Toxicology Profile of N-Methyl-d-aspartate Antagonists Delivered by Intrathecal Infusion in the Canine Model

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Background: Intrathecal N-methyl-d-aspartate antagonists have antihyperalgesic efficacy. The authors examined toxicity in a canine model of chronic lumbar intrathecal infusion.

Methods: Dogs (10–16 kg) were prepared with lumbar intrathecal catheters connected to vest-mounted pumps (100 μl/h). In phase 1, stepwise increments in infusion concentration were performed at 48- to 72-h intervals to determine an infusion dose with minimal but detectable behavioral effects. In phase 2, the dose/concentration defined in phase 1 was infused for 28 days. Behavioral function during infusion and histopathology at sacrifice was assessed. Drugs examined were 2-amino-5-phosphono-valerate (AP5), MK801, memantine, amitriptyline, S-methadone, and saline.

Results: In the phase 1 dose ranging, the minimum effect doses for the several agents were as follows: AP5, 1 mg/day; amitriptyline, 1 mg/day; ketamine, 10 mg/day; MK801, 1 mg/day; and memantine, 4 mg/day. In phase 2, infusion of these doses typically resulted in mild hind limb weakness by 3–5 days after initiation of infusion, which progressed over the 28-day infusion interval. In a limited number of animals, a similar effect was observed with S-methadone. Histopathologically, vehicle-infused animals displayed a minor local catheter reaction. With the drug treatments, a gradient of increasing pathology from cervical to lumbar segments was noted. Pathology ranged from local demyelination to necrotizing lesions of spinal parenchyma near the catheter tip. All drugs given at their respective doses produced pathology scores significantly worse than saline controls.

Conclusions: These drugs given for 28 days at acutely tolerable doses lead to spinal pathology. These data suggest a reevaluation of the use of these agents in chronic spinal delivery.

ELECTROPHYSIOLOGIC and behavioral studies have shown that N-methyl-d-aspartate (NMDA) receptors play a pivotal role in the development of facilitated states of dorsal horn processing after tissue and nerve injury. Intrathecal delivery of agents that competitively block the glutamate binding site, such as (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK801), dextrorphan, memantine, and amitriptyline, possess potent antihyperalgesic properties in preclinical models. These observations stress the potential of NMDA receptor antagonism in persistent human pain states. The adverse effect on higher-order function after systemic delivery has motivated suggestions for their spinal delivery. In humans, the therapeutic activity of ketamine and CPP after epidural or intrathecal delivery has been reported.

An important consideration in the intrathecal delivery of novel agents relates to their safety. Here, safety explicitly considers not only the potential effect on function (e.g., motor, sensory, autonomic) but changes in spinal morphology. Assertions of safety should be based on the use of robust assessments that involve validated animal models, concentrations equaling or exceeding those in humans, and preferably multiple or continuous deliveries through routes used in human therapy. With these criteria in mind, a number of studies have been reported and have yielded a complicated picture. Studies in rabbits and pigs with ketamine report little or no pathology, even after multiple bolus deliveries. In other rabbit studies, significant pathology, including necrotizing subpial lesions with cellular infiltrates, has been reported after once-daily ketamine injections. CPP was examined after multiple bolus intrathecal delivery in rats, and no untoward histologic findings were noted. However, later studies have shown that intrathecal infusion of memantine, dextrorphan, and dextromethorphan in sheep leads to parenchymal necrosis of the spinal cord. In humans, several case reports have suggested severe histologic abnormalities, although in most cases, the use of adjuvants, chemotherapy and radiation therapy, or metastatic disease may have contributed to injury.

In the current study, we examined the effects in a validated canine model of continuous intrathecal infusions of conventional competitive (AP5), noncompetitive (MK801, memantine), and nonconventional (amitriptyline, S-methadone) NMDA antagonists. This work, performed over several years, used a two-phase paradigm. In the first phase, we examined the effects of periodic incrementation of infusate concentration to define the maximum infusion dose that showed just detectable behavioral effects. In the second phase, this infusion dose was delivered for up to 28 days, at which time systematic spinal histopathology was performed.
Materials and Methods

These studies were conducted according to protocols approved by the Institutional Animal Care and Use Committee of the University of California, San Diego.

Animals

Male and female beagles were obtained from Harlan Sprague-Dawley (Mt. Horeb, WI) and Marshall Farms (North Rose, NY). The animals were aged approximately 8–24 months and weighed 10–16 kg (male weights were 10–16 kg; female weights were 11–13 kg) at the initiation of treatment. Dogs were housed in individual runs in a vivarium accredited by the American Association of Laboratory Animal Care. Water and food were provided *ad libitum*, except during food fasting periods as specified.

Intrathecal Catheter Placement

To permit continuous infusion in the intrathecal space, a chronically placed intrathecal catheter was placed as described previously in detail. After an initial 5- to 7-day acclimation period and treatment with sulfamethoxazole trimethoprim (480 mg/tablet, oral twice daily, from 2 days before to 2 days after surgery), animals were treated with atropine (0.4 mg/kg, intramuscular) and anesthetized with xylazine (1.5 mg/kg, intramuscular) or a combination of ketamine and diazepam (10/0.5 mg/kg, intravenously). After tracheal intubation, anesthesia was maintained using either 1–2% halothane or isoflurane and 60% N<sub>2</sub>O–40% O<sub>2</sub> and spontaneous ventilation. Saturation, anesthetic gases, carbon dioxide, and heart and respiratory rates were continuously monitored. The back of the neck was prepped and draped. A 2-cm midline incision was made from the occiput, and the muscles were retracted to expose the cisternal membrane. A PE-10 intrathecal catheter with stylette, sterilized by e-beam radiation, was then inserted through a small cisternal incision and passed approximately 43 cm. Placement in the intrathecal space was determined by sampling lumbar cerebrospinal fluid (CSF) from the catheter. After catheter placement, dexamethasone sodium phosphate (0.25 mg/kg, intramuscular) was given. The externalized end of the catheter was tunneled subcutaneously to exit the skin in the scapular region. The surgery required approximately 30–45 min to complete. During recovery, butorphanol tartrate (Torbugesic<sup>®</sup>, 0.04 mg/kg, intramuscular; Fort Dodge Animal Health, Fort Dodge, IA) was administered to relieve postoperative pain. After recovery, a nylon vest (Alice King Chatham Medical Arts, Hawthorne, CA, or equivalent) was replaced (animals having been acclimated to the vest before surgery), and an infusion pump was placed in the vest pocket, where it was connected to the externalized end of the intrathecal catheter. The intrathecal catheter was continuously infused until initiation of test article treatment with 0.9% (wt/vol) sodium chloride for injection, USP (saline), at approximately 20 µl/h. Dogs were typically allowed a 3- to 5-day postoperative period before being assigned to a drug treatment.

Drugs

AP5, RSketamine HCl (ketamine), and (+)-MK801 hydrogen maleate (MK801; Dizocilpine, [5R,10S]-(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine) were obtained from Research Biochemicals International (Natick, MA), and amitriptyline HCl was obtained from Stuart Pharmaceuticals (Wilmington, DE). Memantine HCl was obtained as a generous gift from Merz & Co. GmbH & Co. (Frankfurt, Germany). S(-)-Methadone was obtained from the National Institute for Drug Abuse, Bethesda, Maryland.

Drug Preparation and Delivery

The powdered drugs were reconstituted for intrathecal dosing preparations using sterile water along with 0.9% sodium chloride for injection, USP (Abbott Laboratories, N. Chicago, IL) adjusted to achieve an osmolality between approximately 275 and 350 mOsm. S-Methadone was dissolved in a small amount (approximately 200 µl) of HCl and then reconstituted with 0.9% (wt/vol) sodium chloride for injection, USP. The pH of the S-methadone was adjusted with NaOH or HCl as needed to obtain an acceptable pH (5.0–7.5). Stock solutions were diluted to the required concentrations using 0.9% (wt/vol) sodium chloride for injection, USP. All solutions were filtered (22-µm sterile filter) before use. Aliquots of the injectate solutions were retained and submitted for culture and were found to be negative. The pH of aliquots of infusate was measured using a pH meter (Corning 250 Ion Analyze; Corning, NY, and AccupHast<sup>®</sup> pH Electrode; Cole Palmer Instrument Company, Vernon Hills, IL), and osmolality was determined by freezing point osmometry (Precision Systems Inc., Natick, MA). Data for test drugs in doses used in the phase 2 study are presented in table 1. Dosing solutions were prepared to be delivered as a continuous infusion. All infusions were performed such that the intended dose was delivered at...
100 μl/h (2.4 ml/day). Infusions were delivered via an infusion pump (Panomat C-10, T-10, or equivalent; Dis- etronic Medical Systems, Inc., St. Paul, MN).

Study Paradigm
The studies described below were performed over an interval of approximately 4 yr. The agents AP5, MK801, memantine, and ketamine were studied in the first 2 yr, and amitriptyline and (+)-methadone were studied during the second 2 yr. Vehicle animals were run concurrently with each treatment series. The same protocols were used for all drugs. Animals were randomly assigned to treatments in order of arrival. After catheter placement, animals were assessed for normal neurologic function and assigned to receive drug or vehicle infusions. Studies were performed in two phases.

In phase 1, catheterized animals were assigned to receive a test drug and undergo stepwise incrementations of infusion concentration at 48- to 72-h intervals. For each drug, we sought to characterize three dose-effect levels.

Effect level 1: Maximum dose with minimum to no observable effect on behavior.
Effect level 2: A range of doses that result in moderate, nondebilitating affects, typically signs of readily detectable motor weakness of the hind limb and blunted placing and stepping responses or asymmetry in ambulation.
Effect level 3: At this dose, effects were observed that were sufficiently severe to require termination of infusion because of dysfunction. Such signs included extreme allodynia, marked agitation/hyperactivity, marked muscle rigidity or weakness, ataxia, paralysis, tremors, and sedation.

Doses used in phase 1 are indicated in table 2. S-Methadone was examined only up to 1 mg/ml because of limited drug availability and limited solubility. In phase 2, the dose defined in phase 1 as having a minimum acutely tolerable behavioral effect was delivered for 28 days. Practically, the dose selected was at or below the lowest doses associated with effect level 2 and/or the highest dose in effect level 1. After completing the drug delivery period, animals were deeply anesthetized, blood and cisternal CSF were sampled, and the dogs underwent whole body perfusion (saline and fixative) for necropsy and histopathologic examination (see Histopathology section).

Study Measures
Observations. Food consumption, rectal temperature, presence of urine and stool, and behavioral function (arousal, muscle tone, and coordination) were recorded daily. Observers were not blinded to treatment. In the safety study, blood pressure and heart rates were taken just before the initiation of drug infusion (day 0) and then again at 4 and 28 days.

Clinical Laboratory Measurements. Blood and cisternal CSF samples were obtained before surgery and on the day of necropsy after an overnight fast. Blood for hematology and clinical chemistry was obtained by jugular or cephalic venipuncture. CSF for clinical chemistry and cell differentiation was obtained by sterile puncture of the cisterna magna.

Necropsy. On the scheduled date of necropsy, each animal was deeply anesthetized with sodium pentobarbital (35 mg/kg or to effect, intravenous). A percutaneous puncture of the cisterna magna was performed to collect cisternal CSF. The chest was opened, the aorta was catheterized, and the blood was cleared by perfusion with approximately 4 1 saline (0.9% sodium chloride) followed by approximately 4 1 formalin, 10%, over approximately 10 min. Methylene blue dye was injected through the catheter to confirm catheter placement and integrity. The spinal cord was exposed by laminectomy.

Histopathology
Four blocks of spinal cord (A: cervical; B: thoracic; C: lumbar [catheter tip region]; and D: lumbar segment just distal to the catheter tip) were taken from the spinal cord, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by light microscopy by a pathologist (L.L.F.) unaware of the experimental treatment. Semiquantitative evaluations were made as to the degree of inflammation and the pathologic changes in the meninges, vessels, nerve roots, and spinal cord parenchyma found in these sections. Observed tissue was assigned an individual pathology score based on the evaluation. The pathology score was on a scale from 0 to 4, where 0 represented no injury or inflammation and 4 represented very severe injury and/or inflammation. In addition, before unblinding the treatments, a forced

Table 2. Summary of Doses and Infusion Concentrations Used in Phase 1 Dose Ranging

<table>
<thead>
<tr>
<th>Infusion Drug</th>
<th>Daily Dose, mg (Infusate Concentration, mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP5</td>
<td>0.1 (0.04) 0.3 (0.125) 1 (0.417) 3 (1.25)</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>0.3 (0.125) 1 (0.417) 2 (0.833) 4 (1.67) 8 (3.33) 16 (6.67)</td>
</tr>
<tr>
<td>Ketamine</td>
<td>1 (0.417) 3 (1.25) 10 (4.17) 30 (12.5) 100 (42.7)</td>
</tr>
<tr>
<td>Memantine</td>
<td>1 (0.417) 2 (0.833) 4 (1.67) 8 (3.33) 12 (5.00)</td>
</tr>
<tr>
<td>MK801</td>
<td>0.1 (0.04) 0.3 (0.125) 1 (0.417) 3 (1.25)</td>
</tr>
<tr>
<td>S-Methadone</td>
<td>0.1 (0.04) 1 (0.417)</td>
</tr>
</tbody>
</table>

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Data Analysis
Continuous normal data were compared using repeated-measures and one-way analysis of variance with independent groups as appropriate. Post hoc Bonferroni tests for multigroup comparisons were used as required. Distribution free testing of data, such as ranked spinal cord pathology, was performed using the Kruskal–Wallis test for comparison of three or more groups with post hoc Mann–Whitney U tests for multigroup comparisons. All statistical comparisons were made at the $P < 0.05$ level of significance.

Results

Phase 1: Dose Ranging

Behavioral Effects. Stepwise incrementation of the infusate concentration of the NMDA antagonist typically resulted in a progression from no evident behavioral effect through the appearance of mild signs of dysfunction (e.g., mild weakness of one or both hind limbs, modest hind limb stiffness and some mild agitation) to severe and required termination of the infusion. These signs included marked hind limb or whole body hypertonicity, paralysis, marked sedation, agitation and/or spontaneous vocalization, extreme sensitivity to light touch applied to the flank (allodynia), and/or marked ataxia. Table 3 summarizes the results observed in the phase 1 dose-ranging work. Typically the onset of changes in behavioral function after a dose incrementation occurred between 24 and 48 h after the dosing change.

Fixed High-dose Infusion. To determine whether there was a confound between the stepwise incrementation of the test drug and the observed pathology, we chose amitriptyline to be delivered at 8 mg/day (an effect level 3 dose). Here, three animals received 8 mg/day amitriptyline from the outset. These animals required sacrifice at 4–10 days because of severe motor and/or behavioral effects. Spinal pathology revealed grade 4 pathology scores in the C and D lumbar spinal blocks.

Phase 2: 28-Day Safety Evaluation

Based on the observations in phase 1, we chose a dose having a detectable but tolerable behavioral effect for a 28-day exposure. These doses were as follows: AP5, 1 mg/day; amitriptyline, 1 mg/day; ketamine, 10 mg/day; MK801, 1 mg/day; and memantine, 4 mg/day.

Behavioral Effect and Survival. In phase 2, all but one animal survived the effect level 1 intrathecal dose until the intended date of sacrifice (table 4). However, mild hind limb stiffness and weakness was typically observed by 3–5 days after initiation of infusion, which progressed slowly over the period of infusion until sacrifice. This was a common finding with all agents (table 4).

Physiologic Measures. Saline-infused animals showed a modest but statistically significant ($P < 0.05$) decrease in blood pressure (121 ± 3 to 108 ± 6 mmHg) and increase in heart rate (90 ± 7 to 121 ± 7 beats/min) over the 28-day infusion, but no change in blood pressure over time was noted for any of the drug treatments, although similar modest increases in heart rate were observed. Body temperature showed no trends over time (data not shown). No significant change in body weight was observed over the 28-day infusion (one-way repeated-measures analysis of variance, $P > 0.05$ for each treatment group; data not shown).

CSF Chemistry. At sacrifice, protein and glucose concentrations in the cisternal CSF of saline-infused animals were 64 ± 5 and 58 ± 10 mg/dl (mean ± 95% confidence interval), respectively. Examination of the data in table 4 indicates that one of three amitriptyline dogs, three of four ketamine dogs, three of three memantine dogs, and two of four MK801 dogs exceeded the 95% confidence interval for CSF protein in vehicle-infused animals. For CSF glucose, only two animals fell below the 95% confidence interval for saline animals.

Typically, in animals showing neurologic signs, cisternal CSF harvested aseptically was cultured and found
negative for gram-negative and -positive bacteria (cultures: Flo Cell Diagnostics, San Diego, CA). Infusate solutions were similarly cultured and found to be negative. Histologic stains for bacteria were found to be negative (data not shown).

Necropsy. At necropsy, the injection of 0.3 ml methylene blue demonstrated that catheter tips were typically located in the intrathecal space around spinal segments L2–L3. All catheters displayed patency and appropriate dye delivery at sacrifice. After laminectomy, vehicle-treated cords showed no evident pathology. Most drug-treated cords in the phase 2 safety study displayed intact dura, but often displayed notable discoloration in the vicinity of the segment containing the catheter tip. In the dose-ranging animals, an evident local erosion of the dura, often less than a centimeter in length, was present at the spinal site adjacent to the underlying catheter tip and pathology.

Histopathology. Examination of saline-treated animals typically revealed a modest investment of the catheter in blocks A (cervical), B (thoracic), and C (lumbar catheter tip). Block D is immediately below the catheter tip. Figure 1 presents low magnification examination of typical spinal sections from the respective treatment groups. In saline-treated animals, inspection of the spinal cord C block revealed no remarkable signs of compression, demyelination, or inflammatory pathology. The majority of saline-treated animals had a minimal, local catheter tract reaction with a sparse, mixed cellular infiltrate (figs. 1A and 2A).

In animals receiving AP5, ketamine, memantine, and MK801, prominent pathology was noted (fig. 1). Figure 2 presents an intermediate magnification of tissue in figure 1, whereas figure 3 presents high-magnification images of material from an animal that received memantine as an example of the observations described herein. A continuum of changes from mild to massive inflammation and necrosis was observed. The mild pathologic changes were characterized by a perivascular to multifocal inflammation within the meninges and, in some animals, within the parenchyma (figs. 2B and C). Figure 2B shows a segment of submeningeal parenchyma of a ketamine-treated animal displaying perivascular inflammation that extends into the Virchow-Robbins space and edema within the white matter parenchyma. Figure 2C shows memantine treatment associated with an exten-

Table 4. Phase 2: Safety Study Summary

<table>
<thead>
<tr>
<th>Drug Dose*</th>
<th>Dog No.</th>
<th>Onset Time of Effects, days</th>
<th>Significant Observations</th>
<th>CSF Protein, † mg/dl</th>
<th>CSF Glucose, † mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitriptyline, 1 mg/day</td>
<td>1</td>
<td>3–5</td>
<td>HL and neck stiffness</td>
<td>55</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>HL and neck stiffness</td>
<td>76</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>Mild HL and neck stiffness</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>AP5, 1 mg/day</td>
<td>1</td>
<td>5–8</td>
<td>Mild to moderate HL weakness</td>
<td>52</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>Mild HL weakness</td>
<td>67</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>Mild HL weakness</td>
<td>60</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3</td>
<td>Mild HL weakness</td>
<td>66</td>
<td>61</td>
</tr>
<tr>
<td>Ketamine, 10 mg/day</td>
<td>1</td>
<td>5</td>
<td>Mild HL weakness, neck stiff, lordosis</td>
<td>143</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1</td>
<td>Mild neck and HL stiffness, ataxia, whole body tremors</td>
<td>100</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>Mild HL weakness</td>
<td>53</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3–5</td>
<td>Mild HL weakness and neck stiffness</td>
<td>214</td>
<td>63</td>
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<tr>
<td>Memantine, 4 mg/day</td>
<td>1‡</td>
<td>6</td>
<td>Whole body tremors, lordosis, ataxia, mild sedation</td>
<td>78</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>Head/neck twitching, ataxia, mild sedation</td>
<td>225</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>HL weakness and ataxia, progressing to partial HL paralysis</td>
<td>297</td>
<td>52</td>
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<tr>
<td>MK801, 1 mg/day</td>
<td>1</td>
<td>2</td>
<td>Mild HL weakness</td>
<td>137</td>
<td>33</td>
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<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>Mild HL weakness and ataxia</td>
<td>40</td>
<td>58</td>
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<tr>
<td></td>
<td>3</td>
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<td>Mild HL weakness and ataxia</td>
<td>109</td>
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<td>4</td>
<td>5</td>
<td>Mild HL weakness</td>
<td>22</td>
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<tr>
<td>S-Methadone, 1 mg/day</td>
<td>1</td>
<td>4</td>
<td>Mild HL stiffness and ataxia</td>
<td>74</td>
<td>71</td>
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<td></td>
<td>2</td>
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<td>Mild HL stiffness and ataxia</td>
<td>91</td>
<td>64</td>
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<td>Saline, 2.4 ml/day</td>
<td>1</td>
<td>23</td>
<td>Mild neck stiff, mild HL ataxia, lordosis</td>
<td>72</td>
<td>59</td>
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<td></td>
<td>2</td>
<td>12</td>
<td>Mild HL ataxia</td>
<td>56</td>
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<td>3</td>
<td>—</td>
<td>None</td>
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<tr>
<td></td>
<td>9</td>
<td>—</td>
<td>None</td>
<td>47</td>
<td>58</td>
</tr>
</tbody>
</table>

* All doses delivered at 100 μH/l, 2.4 ml/day. † Samples taken at sacrifice. ‡ Animal was killed on day 10 of infusion. Pretreatment cerebrospinal fluid (CSF) range (median) values: protein, 10–33 (20); glucose, 53–83 (68). Saline control animals mean ± 95% confidence interval posttreatment CSF protein 64 ± 5 and CSF glucose 58 ± 10.

HL = hind limb; ND = not determined.
sive coagulative necrosis and fibrinoid vasculitis surrounding the parenchymal infarct seen in figure 1C. Figure 2D displays an amitriptyline-treated cord with a significant perivascular inflammation surrounding the meningeal and parenchymal vessels. Note the expanded meninges with necrosis and inflammation.

Small and medium vessels were often rimmed by single to multiple layers of lymphohistiocytes and variable numbers of neutrophils (fig. 3C). Endothelial cells were flat to plump and rounded (reactive). The inflammation was occasionally accompanied by edema (fig. 2B). This degree of pathologic change was occasionally characterized by mild axonal degeneration and swelling. Rarely, there was increased microgliosis in the gray matter neuropil and a scattered inflammatory infiltrate within the white matter (fig. 3D). In some animals, the meningeal inflammatory foci were mass-like, causing mild compression of the adjacent white matter and parenchyma (figs. 1F and 2F). In the most evident cases, fulminant vasculitis, inflammation, and necrosis (figs. 1B–E and 2C–E) were present. Figure 2E shows a methadone-treated cord with a large, focal infarct within the white matter parenchyma. A dense accumulation of inflammation surrounds the necrotic tissue. There was widespread, necrotizing meningitis and vasculitis with and without fibrinoid changes (fig. 3B). A densely cellular, coalescing suppurative to pyogranulomatous inflammation markedly and irregularly expanded the meninges, extending deeply into the spinal cord parenchyma. The cellular components were primarily degenerative neutrophils and plump, epithelioid macrophages with fewer lymphocytes and plasma cells. Large central cores of coagulative necrosis with variable fibroplasia and neovascularization were characteristic (figs. 1D, 1E, and 2E). Prominent dural thickening and extensive meningeal fibrosis were also commonly seen (figs. 1C and 2C). The inflammation frequently effaced the adjacent white matter (fig. 1C). Within the white matter tracts, there was variable axonal degeneration and loss with swollen axonal sheaths, axonophagia, and occasionally axonal sheaths containing gitter cells (fig. 3F). A modest astrocytosis was also present. The gray matter had extensive vasculitis, microgliosis, and inflammation (fig. 3B). In rare cases, there was neuronal chromatolysis and neuronophagia (fig. 3E). Occasionally, the inflammatory response extended out to the epidural tissue (figs. 1C and E). Peripheral vessels
in the meninges were dilated, and hemorrhage was present (fig. 2F). Peripheral dorsal nerve roots entrapped within the inflammation have similar axonal and inflammatory changes as observed in the white matter parenchyma. These findings were most evident in the animals examined from the phase 1 dose escalation series and in the three animals that received the 8-mg/day intrathecal amitriptyline infusions (table 3). The effects of S-methadone were examined in two animals. Studies were limited because of limited solubility and drug availability. However, as indicated in figure 1E, this dose resulted in prominent signs similar to those outlined above with evident parenchymal destruction. It should be noted that the pathology noted with all drug treatments bore a close similarity. However, inspection of the AP5-treated animals typically revealed more modest parenchymal necrosis and a more evident pericatheter mass.

The overall severity of the pathology was broadly quantified in terms of a general pathology score observed in each of the four blocks for each animal. These observations are summarized in figure 4, where the mean pathology scored by block is presented. As indicated, blocks distant from the catheter tip (A: cervical; B: thoracic) show relatively little pathology as compared with saline vehicle. The most severe pathology was variably observed in the lumbar blocks C and D proximal to the infusion site. This scoring and distribution coincided well with the initial impression observed in meningeal pathology noted at necropsy. Comparing the magnitude of the spinal pathology reaction observed with the several drugs in the phase 2 study versus that obtained with the saline reveals a statistical difference for all drugs versus saline, typically at the lumbar catheter tip (block C).

Discussion

Intrathecal Infusions and Dose-dependent Spinal Pathology

The current studies consider the effects of chronic intrathecal infusions of several NMDA antagonists on behavior and on spinal histology. Phase 1 allowed us to...
initially determine the range of doses that could be tolerated given the likelihood of motor dysfunction secondary to spinal NMDA receptor blockade. In these initial dose incrementation studies, we found that there were doses/concentrations that could be readily tolerated by the animal (effect levels 1 and 2) for 24–48 h and then doses wherein prominent and typically persistent neurologic signs were noted (effect level 3) that upon necropsy were invariably associated with evident histopathology. To further define the potential safety profile, we undertook to deliver for 28 days concentrations that had minimal behavioral effect over an interval of 48–72 h. These doses were approximately 0.1 times lower than those doses defined as level 3 doses. Upon infusion of these doses for 28 days, AP5, MK801, memantine, and ketamine animals displayed moderate changes in neurologic function, although not of sufficient severity to necessitate sacrifice. However, after necropsy, it was noted that many of these animals displayed evident components of the pathology that had been observed after the very high dose. Systematic grading revealed that these doses of these agents resulted in distinct spinal pathology that was greatest proximal to the catheter tip and statistically more severe than the vehicle controls.

The pathology observed in the current experiments parallel remarkably that previously reported in sheep after infusion of memantine (2.2 mg/day, 1.3 mg/ml), dextrophan (8.9 mg/day, 5.4 mg/ml), and dextromethorphan (2.2 mg/day, 1.3 mg/ml) and in rabbits after seven daily bolus injections of preservative-free S(+)ketamine (5 mg/day, 10 mg/ml). In the sheep, the lesions were described as "Necrosis: Purulent inflammation infiltration with neutrophil/macrophages." Importantly, the pathology picture observed here is distinctly different from that reported to occur after high concentrations of several intrathecal opiates in dogs, sheep, and humans. Here, the infusion-related pathology is an aseptic mass that arises from the meninges and compresses spinal parenchyma. It is interesting to note that AP5, while displaying necrotic pathology, also

Fig. 3. Spinal cord lumbar segment proximal to catheter tip of a memantine-treated animal. Hematoxylin and eosin staining. (A) Widespread vasculitis and necrosis of the white and gray matter. Bar = 25 mm. (B) Abundant perivascular infiltrate with fibrinoid necrosis of small veins and arterioles with prominent mononuclear infiltration in the gray matter. Bar = 25 mm. (C) Extensive perivascular cuffing of a small vessel in the gray matter. The inflammatory cuff is composed of numerous mononuclear inflammatory cells mixed with neutrophils; note the perivascular edema and gliosis. Bar = 10 mm. (D) Spinal cord gray matter with mononuclear and microglial infiltrate adjacent to small vessels. Bar = 10 mm. (E) A neuron in the dorsal horn shows pronounced degenerative changes within a densely cellular inflammatory infiltrate. Bar = 10 mm. (F) White matter with inflammatory degeneration showing axonal swelling and necrosis. Bar = 10 mm.
tended to show a more evident tendency to form defined pericatheter masses.

**Nature of Pathology**

The current study, with several drugs and dose levels, seems to represent a continuum of changes ranging from mild inflammation characterized by perivascular cuffing and meningeitis to widespread necrotizing vasculitis and meningomyelitis. If we propose that one event led to tissue necrosis that incited the invasion of leukocytes, this picture presents a profile resembling that of an immune-mediated vasculitis. Looking at it as a single process, the frank necrosis in the spinal cord may result from the ischemia caused by vasculitis with fibrinoid necrosis and endothelial or intimal proliferation of vessel walls. The underlying immunologic mechanisms responsible for central nervous system vasculitis are poorly understood. Several proinflammatory agents such as cytokines, chemokines, matrix metalloproteinases, and molecules associated with nitric oxide production are up-regulated in models of meningeitis caused by infectious agents as well as other pathologic processes affecting the central nervous system.28,29

**Mechanism of Pathology**

**Infusion System and Infusate.** The pathology was evident in all groups but those receiving saline. There has been an extensive history of intrathecal infusion using this canine model, and this pathology has not been noted after baclofen,30 adenosine,31 neostigmine,32 brain-derived nerve growth factor,33 clonidine,25 or an extensive series of opiates as noted above at the highest dose thus far examined. Accordingly, it is not a question of a simple reaction to the catheter and infusion. Examination of the properties of the drug infusate reveal that the drug solutions used in the safety study were within pH and osmolarity ranges considered appropriate for continuous spinal delivery.34 There were no adjuvants in the injectate. The effects do not likely arise from an infectious source. Formulated infusates and CSF from animals showing prominent pathology were cultured and found negative for gram-negative and -positive bacteria. Histologic stains for bacteria were found to be negative. CSF glucose concentrations were found to be in the normal range. Accordingly, we do not believe the pathology arises from an infection, or a general reaction to the infusion or to the physical properties of the infusate.

**Drug Target.** The agents used here are characterized by no single structural motif; the single pharmacologic property, however, which seems to characterize their actions, is their affinity for blocking the NMDA receptor. The effects observed here were prominently noted with an agent that competitively blocks the glutamate binding site (AP5) and that serves as a channel blocker (memantine, MK801, ketamine, and amitriptyline).4 Importantly, S(+) -methadone has potent activity as an NMDA channel blocker22 but not as an opiate agonist and evokes a similar pathology. Importantly, opiate agonists produce space-occupying granulomas, but not the parenchymal effects observed in these studies. Although other mechanisms may apply, this pervasive commonality suggests the NMDA ionophore as a potential mechanistic link.

N-Methyl-D-aspartate receptors are abundantly expressed in dorsal horn neurons35 and on nonneuronal cells including astrocytes36 and oligodendroglia.37 NMDA receptors are calcium ionophores, which initiate excitatory cascades. NMDA receptor blockade attenuates facilitated spinal states and has neuroprotective effects in the face of injury and hyperexcitability.38,39 Despite these protective effects, Olney et al.40 showed that systemic NMDA antagonists, including MK801, AP7, AP5, ketamine, memantine, dextromethorphan, and CPP, produce vacuolization in the central nervous system, notably in the retrosplenial cortex. These effects are accompanied by a reduction in mitochondria and endoplasmic reticulum.40 The relative systemic potency of the several agents in producing these effects approximately corresponds with the estimated affinity of these agents, both competitive and noncompetitive, for the NMDA site, suggesting that site as a common mechanism of action.40 More recently, it has been shown that transient (as little as 4 h) blockade of NMDA but not non-NMDA receptors in the newborn rat activate programmed cell death,41 although it is not clear whether this process is relevant to the spinal effects in the adult.

**Fig. 4.** Pathology score (µm ± SE) plotted by spinal block (A: cervical; B: thoracic; C: lumbar catheter tip; D: lumbar below catheter) for groups of animals receiving saline, amitriptyline (1 mg/day), ketamine (10 mg/day), memantine (4 mg/day), MK801 (1 mg/day), or AP5 (1 mg/day). One-way repeated-measures analysis of variance displays a significant main effect for all treatments except saline (P < 0.05). *Post hoc comparisons show significant pathology as compared with the respective A block (P < 0.05).
animal. How this in vivo toxicity arises from NMDA antagonism is not certain. Several possibilities are plausible. (1) Excitotoxicity. Block of NMDA receptors on inhibitor interneurons (GABAergic) modulating downstream excitatory (glutamatergic) neurons would lead indirectly to an increased glutamate release. Therefore, ketamine enhances amphetamine-evoked dopamine release. Given the presence of the NMDA antagonist, the origin of the developing toxicity would then arise from an action of glutamate mediated by other receptors, such as the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor. (2) Trophic role of NMDA receptor function. NMDA receptors may play a trophic role in cell function, which would be revealed by persistent NMDA receptor antagonism. Therefore, cultures exposed to NMDA but not non-NMDA antagonists display neuronal loss, suggesting a need for Ca2+ influx through the NMDA receptor.

Other effects of NMDA blockade include interference with neuronal migration which is dependent on NMDA receptor-mediated Ca2+ flux. In addition, NMDA receptor antagonists, by blocking calcium influx, rapidly increase neurite initiation and elongation, consistent with an increase in presynaptically located SNAP-25.

(3) Metabolic alterations. Systemic delivery of MK801 resulted in an increase in brain glutathione, glutamate, and glutamine. In these studies, MK801 was shown to disrupt the astrocyte glutamate-glutamine cycle and promote glial cell swelling. Whether any of these mechanisms contribute to the observed toxicity profile remains to be seen.

An additional intriguing alternative is one of direct membrane toxicity. At high concentrations, amphiphilic drugs can form molecular aggregates that lead to a detergent-like action of the formulation. Local anesthetics form self-aggregations (e.g., micelles) that have detergent properties. At the concentrations where such aggregation occurs, membrane disruption and local neurotoxicity can be demonstrated after spinal delivery. Amitriptyline has local anesthetic actions after spinal delivery, and a number of studies have demonstrated a concentration-dependent toxicity when applied to peripheral nerve or intrathecally in vivo. Therefore, 0.1% amitriptyline acted as a local anesthetic, whereas at concentrations of 0.3% or greater, amitriptyline produced irreversible neural impairment. In those studies, it was shown that amitriptyline underwent self-aggregation at approximately 15 mM (0.46%/4.6 mg/ml) in phosphate-buffered saline. This covariance strongly suggests a potential link between amitriptyline toxicity and its detergent activity. A similar argument has been made as a potential mechanism for local anesthetic toxicity. As noted in the current canine study, intrathecal infusion of amitriptyline at concentrations of 1 mg/day (0.4 mg/ml) had a modest effect, whereas at doses of 8 mg/day (3.5 mg/ml), there was a potent toxicity. Examination of the high degree of localization of the toxicity strongly suggests a phenomena dependent on a steep concentration gradient around the catheter tip. It is interesting that methadone, but not morphine, has been reported to form micelles, a finding consistent with current and previously reported pathology. Further studies are required to consider the applicability of this potential mechanism of toxicity.

Human Correlations

Some case reports have indicated that human patients who have received intrathecal ketamine have displayed at autopsy serious pathologic observations similar to those reported in the dog studies. Others have not. These clinical studies are complex and may reflect false-positive observations because of the chemotherapy and radiation therapy as well as the complex spinal polypharmacy to which these patients had been exposed.

Caveats to the Interpretation of the Results

The current studies, in accord with those previously reported by other groups in other spinal delivery models, provide strong support that under certain conditions, these agents with a propensity for NMDA receptor blockade can lead to significant toxicity with chronic exposure. These results thus contribute to the accumulating data that the intrathecal delivery of current NMDA antagonists should not be considered as a viable therapeutic alternative. Several issues should be noted.

First, although these data indicate that these specific drugs have a risk of serious spinal toxicity when used chronically, we recognize that there may be relatively safe doses/concentrations. What those doses are we cannot say.

Second, it should be stressed that we have little information as to the therapeutic ratio (e.g., dose producing toxicity vs. the dose producing analgesia). The canine models of acute nociception are not altered by agents such as NMDA antagonists, and it may be that even the infusion paradigm producing minimal behavioral effects in fact exceeds to some marked degree those doses required to produce an antihyperalgesic effect. We have argued that it is the local CSF concentrations that lead to toxicity. To address this issue, systematic comparative pharmacokinetic studies will be required to provide an algorithm whereby the concentrations associated with toxicity in dogs can be translated to dosing regimens in humans which might yield those same concentrations.

Third, it is possible that single bolus delivery would display a different toxicity profile. This is certainly the case for morphine, where daily bolus delivery for 28 days did not produce a granuloma while comparable doses given as an infusion did. It should be stressed...
that toxicity has now been reported in two large animal models with continuous infusion, whereas in studies with bolus delivery, disparate results have been noted (see above discussion). Interestingly, this consideration makes the case that acute exposure is not an adequate test of safety for continuous infusion use.

Fourth, complicating the problem of the NMDA-targeted agents is that the specific mechanisms of toxicity are not clear, so we do not know what to avoid. Although the safety of other approaches such as the use of NMDA-glycine site antagonists have not been studied, if the ionophore block is responsible, then such agents might have similar actions. Whether other ionophore subunits having a pharmacology distinct from this profile have toxicity remains to be determined.

Fifth, we believe that the detergent–micelle hypothesis put forth by Kitagawa et al. is provocative and provides an important guideline for the development of future spinal drugs. Therefore, should the detergent micelles possess a propensity to undergo micelle formation may in fact be the case.

In conclusion, given the current data set and the above considerations, we believe strongly that advocation of NMDA receptor antagonists with very high affinity for the receptor and low propensity to undergo micelle formation may in fact be relatively safe after local delivery.

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References

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