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Case Study

Neurologic Complications Associated with Transdermal Placement of Intrathecal Catheters in Sheep

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A study using an ovine model of transdermal intrathecal catheterization was planned to investigate the neurotoxicity of magnesium sulfate. Nonpregnant Merino cross ewes (n = 8; age, 5 y; weight, 55.0 ± 6.5 kg) were anesthetized for placement of a lumbar intrathecal catheter. The study protocol defined a 5-d recovery period after introduction of the catheter before the administration of test substances (2 mL of 0.9% saline or 50 or 150 mg $MgSO_4$) followed by euthanasia 1 wk later. Although 3 sheep successfully completed the study as planned, one of the remaining 5 sheep was withdrawn when the catheter was accidentally dislodged 2 d after anesthesia; another was withdrawn because of persistent neurologic deficits of the left hindlimb and intense pruritus during the first 24 h after placement of the catheter; and the remaining 3 animals experienced unacceptable complications within the first 4 h of administration of the test substance. These complications included hindlimb weakness, intense irritation or pruritus of the hindlimbs, recumbency, inability to stand, spasm of the hindlimb, and arching of the back. Postmortem examination of 4 sheep with clinical signs revealed similar gross findings: acute, segmental myelomalacia and hemorrhage within the spinal cord parenchyma in the region of the catheter. Histologic changes included segmental areas of acute myelomalacia, consistent with the intraparenchymal placement of the catheter. Postmortem CT imaging of 3 sheep confirmed the location of the catheter within the spinal cord. Procedural refinement for the placement of intrathecal catheters in sheep by avoiding an invasive surgical procedure was unsuccessful. We therefore recommend a complete or partial surgical approach for the insertion of an intrathecal catheter in sheep or fluoroscopy or ultrasonography intraoperatively to confirm correct placement of the catheter.

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To deliver pharmacologic agents directly to the CNS or the CSF, either catheterization or direct injection into the subarachnoid space (that is, intrathecal administration) is necessary. The advantages of administering therapeutic agents by this route include bypassing the blood–brain and spinal cord barriers; limiting systemic toxicity; and achieving high local concentrations of drugs near the intended target.[3,4] Prior to the clinical use of therapeutic agents, neurotoxicity studies are required for determining the safety of the agent in question. Animal models are often used for this purpose.[4]

Many animal models have been used to assess the neurotoxicity of neuraxially administered agents before clinical use. The ovine model has been extensively reported, with the majority of studies involving intrathecal catheter placement via a laminectomy or hemilaminectomy.[4,5,15,18] Placement of an intrathecal catheter in sheep may also be performed by a partial surgical approach whereby the lumbodorsal fascia is exposed through a midline skin incision to allow insertion of a catheter at L7-S1[7,9,10] A series of studies using an ovine model of transdermal intrathecal catheterization were planned to investigate the potential neurotoxicity of intrathecal $MgSO_4$ for application in human anesthesia and analgesia. To avoid a surgical procedure, a minimally invasive approach was planned but resulted in an unexpectedly high incidence of neurologic complications.

Case Study

The study was approved by the Animal Ethics Committee of the University of Western Australia in accordance with the *Australian Code of Practice for the Care and Use of Animal for Scientific Purposes*.[2] Sheep housed in shared raised pens were acclimated to the research facility for 1 wk prior to surgery. Two days prior to surgery, they were moved to individual raised pens (1.2 m^2). Rooms were controlled for temperature (20.5 to 21.5 °C).

The study involved nonpregnant Merino cross ewes (n = 8; age, 5 y). The ewes were premedicated with a combination of acepromazine (0.03 mg/kg; ACP 2 injection, 2 mg/mL, Ceva Delvet, Asquith, New South Wales, Australia) and buprenorphine (0.01 mg/kg; Temgesic, 0.3 mg/mL, Reckitt Benckiser, West Ryde, New South Wales, Australia) by intramuscular injection 30 to 40 min prior to induction of anesthesia. An 18-gauge catheter was placed in a cephalic vein, and anesthesia was induced with a combination of midazolam (0.25 mg/kg; Midazolam injection, 5 mg/mL, Pfizer Australia, West Ryde, New South Wales, Australia) and ketamine (5 mg/kg; Ketamil, 100 mg/mL, Troy Laboratories, Smithfield, New South Wales,

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Australia) by intravenous injection. The trachea was intubated (internal diameter, 8.5 mm; Cuffed tracheal tube, Portex, Hythe, United Kingdom) and a circle-breathing system was connected for the delivery of isoflurane in 100% oxygen through an anesthetic machine (Datex Ohmeda ADU anesthetic machine, GE Healthcare, Uppsala, Sweden). The sheep were positioned in sternal recumbency with the hindlimbs pulled forward to flex the hips and spine. The isoflurane vaporizer was adjusted, as judged by an experienced veterinary anesthetist, to maintain an adequate depth of anesthesia. Anesthesia was maintained and monitored as previously described.[6]

With the sheep positioned in sternal recumbency, the wool over the catheter insertion site in the lumbosacral area was clipped, and the skin was prepared by using 0.5% chlorhexidine in 70% ethanol. Under strict aseptic conditions, the skin overlying the insertion site was infiltrated with local anesthetic (2 mL per sheep; 0.5% bupivacaine in adrenaline 1:200 000, Marcain Hospira, Lake Forest, IL). An 18-gauge catheter (RapID Portex Spinal/Epidural Minipack, Smiths Medical, Brisbane, Queensland, Australia) was inserted through a Tuohy needle at the lumbosacral junction in a 'loss of resistance' technique, with the position of the catheter confirmed by the aspiration of CSF. The catheter was sutured into place, and the exit site was covered with a chlorhexidine-impregnated sponge (Biopatch, Ethicon, Somerville, NJ) and a clear adhesive dressing.

The animals were closely monitored during the postoperative period. After the procedure, each sheep was individually housed, with visual, olfactory and auditory communication with at least one other sheep. Animals were observed twice daily to determine postoperative wellbeing. Food and water intake, urine and feces production, rumination, demeanor, gait and posture, hindlimb function, mentation, teeth grinding and the appearance of the catheter exit site were scored. The study protocol defined a 5-d recovery period after introduction of the intrathecal catheter before the administration of test substances, with euthanasia 1 wk thereafter.

The test substance for intrathecal injection was $MgSO_4$; 2 mL of 0.9% saline (vehicle control) or 50 or 150 mg $MgSO_4$ was administered into the intrathecal catheter 5 d after placement. Euthanasia after placement of the intrathecal catheter or after the administration of test substance was considered in the event of sustained moderate-to-severe deviations from normal in the categories for wellbeing (as listed previously). Sheep were euthanized through intravenous injection of pentobarbital (160 mg/kg). Postmortem examination, including CT imaging, of the animals with adverse reactions was performed at an independent laboratory (Murdoch University Pathology Service, Murdoch, Western Australia, Australia).

Results

Eight ewes weighing 55 ± 6.5 kg underwent general anesthesia for transdermal placement of an intrathecal catheter. The induction, maintenance, and recovery from anesthesia were uneventful in all cases. Three animals successfully completed the study as planned. The remaining 5 animals were withdrawn from the study due to accidental dislodgement of the catheter at 2 d after anesthesia (n = 1), persistent neurologic deficits of the left hindlimb and intense pruritus for the first 24 h after placement of the catheter (n = 1), or unacceptable complications within the first 4 h of administration of the test substance (n = 3; Figure 1). These complications included hindlimb weakness, intense irritation or pruritus of the hindlimbs, recumbency, an inability to stand, spasm of the hindlimb, and arching of the back.

Postmortem examination of the 4 sheep that had clinical signs associated with the administration of test substances (sheep 1, 7, and 8) or placement of the catheter (sheep 5) revealed similar gross findings. Acute, segmental myelomalacia and hemorrhage of the spinal cord was present in the region of the catheter, which was within the spinal cord parenchyma. The histologic changes were also similar among these 4 animals, with segmental areas of acute myelomalacia consistent with intraparenchymal placement of the catheter. Malacic tracts extended from the dorsal aspect of L6, where the catheters entered the spinal cord, through the central and ventral gray matter to the level of L2. Wallerian degeneration within the ascending spinal cord tracts cranial to the injury and degenerative changes within the sciatic nerves (sheep 1, 7, and 8) were considered secondary to the L6 spinal cord lesions (Figure 1).

Postmortem CT imaging of 3 sheep that were euthanized before the end of the experiment was performed. Sheep 1 had clinical signs associated with the right hindlimb, and the CT images confirmed that the catheter was within the right ventral region of the spinal cord (Figure 2). Sheep 7 displayed bilateral hindlimb signs, and the catheter was within the parenchyma of the spinal cord (Figure 3). The catheter was dislodged in sheep 8 prior to CT examination (Figure 4). In each of these animals, the space around the spinal cord at the catheter insertion site was no more than 1 mm.

Discussion

Investigating the neurotoxicity of intrathecal magnesium for application to human anesthesia and analgesia practice required the placement of intrathecal catheters in a sheep model. To avoid a surgical procedure, transdermal placement of the intrathecal catheter was performed in 8 anesthetized sheep. Given the frequency of adverse events, this attempt at procedural refinement was considered to be unsuccessful.

As argued in Russell and Burch's landmark work, *The Principles of Humane Experimental Technique*, the continual refinement of practices in animal-based research is key to minimizing the burden of research on animal welfare and to optimizing the integrity of scientific data.[16] With this ethos in mind, we planned to use a minimally invasive approach to place intrathecal catheters in sheep. The potential advantages of a less-invasive approach included decreased risk of postoperative infection; decreased trauma to the spinal cord; decreased requirement for specialized surgical equipment and expertise; decreased time under anesthesia; and decreased postprocedural pain.[11] To our knowledge, this less-invasive approach to the placement of intrathecal catheters in sheep has not been described previously.

Various sheep models using intrathecal catheters have been reported.[5,7,9,10,15,18] In one study exploring the measurement of CSF pressure as an indication of the patency of an intrathecal catheter, catheters were placed by using laminectomy L2.[5] In a study investigating the pharmacokinetics of ropivacaine, both epidural and intrathecal catheters were placed after laminectomy at the level of L5-L6.[15] Furthermore, laminectomy at L6-S2 was used to determine the efficacy of intrathecal morphine for the management of neuropathic pain.[18] A series of older studies reported success with a technique involving surgical exposure of the fascia at the site of insertion of the intrathecal catheter with a Tuohy needle.[7,9,10] These studies,[7,9,10] performed by the same group of authors, represent a less-invasive surgical approach to the intrathecal space, although exposure of the site was required. A retrospective review of complications associated with indwelling intrathecal catheters in a range of species, including sheep, reported that all the catheters placed in sheep

Sheep[a]	Outcome	Test substance	Postmortem examination and histopathology results	CT findings
1	Euthanasia after administration of test substance, 5 d after catheter placement. Right hindlimb weakness within 15 min of administration of test substance.	150 mg $MgSO_4$	Spinal cord L6-L5: Severe, acute, segmental hemorrhage and malacia with intralesional catheterization. Catheter extended through the L6-S1 intervertebral space and entered the spinal cord parenchyma. Hemorrhage present around the catheter at the entry site. Lumbar spinal cord segmentally softened (malacia), and catheter extends cranioventrally. Hemorrhage present in surrounding neuroparenchyma and in the tissue around the left sciatic nerve. Spinal cord L1-L5: Severe, acute, segmental myelomalacia with Wallerian degeneration and fibrinosuppurative meningitis. Sciatic nerves: mild, multifocal, acute vacuolar degeneration.	The hyperattenuating cylindrical structure of the intrathecal catheter is visible within the right ventral region of the spinal cord. The catheter extends from the dorsal intervertebral space of L5-L6 and extends cranially to the cranial endplate of L4. Both subarachnoid and intraparenchymal contrast agent is present. Gas-attenuating regions present at the cranial end of the catheter within the spinal cord, but might be postmortem changes.
2	Catheter dislodged 2 d after anesthesia; removed from study	–	–	–
3	Completed study	150 mg $MgSO_4$	–	–
4	Completed study	150 mg $MgSO_4$	–	–
5	Euthanized the day after catheter placement. Neurologic signs evident immediately after recovery from anesthesia. Catheter was removed the following day, because left hindlimb neurologic deficit and pruritus did not resolve.	–	Spinal cord L4-L6: Severe, acute, focally extensive necrosis with hemorrhage. Loss of spinal cord tissue, primarily restricted to left dorsal horn of the gray matter, which contains the motor neurons for the hindlimb, thus explaining the antemortem motor deficits in this limb. Associated inflammatory response is relatively mild, consistent with the acute nature of the insult. Spinal cord: Severe, acute, focally extensive necrosis with hemorrhage	–
6	Completed study	150 mg $MgSO_4$	–	–
7	Euthanasia after administration of test substance, 5 d after placement of the catheter. Bilateral hindlimb weakness within 15 min of administration of test substance; progressed to recumbency and inability to stand with extension of hindlimbs after 4 h. Patella, withdrawal, and panniculus reflexes normal.	50 mg $MgSO_4$	Spinal cord L2-L6: severe, acute, segmental hemorrhage and malacia with intralesional catheterization. The catheter tracks through the L6-S1 intervertebral space, entering the spinal cord parenchyma, where there is hemorrhage. The catheter tracks cranioventrally through the ventral horn white matter for approximately 5 cm. Hemorrhage and malacia of the neuroparenchyma surrounds the catheter site. Spinal cord L2-L5: Severe, acute, segmental myelomalacia with Wallerian degeneration and fibrinosuppurative meningitis. Sciatic nerves: Mild, multifocal, acute vacuolar degeneration.	The catheter enters the dorsal intervertebral space at L6-S1 and travels in the left ventromedial spinal cord or subarachnoid space, deviating dorsally in the midbody of L5, where it appears to terminate. Cranial to this lesion is a long area of intraparenchymal hyperattenuating contrast agent within the left dorsal spinal cord (and potentially the central canal), extending from the midbody of L5 to that of L1.
8	Euthanasia after administration of test substance, 5 d after catheter placement. Intermittent arching of the back and spasm of hindlimbs within 60 min of administration of test substance. No resolution of clinical signs after 4 h.	Saline	Spinal cord L4-L2: Severe, acute, segmental hemorrhage and malacia. Segmental malacia and focal hemorrhage of the lumbar spinal cord on transverse section. On longitudinal section of L4, a linear area of brown discoloration extends cranioventrally. At the level of L2, a poorly demarcated, circular area of hemorrhage and malacia distorts the ventral gray and white matter. Spinal cord L2-L5: Severe, acute, segmental myelomalacia, with Wallerian degeneration and fibrinosuppurative meningitis. Sciatic nerves: Mild, multifocal, acute vacuolar degeneration.	The catheter was accidentally removed prior to CT and cannot be visualized deep to the surface of the skin or within the spinal canal. Pooling of contrast agent in the dorsal subarachnoid space.

[a]In chronologic order

Figure 1. Summary of complications of 8 sheep after placement of an intrathecal catheter: clinical, postmortem examination, histology, and computed tomography. Sheep are numbered in chronologic order.

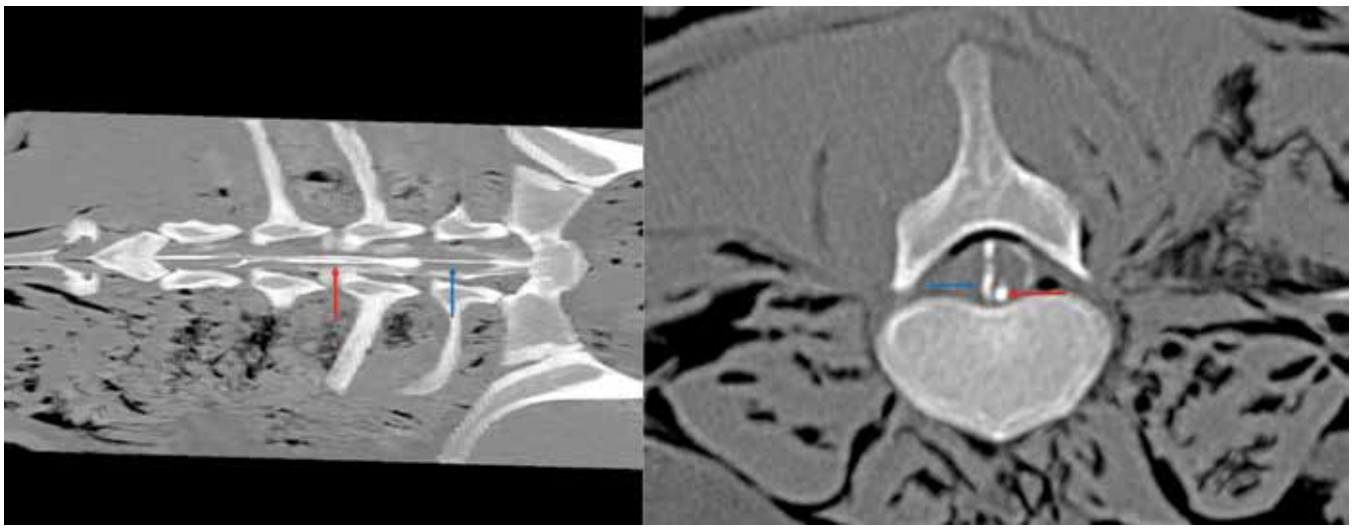

Figure 2. Postmortem CT images of sheep 1. The catheter is within the spinal cord (red arrow) to the right of the ventral midline fissure (blue arrow). The hyperattenuating cylindrical structure of the intrathecal catheter is present within the right ventral region of the spinal cord. The catheter extends from the dorsal intervertebral space of L5-L6 and extends cranially to the cranial endplate of L4. Both subarachnoid and intrapernchymal contrast agent are present. Gas-attenuating regions are present at the cranial end of the catheter within the spinal cord, but these may be postmortem changes.

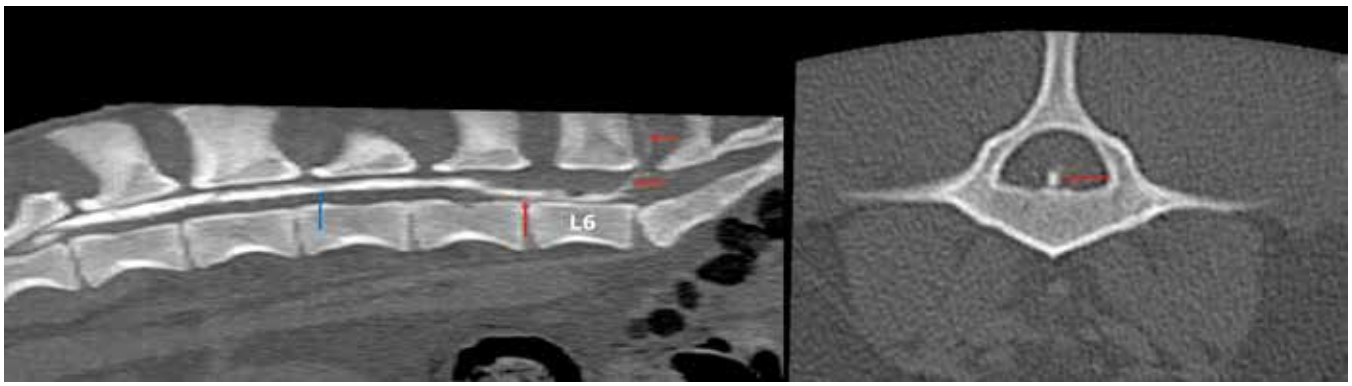

Figure 3. Postmortem CT images of sheep 7. The catheter enters the dorsal intervertebral space at L6-S1 and travels in the left ventromedial spinal cord or subarachnoid space, deviating dorsally in the midbody of L5 where it appears to terminate (red arrows). Cranial to this is a long area of intraparenchymal hyperattenuating contrast agent within the left dorsal spinal cord (and potentially the central canal), extending from the midbody of L5 to the midbody of L1 (blue arrow).

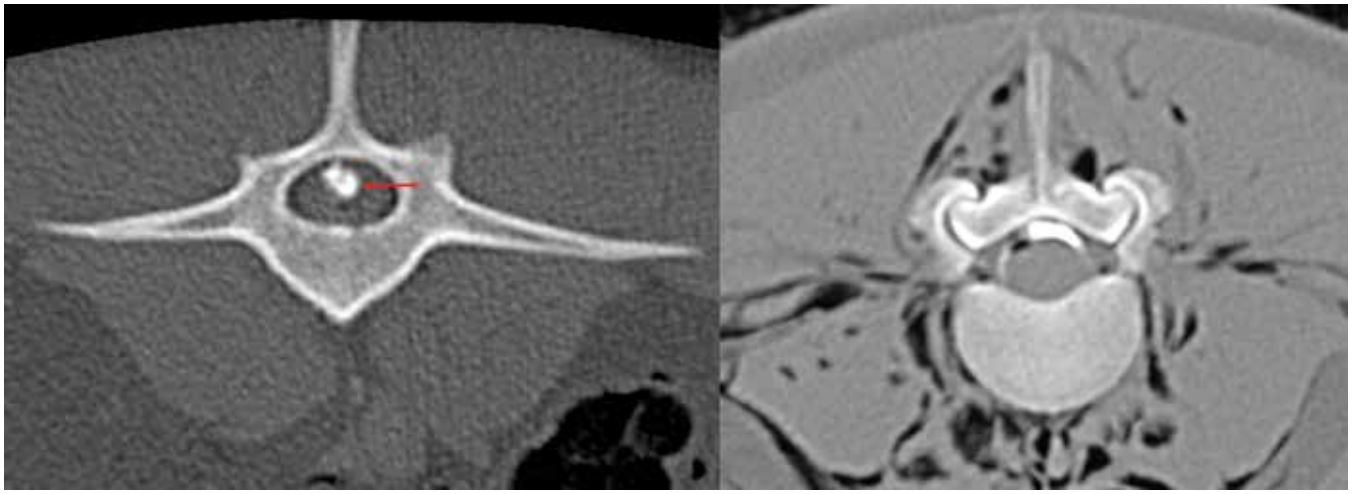

Figure 4. Postmortem CT images of sheep 8. Intraparenchymal hyperattenuating contrast agent is present within the left dorsal spinal cord (red arrow). Hyperattenuating contrast agent is present within the dorsal subarachnoid space. The catheter cannot be visualized.

were implanted through laminectomy or hemilaminectomy procedures in the caudal lumbar region.[4] Despite the variations in the location of catheter placement and the study designs, complications associated with surgical placement of intrathecal catheters either did not occur—or were not disclosed. This conclusion suggests that a surgical approach for the placement of intrathecal catheters in sheep is associated with low morbidity.

The factors that can contribute to local tissue changes after the placement of a foreign body such as an intrathecal catheter include, but are not limited to, the technique used to insert the catheter; location of the catheter; catheter material; rate of administration of the test substance; length of time the catheter is in place; aftercare of the insertion site; infection; and concentration of the test substance.[4] In the current study, there were significant clinical signs associated with spinal cord trauma. This trauma is attributed to the inadvertent placement of the catheter within the parenchyma of the spinal cord, with subsequent hemorrhage and inflammation, followed by myelomalacia, degeneration, and meningitis.

The anatomy of the ovine lumbar spine is well described. MRI of 10 lumbar segments of cadaveric ewes revealed that sheep have 6 or 7 lumbar vertebrae and 4 sacral vertebrae, and the spinal cord ends between S1 and S2.[14] In 62-kg sheep, the width of the spinal canal at L6 is 18.9 ± 1.6 mm and, as in humans, the canal was wider than it was deep, according to CT images.[12] In large sheep (80.6 ± 28.7 kg), the dural sac represented 49% of the vertebral canal area, and the space available for the dural sac was greatest at L6, on the basis of CT images.[13] These details all support a lumbar location for the placement of an epidural or intrathecal catheter in sheep.

Success with a nonsurgical approach for placement of intrathecal catheter is described in a single study in 30-d-old piglets.[11] The authors inserted an intrathecal catheter transdermally at L2-L3 in anesthetized piglets and left the catheter in place for only 15 min. The authors did not report any complications such as bleeding, CSF leakage, neurologic injury, or ataxia in during the week after the procedure. Confirmation of correct placement was by observation of CSF leakage from the needle, although fluoroscopy was used in some animals to avoid failure of the procedure.[11] The authors concluded that the technique could be performed safely and that it provided an easier and less-invasive approach for intrathecal catheter insertion. Unfortunately, this approach was unsuccessful in the current study using adult sheep. There are many potential explanations for this difference: the site of insertion of the catheter was more cranial in the piglets; the catheters were not left in situ in the piglets; and species-specific anatomic variations.

The original aim of the current study was to investigate the neurotoxicity of intrathecal $MgSO_4$ in a sheep model and to describe any micro- or macroscopic evidence of neurotoxicity due to repeated exposure to this substance. The methodology required the introduction and maintenance of an intrathecal catheter in the caudal lumbar region of anesthetized sheep by using a transdermal approach. Complications were observed either during the immediate postanesthetic period or after the injection of saline or $MgSO_4$ into the catheter. The side effects cannot, therefore, be attributed solely to the test substance $MgSO_4$. Given the postmortem findings, the adverse events described in the current study are attributable to the location of the intrathecal catheter within the spinal cord parenchyma. The immediate development of clinical signs in sheep 5 may be due to the severity of the spinal cord injury. The delayed manifestation of clinical signs in sheep 1, 7, and 8, which were apparent when saline or $MgSO_4$ was injected into the catheter, is difficult to explain. Perhaps the confined space of the holding pens precluded the identification of subtle gait abnormalities.

Eight sheep were enrolled in this study, and only 3 completed it as intended. Postmortem examination of the animals with unexpected clinical signs associated with the placement of the intrathecal catheter revealed a range of lesions. These lesions are attributed to the inadvertent placement of the catheter within the spinal cord parenchyma. The ease with which the spinal cord was penetrated is surprising, and in all cases, there was a flow of CSF, which, along with identification of anatomic landmarks, was used to confirm correct placement. Diagnostic imaging modalities such as fluoroscopy,[1] CT,[8] and ultrasonography[17] provide tools to increase accuracy and confidence in intrathecal catheter placement in a less-invasive manner. The use of ultrasonography to mark the insertion site of a catheter and real-time ultrasound guidance is associated with a higher success rate for performing lumbar puncture in humans, compared with relying on the identification of landmarks alone.[17] In addition, the CSF pulse pressure can be used to confirm correct placement.[5] These methods all require specialized equipment and expertise, but given the complications reported in the current study, incorporation of these techniques into future studies of this nature should be considered.

This report must be interpreted with consideration of the limitations of the study. The initial study design did not accommodate for the frequency of complications, which were unexpected. Consequently, an independent pathologist assessed only animals with clinical abnormalities. Independent postmortem examinations of every animal may have provided additional information regarding local tissue changes in the vicinity of the intrathecal catheter in animals without clinical signs. However, some morphologic change can occur due to the insertion of an intrathecal catheter, and although these changes are undesirable, they may not be avoidable.[4] Furthermore, clinical signs seldom occur when spinal cord compression or nerve fiber degeneration is mild.[4] Evaluation of successful catheter placement in the current study was made entirely in the absence of clinical signs. Mild manifestations of neurologic impairment of sheep in a research facility environment might be difficult to detect solely by observation. An additional limitation of this study is that it was small, involving only 8 animals.

In the current study, procedural refinement for the placement of intrathecal catheters in sheep by avoiding an invasive surgical procedure was unsuccessful. We therefore recommend a surgical approach to allow direct visualization of the catheter in situ, partial surgical exposure, or the use of an imaging modality such as fluoroscopy or ultrasonography before a sheep recovers from anesthesia, to ensure confidence in correct placement of the catheter.

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