, + + + | 3, 14 |
| Control | | | | | | | | |
| | Blinded–1.636 ml/ day | 4 | 0, 0, 0, 0 | na, na, na, na | +, 0, 0, 0 | +, 0, 0, 0 | 0, 0, 0, 0 | 43, 43, 43, 43 |
| | High Rate–7.9985 mg/day | 1 | 0 | na | + | 0 | 0 | 43 |
| | Low Rate–1 ml/day | 1 | 0 | na | + | 0 | 0 | 43 |

[a]0, No Changes; +, Mild Change; + +, Mild Change; + + +, Severe Change; na = Not Applicable.
[b]This animal began to regain the righting reflex on day 9, and was completely normal on day 13.

is stable in solution. The doses of the NMDA antagonists were initially chosen in this toxicologic assessment to represent a four-fold increase per dose as the doses escalated (levels 1, 2, 3, 4). Dose 3.5 was added to delineate moderate changes (neurologic, histologic) between levels 3 and 4. We also added level 5 as a supramaximal dose (three times higher than dose 4) to see maximal neurologic changes. The high flow rate of the control animal receiving 7.9985 ml/day was administered to demonstrate that behavioral changes were caused by the toxic effects of the drugs and not by the added fluid pressure of larger injection volume at higher known doses.

Prior to the study, previously published literature was reviewed and criteria were developed for the behavioral, neurologic and histopathologic changes (Table 1 and Table 2). However, since there were not any reported data for long-term continuous infusion of these NMDA antagonists, known doses were administered to establish dose-related behavioral, gross anatomic and histopathologic changes. The different doses in these studies were obtained by changing the flow rates (0.051–13.25 ml/day) and the concentrations of drug remained constant (10 mg/ml). These studies were then followed by a double-blinded study in which the experimenters had no knowledge of the 21 different treatment conditions which were actually six dosing levels plus controls. In the blinded studies the flow rate remained constant and the drug concentrations varied so the investigators would be unaware of the dose being administered. A flow rate of 1.636 ml/day was chosen for the blinded studies because it allowed for the different doses to be formulated without the drug falling out of solution at the higher doses.

## Histopathology

Animals were euthanized on day 43 or when the sheep became paraplegic and underwent necrop-

sies. Immediately after each animal was euthanized, we collected approximately 2–3 ml of CSF from the L7/S1 intravertebral space for fluid analysis and for bacteriologic cultures to assure that the changes seen upon histopathologic examination were due to drug-related effects and not bacteriologic considerations.

A laminectomy was performed from S-1 cephalad to C-1, and the location of the catheter insertion and termination site documented. Removal of the entire brain and spinal cord allowed for gross examination to detect significant lesions. Blocks of tissue from the following CNS areas were embedded in paraffin wax for histopathologic examination: cervical, thoracic, and lumbosacral segments of the spinal cord and the olfactory; frontal lobe; posterior cingulate gyrus; amygdala; hippocampus; retrosplenium cortex; cerebellum; pons; and medulla regions of the brain. The spinal cord, brain, and samples of the liver, spleen, heart, kidney, and intestines were fixed for two weeks in 10% neutral formalin. Sections were cut transversely at a thickness of 4 μm to 5 μm, deparaffinized, then stained by conventional methods that included hematoxylin and eosin and Luxol fast blue-Holmes' silver nitrate stains, and examined for toxic reaction. In the double-blinded studies, evaluation of the gross and histopathologic changes was performed by a pathologist blinded to the different drugs and their doses.

### Statistical Analysis

Comparisons across two groups (ie, low dose vs. high dose) were analyzed using the nonparametric Mann–Whitney U statistic (U). Comparisons across three or more groups (ie, dextrorphan vs. dextromethorphan vs. memantine) were analyzed using the nonparametric Kruskal–Wallis statistic (H). Systat 5.1 for DOS performed the analysis.

## RESULTS

All 48 sheep randomly received continuous intrathecal administration of dextrorphan, dextromethorphan, or memantine via subcutaneous, programmable continuous-infusion pumps (Medtronic Synchromed, pump models 8615 and 8617). Of these 48 animals, 41 were actually used in the study groups. Of the seven animals excluded from the study, three animals had questionable patency of the intrathecal catheters; one animal had a neurologic deficit caused by intraoperative trauma; another animal developed a seroma over the area of the implanted pump; and catheters in two animals migrated into the epidural space. The mean duration of implantation was 29.1 days ($\pm$ 16.6) for all sheep receiving study drugs. All the control sheep survived for the entire 43 days, which was significantly different from the animals that received dextrorphan, dextromethorphan, or memantine. The results did not differ between male (39 sheep) and female (2 sheep) animals, and their data were merged for subsequent analysis.

### Physiologic Effects

In all of the studies, there was no deviation from normal values with respect to body temperature, blood pressure, heart rate, or blood chemistries. Bacterial cultures taken of the saline infusate and CSF at sacrifice showed no growth after seven days of observation.

#### *Behavioral and Motor Observations*

The behavioral/motor changes and the number of days the animal received the antagonists are summarized in Table 2. The saline-infused animals did not exhibit any behavioral or motor changes. All motor deficits affected the hind limbs only. The sheep that developed hind limb paralysis were unresponsive to noxious cutaneous stimulation, however, bowel and bladder control were normal. Micturition and defecation in these paraplegic animals was monitored by assisting the animal in rising and supporting them in the standing position for a period of 5 min. Sheep normally urinate and defecate after rising from the prone position. We speculate that the animals' autonomic systems were still intact (bowel, bladder) while the somatic systems (neuromuscular and sensory) were affected by the histopathologic changes (inflammation, necrosis, etc.). This is sometimes seen in humans with spinal cord compression injury. Responses to high doses of all three drugs differed significantly from responses to dose level 2 and lower in regard to the behavior and motor grade and the number of days the animal received the test agents ($U = 24$, $p$ 0.001 and $U = 240$, $p$ 0.001 respectively; Mann–Whitney U statistic). The

neurologic symptoms the sheep developed typically began with the animal biting the area of the catheter tip location, progressing to stiffness in the rear legs, limping on one rear leg, loss of righting reflex in the effected leg, dragging of the leg, guarded posture, difficulty rising, and paraplegia. These symptoms appeared as early as the first day after the animal was started on drug or as late as day 36. However, these symptoms varied in severity and extent of toxicity based on the dose level and the drug the animal received. Only one animal (dextrorphan dose 3) recovered from a drug-induced neuroneal deficit. This sheep lost the righting reflex in the right rear leg on day 6 of the drug study, which continued until day 9 when the sheep began to regain the righting reflex and continued to improve until normal behavior returned on day 13.

The threshold doses for neurotoxicity are dextrorphan dose 3, dextromethorphan dose 1, and memantine dose 1. Behavior and motor grades statistically differed across the three different drugs plus the control group ($H = 9.33$, $p < 0.05$, 3df, Kruskal–Wallis statistic). Dextromethorphan produced the highest behavior and motor grades and dextrorphan the lowest; the control animals exhibited no change in behavior or motor activity.

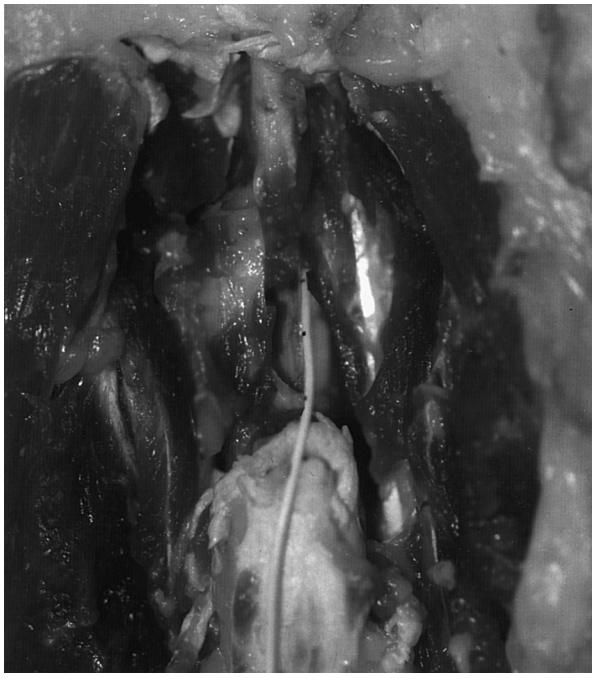

**Figure 2.** Necropsy dissection of the intravertebral space L7-S1 showing catheter entering the intrathecal space.

### *Gross Post-mortem Observations*

Gross examination of the spinal cord confirmed the catheter insertion site at L7-S1 (Fig. 2) and catheter termination sites (Fig. 3) ranging from L-6 to T-10 with the majority between T-13 and L-2. The course of each catheter varied (dorsal > ventral > right lateral > left ventral > right dorsal lateral > left lateral = right ventral) and appeared to have no effect on any of the gross changes observed. In contrast, the presence of gross lesions in the spinal cord were consistently associated with the catheter tip location.

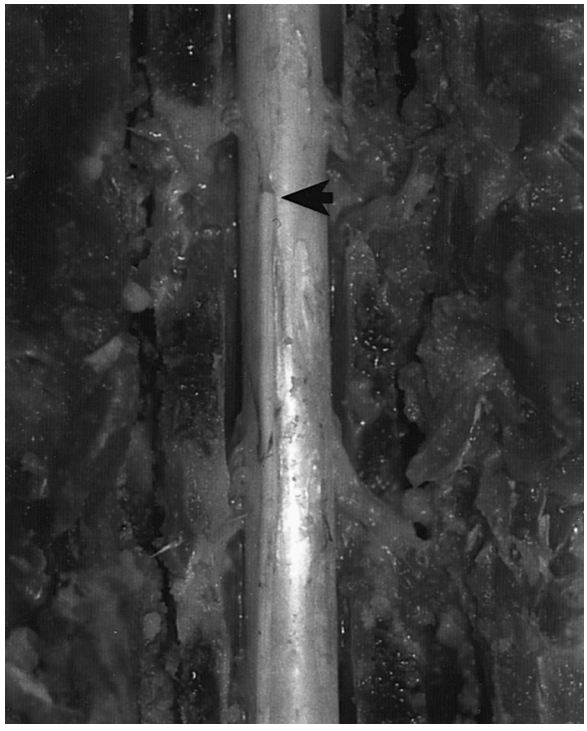

**Figure 3.** Catheter tip is seen through the translucent dura. Catheter is lying on the dorsal surface of the spinal cord.

## Histopathologic Assessment

A summary of the spinal histopathology for all the sheep in these studies are presented in Table 2. At high dosing levels all of the agents studied statistically differed from dose level 2 and lower in regard to the presence of inflammatory cells and necrosis ($U = 3\ 1$, $p < 0.001$ and $U = 23$, $p < 0.001$, respectively; U = Mann–Whitney U statistic). The occur-

rence of necrosis differed significantly between the different dose levels of all the drugs ($H = 23.97$, $p < 0.001$, 5df). Purulent inflammation and infiltration of neutrophils, macrophages, and an occasional giant cell characterized the necrosis. Axonal changes included axonal swelling and axonal loss. High CSF values of total protein were associated with spinal lesions in all but one sheep. One of the control animals had a total protein level of 284 mg/dl. However, histologic examination of this animal did not reveal any microscopic lesions. The regions of the brain that were examined did not reveal any significant microscopic lesions. Histologic evaluation of the various organs did not reveal any drug-related changes. The histologic changes were dose-dependent with the exception of four animals. One sheep that had received memantine level 2 showed no behavioral, motor, or neurologic symptoms. Gross post-mortem examination revealed a lesion measuring 1 cm † 0.5 cm at the site of catheter termination. Histologic exam of this sheep revealed moderate to severe changes. Conversely, the other animal receiving the same dose exhibited mild clinical symptoms yet had no histopathologic damage to the spinal cord. One of the animals that received memantine level 3 revealed severe behavioral and motor effects and had to be euthanized on day 10. Gross examination at necropsy revealed a ventral lesion at L1-T13 with purulent inflammation, discoloration, and necrosis. The sheep that had dextromethorphan at dose level 2 exhibited moderate behavioral changes and appeared grossly normal at necropsy. However, upon sectioning the formalin-fixed cord, areas of discoloration and slight swelling were associated with the catheter termination site. One of the sheep that received dextrorphan level 3 recovered from moderate behavior changes and appeared grossly normal at necropsy. Histopathologic examination of this animal revealed moderate and severe histologic changes. The histopathologic changes noted in these four sheep were similar to those seen in sheep that were given higher doses of the drugs. No significant gross anatomic changes were noted in control animals. However, upon histopathologic examination, three of the seven control animals showed a very mild catheter reaction consisting of a mild fibrosis around the catheter. The occurrence of intrathecal fibrous reaction did not differ between the dose levels ($H = 4.75$, $> 0.05$, 5 d.f.).

## DISCUSSION

The administration of intrathecal or epidural morphine for chronic pain states is widespread and well-established (15,18). However, unpredicted side effects such as ventilatory depression, itching, and urinary retention have been reported (16) and the use of spinally administered morphine has been accompanied by reports of tolerance (43). Common clinical practice including our experience and that of others (44) found that morphine tolerance could develop to a point where intrathecal doses of 50 mg/day were ineffective in controlling pain. Additionally, some neuropathic pain syndromes may be unresponsive to morphine (15,16). Therefore, it is believed by many researchers that morphine may not be the drug of choice for intrathecal administration (18). In response to the need for developing new agents for spinal administration in the clinical setting (45), this preliminary investigation of the toxic effects of dextrorphan, dextromethorphan, and memantine was conducted. Recent studies in rats showed these agents to be promising in the treatment of hyperalgesia and allodynia (11). These NMDA antagonists have been in use clinically for many years, dextrorphan and dextromethorphan as antitussives and more recently as anticonvulsants in the treatment of epilepsy (46) and memantine for the treatment of Parkinson's disease (34) and dementia (39,47). Presently, there are no data on the safety, side-effect profiles, or toxicity of long-term intrathecal administration of dextrorphan, dextromethorphan, or memantine in animals or man (14). Previous researchers expressed concern over the use of experimental agents and clinically approved agents administered via a different route (for example, approved for oral administration but delivered intrathecally) without behavioral, toxicologic, and histopathologic data from animal studies (14,21,22,48,49). It has been proposed that the neurotoxic potential of spinally administered drugs may not be adequately detected when morphologic analysis does not include functional studies (20). Therefore, the lack of behavioral changes alone is not sufficient to eliminate the possibilities of a drug having neurotoxic effects (21). Also, it is feasible that toxicity can be present in the absence of neurologic symptoms. For these reasons, in the present study, histopathologic changes as well as neurologic, motor, and behavioral changes were examined.

The sheep was chosen as the animal model because its neural axis is similar to the human's in relation to spinal cord length, CSF volume, CSF production, and body weight; thus providing in the study animal a body cavity and anatomic spaces close to what would be encountered in a human patient (50). In addition, the volume of the spinal CSF compartment in the sheep is large enough to allow some dilution of the test agent and therefore more closely approximate lumbar intrathecal administration in the human (51). Previous studies of local anesthetics in sheep indicated that this animal model probably has the same milligram-per-kilogram dose requirements as humans do (52). Furthermore, utilizing the sheep, our surgical protocol is analogous to the surgical protocol used in humans (semipercutaneous placement of the intrathecal catheter), which is very difficult in other large animal models like the dog in which a mini-laminotomy must be performed (53). The sheep has been routinely used by investigators to study the effects of various substances administered into the subarachnoid space (54). Others have investigated the neurotoxicity of intrathecal local anesthetics (55), enkephalinase inhibitors (51), pharmacokinetic distribution of opiates given epidurally, intrathecally, or intraventricularly (56–59), and antinociception after epidural administration of clonidine (60,61).

Like Rawal et al. we noted a dramatic difference in the behavioral responses between animals receiving similar volumes of saline or test drug (18). The spinal administration of dextrorphan, dextromethorphan, and memantine showed dose-dependent onset and duration of behavioral, motor, and neurologic changes. These changes were so sensitive that we repeatedly noticed biting of the area associated with the catheter tip location as an early sign of toxicity. This behavior was also noted by Atchison et al. in a dog that received 30 mg of intrathecal morphine. This animal went into convulsions 15 min after exhibiting this behavior, and died shortly thereafter (62). Our findings and those by others (11,14) have demonstrated that high doses of these agents produce obvious motor deficits.

There was no correlation between weight and clinical symptoms or histopathologic results within the dose groups. In the known dose studies where the concentration of drug remained the same(10 mg/ml), the flow rates did vary between sheep depending on dose and the weight of the animal. The infusion volume range in these studies ranged between 0.051 ml/day and 13.25 ml/day. However, in the blinded studies, all the volumes remained the same (1.636 ml/day) and the concentration varied. Comparing the different volumes administered within a drug and within one dose rate, the higher volumes of injectate did not statistically increase the incidence of pathology.

Dextrorphan, the more potent active metabolite of dextromethorphan (63), appears to be the least toxic of the study drugs at dose levels 3 and lower. However, one animal that received dextrorphan dose level 3 displayed moderate behavioral changes and severe to moderate histopathologic changes. Two other sheep receiving the same dose exhibited no behavioral or motor changes and minimal to no histopathologic change.

Conversely, dextromethorphan was clearly the most irritating to the spinal cord tissue. Toxic reaction was present at all dosing levels upon histologic examination with the exception of two animals; tissue samples from two sheep dosed at levels 1 and 2 revealed no histologic changes. Additionally, all dose levels of dextromethorphan exhibited behavioral and neurologic changes (with the exception of the same two animals that did not show such changes).

Slightly less toxic than dextromethorphan, memantine at dose level 1 presented no behavioral, motor, or neurologic changes. However, all animals that received the drug at dose level 3 and above displayed behavioral, motor, neurologic, and histologic changes, except for one sheep at dose level 3 that showed no such changes.

No significant behavioral, motor, neurologic, or gross anatomic changes were noted in control animals. Histopathologic examination revealed three of the six control animals to have a minimal catheter reaction consisting of a mild fibrosis around the catheter. This reaction has been noted in other animal models and may be unavoidable to some extent due to the presence of the catheter and simple mechanical irritation (44,64). Our study presented a general correlation between degree of behavioral, motor, and neurologic change and the degree of histopathologic change. However, we also showed that neurotoxic effects can be present without an animal showing any clinical symptoms. The severe neurotoxic effects of the higher doses of the NMDA antagonists studied in this investigation and the case

findings of another agent of the same pharmacologic type (65) further warrants thorough testing of these agents prior to clinical use. The lower doses of these agents have been shown by others (4,11,14) to produce antihyperalgesic or analgesic properties in the neuropathic rat. To provide appropriate preclinical background for these antagonists, future studies are being planned to determine the analgesic or antihyperalgesic efficacy of the lower doses of these agents.

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