Safety Assessment and Pharmacokinetics of Intrathecal Methylprednisolone Acetate in Dogs

Mienke Rijsdijk, M.D.,* Albert J. M. van Wijck, M.D., Ph.D.,† Cor J. Kalkman, M.D., Ph.D.,‡ P. C. Willem Meulenhoff, M.Sc.,§ Marjorie R. Grafe, M.D., Ph.D.,‖ Joanne Steinauer, B.S.,# Tony L. Yaksh, Ph.D.**

ABSTRACT

Background: Intrathecal methylprednisolone acetate (MPA) has been used in patients with chronic pain syndromes. Its safety has been debated after reports of adverse events. No systematic preclinical evaluation of MPA has been reported. In the current study, the acute and long-term effects of intrathecal MPA on dog spinal tissue was studied with the injectate reformulated to include minimal adjuvants.

Methods: Seventeen dogs were implanted with intrathecal catheters and randomized to three groups: vehicle (lidocaine; 4 dogs), MPA 20 mg/ml (human dose; 7 dogs), and MPA 80 mg/ml (maximum deliverable dose; 6 dogs). In parallel with the human protocols, dogs received four injections at 7-day intervals. Clinical observations and plasma methylprednisolone measurements were done before and at intervals after intrathecal delivery. One week (acute) or 6 weeks (long-term) after the last injection, animals were sacrificed and spinal tissues harvested for histopathology.

Results: Other than a brief motor block, no adverse clinical event occurred in any animal. Group A (vehicle) showed minimal histologic changes (median histology-score; acute: 1.3, long-term: 1.0). Group B (MPA 20 mg/ml) had a diffuse inflammatory reaction (acute: 2.0, long-term: 3.0), group C (MPA 80 mg/ml) a severe inflammatory response, with large inflammatory masses (acute: 4.0, long-term: 7.0) The severity of the inflammatory reaction increased significantly with increasing dose at long-term sacrifice (acute $P = 0.167$, long-term $P = 0.014$). No neuronal injury, demyelination, or gliosis was seen in any animal.

Conclusion: These results, showing dose-dependent intrathecal inflammatory reactions at MPA doses and injectate concentrations comparable to those used in humans, indicate that the continued use of this modality in humans is not recommended.

What We Already Know about This Topic

- Subarachnoid administration of methylprednisolone acetate (MPA) is used experimentally in patients with postherpetic neuralgia and chronic complex regional pain syndrome
- Safety of subarachnoid administration of MPA is unknown

What This Article Tells Us That Is New

- In dogs, subarachnoid administration of MPA caused meningeal inflammation, demonstrating that subarachnoid MPA should not be administered to patients

THE use of intrathecal corticosteroids began in the 1960s as treatment for patients with sciatica, multiple sclerosis, or arachnoïditis.[1] The rationale to administer steroids *via* the intrathecal route was the direct action of the drug on inflamed nervous tissue, presumably decreasing inflammation and edema, and thereby alleviating pain and neurologic symptoms. Other advantages of intrathecal administration of steroids were considered to be the lower therapeutic dose required compared with systemic administration, leading to a decrease in adverse effects. In addition, compared with epidural administration, the intrathecal route was thought to yield a more predictable spinal spread of the drug, a longer duration of action, and a lower risk of neuraxial hematoma or drug overdose.

A randomized controlled trial (RCT) performed in Japan in 2000 reported extraordinary efficacy of intrathecal meth-

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* Resident Anesthesiology and Ph.D. Student, Anesthesiology and Critical Care Department, University Medical Center Utrecht, Utrecht, The Netherlands. † Staff Anesthesiologist and Head of the Pain Clinic, Anesthesiology and Critical Care Department, University Medical Center Utrecht. ‡ Professor of Anesthesiology, Anesthesiology and Critical Care Department, University Medical Center Utrecht. § Staff Pharmacologist, Pharmacy Department, University Medical Center Utrecht. ‖ Professor of Pathology, Pathology Department, Oregon Health and Science University, Portland, Oregon. # Laboratory Manager and Director of Anesthesiology, Core Confocal Imaging Facility, Anesthesiology Department, University of California, San Diego, San Diego, California. ** Professor in Anesthesiology and Pharmacology and Vice Chairman for Research in Anesthesiology, Anesthesiology Department, University of California, San Diego.

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Address correspondence to Dr. van Wijck: Pain Clinic, Department of Anesthesiology, University Medical Center Utrecht, P.O. Box 85500, 3508 GA Utrecht, The Netherlands. a.vanwijck@umcutrecht.nl. Information on purchasing reprints may be found at www.anesthesiology.org or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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ylprednisolone acetate (MPA) in patients with severe postherpetic neuralgia (PHN).[2] After 4 intrathecal injections with MPA and lidocaine, 82 of 89 PHN patients had good or excellent pain relief, compared with only 5 of 91 and 3 of 90 patients after lidocaine or no treatment, respectively. No side effects or complications were reported. However, despite these excellent reported results, intrathecal MPA injection has never become part of standard care for intractable PHN. Concerns about safety of intrathecal MPA and/or a lack of confirmation of the results may have played a role.[3–6] Indeed, a variety of adverse events have been reported with intrathecal corticosteroids, including chemical meningitis, transverse myelitis, cauda equina syndrome, lumbar radiculitis, intractable headache, urinary retention, and adhesive arachnoiditis.[1,7] Several explanations may be offered for these adverse events, including the presence of formulation adjuvants (benzyl alcohol, benzalkonium chloride or myristylgamma-picolinium chloride) in corticosteroid injections fluids; the use of particulate material (as with MPA) that may represent a physical stimulus; or an unknown mechanism resulting from the high concentrations of the steroid itself. Preclinical safety studies with other intrathecal steroids such as bethamethasone have been associated with development of arachnoïditis.[8,9] However, in these studies, preservatives were again present in the studied formulation and may represent a possible alternative explanation for the inflammatory response observed. To the best of our knowledge, there has been no systematic preclinical safety study on the use of repeated intrathecal dosing of MPA following the paradigms and dosing ranges used in previous human studies. To minimize the concerns related to the potential effects of preservatives, such an evaluation should be carried out in their absence. Accordingly, the aim of the current study was to provide information on the safety and kinetics of the effects of multiple intrathecal injections of methylprednisolone acetate after minimization of the preservatives in which it is commercially formulated (Depo-Medrol®, Pfizer), using a well-defined canine model.

Materials and Methods

The protocol of the study has been approved by the International Association for Assessment and Accreditation of Laboratory Animal Care accredited Institutional Animal Care and Use Committee of the University of California, San Diego.

Drug Preparation

The test article was prepared from the commercially available MPA formulation (Depo-Medrol®, 40 mg/ml MPA, Pfizer). This suspension of MPA also contains the preservative myristylgamma-picolinium chloride and the adjuvant polyethylene glycol. To minimize the presence of these soluble adjuvants from the commercial formulation, we took the following practical steps. The contents of each vial were centrifuged in a minicentrifuge (Fisher Scientific, Hampton, NH, Cat no 05-090-128, 14,000 rpm) for 10 min. The supernatant was aspirated following rigid aseptic precautions with a needle and syringe. The residual pellet of 10 mg, 20 mg, or 40 mg (depending on the dose) MPA was resuspended in a mixture with 0.4 ml lidocaine 2% and 0.1 ml glucose 50% (= vehicle) to a total volume of 0.5 ml. Lidocaine was added to confirm that the needle tip was indeed located within the intrathecal space by eliciting a brief sensory and motor block. To summarize, the dose of MPA 20 mg/ml contains 10 mg MPA in a volume of 0.5 ml with a lidocaine concentration of 1.6% and glucose 10% and the dose of 80 mg/ml 40 mg MPA, also in a volume of 0.5 ml with similar lidocaine and glucose concentrations. The vehicle had the same constituents in which the formulation was resuspended including lidocaine. This preparation was made immediately before use. The test article had a pH of 6.5 and a measured relative density of 1.04 kg/L. Methylprednisolone, MPA, and myristylgamma-picolinium chloride concentrations were determined using high-performance liquid chromatography and an ultraviolet detector. The measured concentration of myristylgamma-picolinium chloride in the unseparated supernatant of the commercial formulation was 0.36 mg/ml and after reformulation that was significantly reduced to 0.023 mg/ml in the MPA 80 mg/ml dosing formulation and below detection limits in the MPA 20 mg/ml formulation. The concentration of the preservative polyethylene glycol was not measured in the test article. However, polyethylene glycol is completely soluble in water, and by removing the supernatant of the commercial formulation, most of the polyethylene glycol was removed from the MPA; based on the myristylgamma-picolinium chloride data the concentration of polyethylene glycol in the reformulated material would be estimated to be approximately 0.1 times that of the commercial formulation.

Animals

Destination-bred beagle dogs (Marshall BioResources, North Rose, NY), 12 males (9–14 kg) and 6 females (6–8 kg, age 11–14 months), were individually housed and given *ad libitum* access to food and water. The lighting in the kennel was set on a 12-h daily light-dark cycle. Animals were acclimated for a minimum of 8 days and adapted to the testing protocols for 5 days before surgery. All dogs were preoperatively screened for normal blood chemistry (IDEXX, Laboratories, West Sacramento, CA), absence of infectious diseases, and normal neurologic status.

Surgical Procedure

A chronic intrathecal catheter was placed for repeated intrathecal bolus injections of MPA. Surgical placement of the intrathecal catheter was accomplished 9–11 days before the first dose. Prophylactic antibiotic treatment (sulfamethoxazole trimethoprim tablet 15–20 mg/kg orally twice daily) was given 24 h before surgery continuing until 48 h after surgery. After administration of atropine (0.04 mg/kg intramuscular)

and xylazine (1.5 mg/kg intramuscular), animals received 4–5% isoflurane in 50% oxygen and 50% air and then the trachea was intubated. Anesthesia was maintained under spontaneous ventilation with 1.0–2.0% isoflurane in 40% oxygen and 60% N_2O. Surgical areas were shaved and prepared with chlorhexidine scrub and solution. Using sterile technique the cisternal membrane was exposed. A cisternal cerebrospinal fluid (CSF) sample of 3 ml was taken for laboratory analysis and the intrathecal catheter (polyurethane 0.012" ID × 0.025" OD, previously sterilized by ethylene oxide gas) was inserted and passed caudally at a distance of approximately 38–45 cm to a level corresponding to the L3–L6 vertebral segment. The external part of the catheter was then tunneled subcutaneously to exit on the upper back and plugged. The incision was closed in layers with 3-0 Vicryl suture (Ethicon Inc., Somerville, NJ). Upon closure, the isoflurane and nitrous oxide were discontinued and a subcutaneous injection with 4.5 mg/kg carprofen was administered for postoperative pain control. The dogs were observed during recovery, and buprenorphine (0.02 mg/kg) was given intramuscularly if necessary to relieve any remaining postoperative discomfort. In six dogs, a saline pump (Medtronic MiniMed 508; Medtronic Inc., Minneapolis, MN) was used to deliver a low volume of saline to ensure intrathecal catheter patency. This was later found to be unnecessary and the use of the pumps was discontinued.

Clinical Observations

After surgery, before the first dose, 7 days after the final dose of MPA and before sacrifice, all dogs underwent a detailed neurologic assessment consisting of general attitude observations, preferred cage position, gait observations, muscle tone, sensation and pain observation, spinal reflexes (quadriceps, extensor carpi, flexor, perineal, extensor thrust, crossed extensor), proprioceptive reflexes (wheelbarrow, extensor postural thrust, proprioceptive positioning, hemistand and hemiwalk, placing response visual and tactile, righting reflex) and cranial nerve reflexes (nerves I to IX and XII).

Daily observations including body temperature and general behavior (arousal, muscle tone, and coordination) were assessed in all dogs. The clinical observations made previous to and at certain time points (1, 3, and 6 h and 1, 3, and 6 days) after administration of intrathecal MPA included indices of arousal, muscle tone, coordination, body weight, body temperature, heart rate and blood pressure (measured at the base of the tail), thermal latencies measured with the Canine Thermal Testing System,[10] and blood glucose values (One Touch Ultra, LifeScan Inc., Main, CA).

Study Design

First, the acutely tolerable dose of methylprednisolone was established using three male dogs; the first dog received a dose of MPA 20 mg/ml, the second dog, 40 mg/ml, and the third dog, 80 mg/ml. Clinical observations were made as described previously, before injection and 1, 3, and 6 h and 1, 3 and 6 days after the injection. At each time point, blood was withdrawn to measure plasma levels of methylprednisolone and blood glucose. The highest tolerable dose (80 mg/ml) was repeated in the same three dogs with a maximum of a total of four intrathecal injections with 7-day intervals between injections. One week after the last injection, the dogs were sacrificed and tissue harvested as described in the Necropsy section.

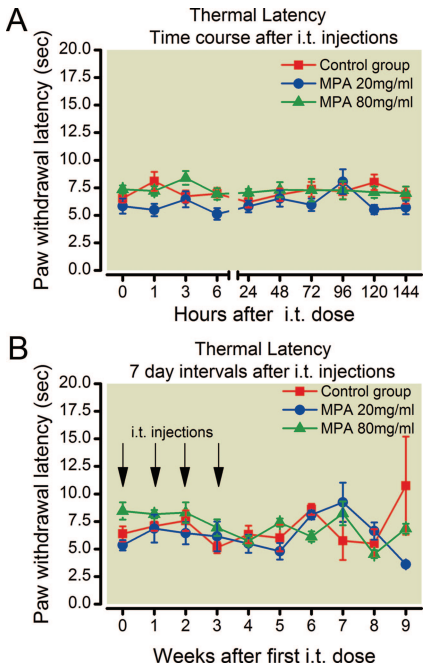

Fig. 1. (*A*) Time course of mean paw withdrawal latencies after first intrathecal injection. (*B*) Time course of mean paw withdrawal latencies at 7-day intervals after intrathecal injections. i.t. = intrathecal; MPA = methylprednisolone acetate.

A second pilot group of three male dogs received four intrathecal injections of MPA 20 mg/ml, the therapeutic dose administered in humans, with 7-day intervals following the same schedule for clinical observations, methylprednisolone plasma levels, and sacrifice.

In the final phase of the preclinical study we randomized 12 dogs, 6 males and 6 females, to 3 groups. The first group of four dogs (two male, two female) received vehicle, lidocaine with glucose (+ 0.5 ml saline 0.9% flush); the second group (two male, two female) received a low- dose MPA 20 mg/ml (+ flush) and the third group a high dose 80 mg/ml (+ flush) during 4 weeks with a 7-day interval between the injections. Clinical observations were made as described in the Clinical Observation section, before injection and 10 min, 1, 3, and 6 h and 1, 3, and 6 days after the injection. In addition, blood was withdrawn at each time point to measure plasma levels of methylprednisolone and blood glucose. Seven days after the last injection, one female and one male dog from the vehicle group, one female from the MPA 20 mg/ml group, and one female from the MPA 80 mg/ml group were sacrificed as described in the Necropsy section. In the remaining eight dogs the external part of the intrathecal

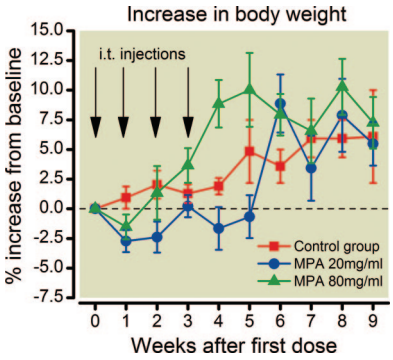

Fig. 2. Percentage increase in body weight from baseline for the three groups. i.t. = intrathecal; MPA = methylprednisolone acetate.

catheter was internalized by cutting the external catheter short, plugging the end, and letting the remaining external section slip subcutaneously. Six weeks after the last injection, the eight dogs were sacrificed and tissue harvested as described in the Necropsy section.

Necropsy

For euthanasia, dogs were deeply sedated with acepromazine (10 mg/ml intramuscularly). Blood samples were taken for chemistry, complete blood count, and methylprednisolone levels. Dogs then were deeply anesthetized (sodium pentobarbital, 30 mg/kg, intravascularly) and cisternal CSF samples for laboratory analysis and methylprednisolone levels and urine, obtained by cystocentesis, for clinical analysis, and creatinine/cortisol ratio were taken. The animals were exsanguinated by perfusion with saline 0.9% followed by 10% neutral-buffered formalin. The spinal cord and brain were exposed and examined, and the presence and localization of MPA plaques noted. The position and integrity of the intrathecal catheter was established by injection of methylene blue dye at necropsy. The spinal cord, including meninges, were resected in sections at specific regions (coded A: Cervical, B: Thoracic, C: Lumbar at catheter tip, D: Low lumbar including cauda equina) and placed in fixative (10% neutral-buffered formalin). The brain was resected by cutting the brainstem, cranial nerves, and vessels, and also placed in fixative.

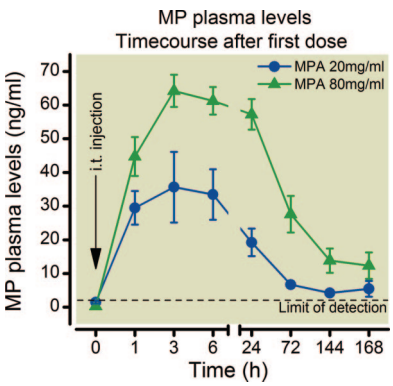

Fig. 3. Time course of methylprednisolone plasma levels after the first intrathecal MPA 20 mg/ml or 80 mg/ml injection. i.t. = intrathecal; MPA = methylprednisolone acetate; MP = methylprednisolone.

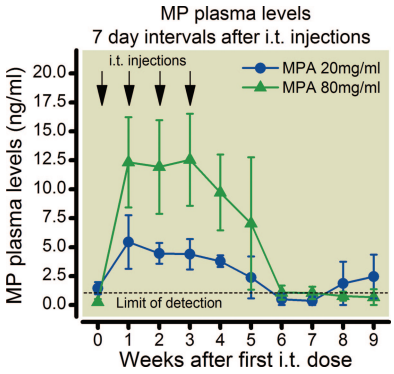

Fig. 4. Time course of methylprednisolone plasma levels before every intrathecal drug administration, at 7-day intervals. i.t. = intrathecal; MPA = methylprednisolone acetate; MP = methylprednisolone.

Histopathology

Paraffin sections of thoracic, lumbar, and sacral spinal cord with surrounding meninges were stained with hematoxylin and eosin. All sections were examined by a neuropathologist (MG) who was unaware of the treatment group assignments until the complete review was accomplished. Sections were examined for the presence, location, and type of inflammatory reaction, including inflammatory cell infiltrates, granulation tissue, and fibrosis. Lumbar and sacral sections were scored on a scale of 0 to 4, with 0 being no inflammatory response and 4 being the maximal response observed in this cohort. Separate scores were given for dura and arachnoid. Further evaluation of spinal cord injury was performed on sections stained with FluoroJade C (degenerating neurons) and Luxol Fast Blue/cresyl violet (myelin), and with immunohistochemical stains for Neuronal nuclear antigen (neurons), Glial fibrillary acidic protein (astrocytes), and Ionized calcium binding adaptor molecule 1 (microglia/macrophages). Spinal cord parenchymal pathology was given a score of 0–4 (normal to severe injury) based on evaluation of all stains. Total histology score was the sum of the scores for dura, arachnoid, and spinal cord (possible score of 0–12).

Methylprednisolone Plasma and CSF Sampling

In dogs, plasma levels of methylprednisolone were measured 1, 3, 6, 24, 72, 144, and 168 h after the first intrathecal injection of MPA. A plasma sample was also taken before the second, third, and fourth intrathecal injections and at sacrifice. In the dogs with a long-term recovery, periodic plasma samples were taken at 7-day intervals after the last intrathecal injection until sacrifice. The methylprednisolone concentrations in blood plasma and CSF samples were measured with an enzyme-linked immunosorbent assay kit (Neogen Corporation, Lexington, KY) with external standards diluted for quantification.

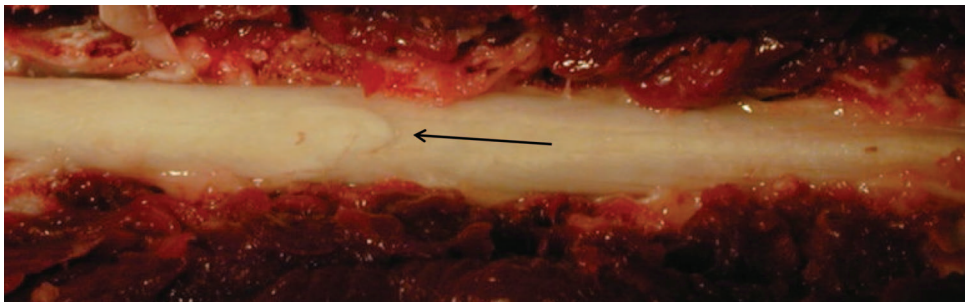

Fig. 5. A white solid deposit of 2 cm on the dorsal side of the spinal cord (*arrow*).

Statistical Analysis

The three groups were compared for the clinical parameters using a two-way ANOVA model. For the plasma methylprednisolone values, areas under the curve were calculated for every animal. Differences in area under the curve between the two dosing groups were calculated using a Student *t* test. Differences between the median histology scores were calculated with a Kruskal-Wallis test. Two-sided *P* values ≤0.05 were considered significant (PASW Statistics version 17.0, Chicago, IL).

Results

Eighteen dogs were included in the study. During dose ranging, a catheter in one of the male dogs receiving his third MPA 80 mg/ml dose became blocked. This animal, although examined, was excluded from further analysis.

The clinical observations (body temperature, heart rate, blood pressure, thermal latencies), methylprednisolone plasma levels and laboratory results were based on the data collected in the final phase of the preclinical study using 12 dogs. The pathology and histopathology results are based on all 17 included dogs.

Clinical Observations

All dogs displayed a brief motor block (10–40 min) after dosing, confirming correct intrathecal delivery of the lidocaine containing vehicle or test article. Other than the brief decrease in muscle tone and coordination, no behavioral changes were observed in any of the dogs during or after intrathecal injection. There were no significant differences in body temperature, heart rate, blood pressure, plasma glucose levels, and thermal latencies between the treatment groups and control group at the time points after drug delivery (fig. 1). In addition, no significant differences were observed after correction for sex or between the first and last injection. Body weight increased to a larger extent in animals treated with high-dose MPA ($P = 0.04$). The per-

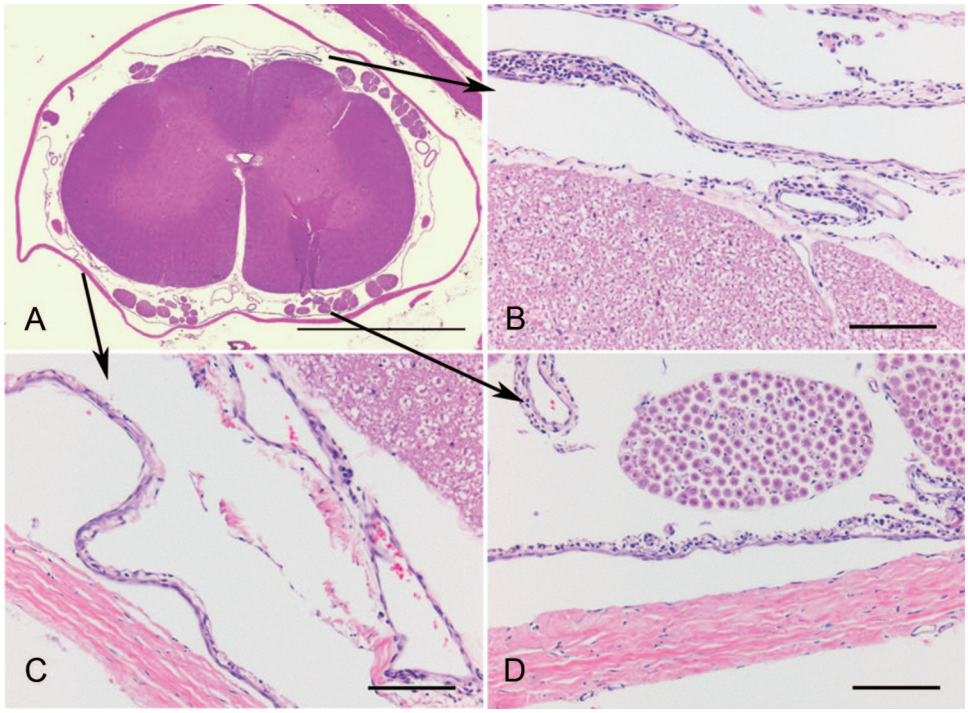

Fig. 6. Vehicle, acute sacrifice. (*A*) Minimal inflammatory infiltrates in arachnoid (*bar* = 3 mm). (*B–D*) *Arrows* indicate regions shown at higher power. (*B*) Mild arachnoid and perivascular inflammatory infiltrates (*bar* = 100 μm). (*C* and *D*) Areas with no to minimal inflammatory infiltrates (*bar* = 100 μm).

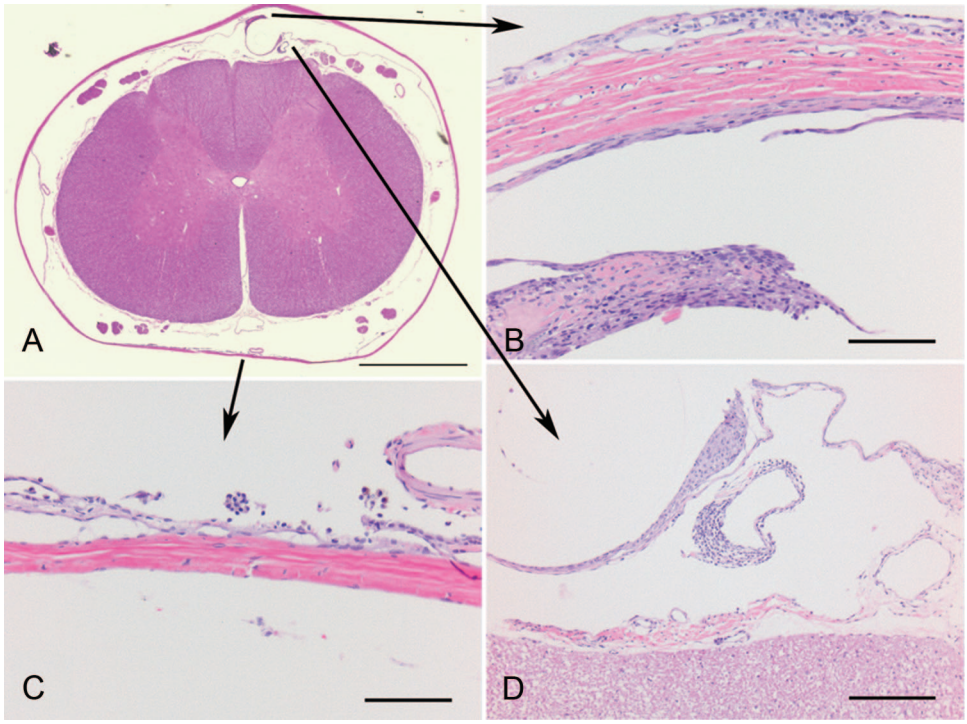

Fig. 7. Vehicle, long-term sacrifice. (*A*) Mild dural and arachnoid inflammation, with catheter site in dorsal arachnoid (*bar* = 3 mm). (*B–D*) *Arrows* indicate regions shown at higher power. (*B*) Mild chronic inflammation and fibrosis on outer and inner surfaces of dura adjacent to the catheter site, with focal fibrosis and chronic inflammation around catheter (*bar* = 100 μm). (*C*) Minimal arachnoidal chronic inflammation, consisting primarily of macrophages (*bar* = 100 μm). (*D*) Chronic perivascular inflammation adjacent to catheter site (*bar* = 200 μm).

centage body weight increase from baseline until after the last intrathecal drug delivery was 8.9% in the high-dose MPA *versus* 1.9% in the vehicle- treated group, suggesting a systemic effect of the intrathecal MPA (fig. 2).

Methylprednisolone Plasma Levels

In all drug-treated animals, peak plasma levels of methylprednisolone were observed 3 to 6 h after the first drug delivery (low-dose MPA (20 mg/ml); 35.6 and 33.5 ng/ml at 3 and 6 h, *vs.* high dose MPA (80 mg/ml); 61.3 and 57.3 ng/ml, respectively. A steep decrease occurred after 24 h, and, 7 days after the first drug delivery, before the second injection, methylprednisolone levels were very low but detectable (low dose MPA; 5.5 ng/ml and high dose MPA; 12.3 ng/ml). The area under the curve for MPA 20 mg/ml was 1,790, for MPA 80 mg/ml 5,227 ($P < 0.001$) (fig. 3). Methylprednisolone levels remained detectable up to 2 weeks after the last drug delivery (fig. 4).

Laboratory Results

All dogs showed normal plasma and urine laboratory results (complete blood count, kidney and liver function, creatinine/cortisol ratio) before surgery. At acute and long-term sacrifice, normal plasma and urine laboratory results were again observed in almost all dogs. Only alkaline phosphatase increased to 328 U/l (normal values: 10–150 U/l) in one acutely sacrificed dog, and leukocyte number was decreased in two acutely sacrificed dogs to 5.6 and 4.9 (normal value; 6.0–17.0 $10^3/\mu l$), all treated with the high-dose MPA.

The cisternal CSF samples showed abnormalities in all dogs, but the severity of the deviation was clearly dose-dependent. Increased nuclear cell count and protein levels were observed, with the highest values in dogs treated with high-dose MPA. At acute sacrifice the elevations in nuclear cell count (normal value: 0–5 cells/μl, vehicle: 27 and 180 cells/μl, low-dose MPA: 310 cells/μl, and high-dose MPA: 1,210 cells/μl) were more prominent than at long-term sacrifice (vehicle: 2 and 10 cells/μl, low-dose MPA: 8, 21, and 22 cells/μl, and high-dose MPA: 193, 299, and 308 cells/μl).

CSF protein levels were also highest at acute sacrifice in dogs treated with high-dose MPA (vehicle: 34.4 and 47.4 mg/dl, low-dose MPA: 41.5, and high-dose MPA: 142.7 mg/dl) compared with levels at long-term sacrifice (vehicle: 20.3 and 21.8 mg/dl, low-dose MPA: 24.3, 38.9, and 44.5 mg/dl, and high-dose MPA: 52.1, 54.2, and 68.9 mg/dl).

CSF glucose levels decreased with increasing dose and were lowest at long-term sacrifice (acute sacrifice; vehicle: 64 and 65 mg/dl, low-dose MPA: 66, and high-dose MPA: 59 *vs.* long-term sacrifice vehicle: 65 and 72 mg/dl, low-dose MPA: 64, 68, and 68, and high-dose MPA: 54, 55, and 58) suggesting the presence of an intrathecal inflammatory process.

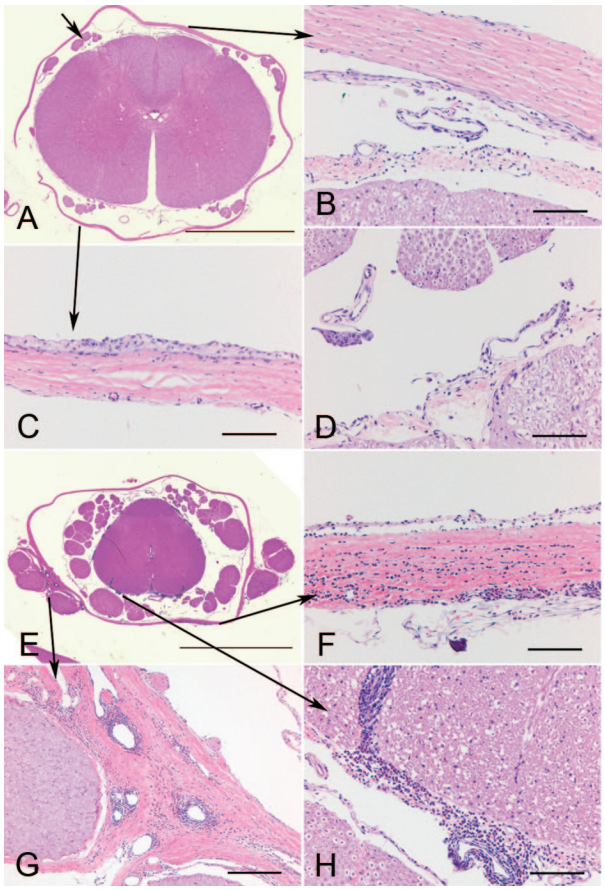

Fig. 8. Methylprednisolone acetate 20 mg/ml, acute sacrifice. (*A*) Mild leptomeningeal inflammation (*bar* = 3 mm). *Long arrows* indicate regions shown at higher power in *B* and *C*. *Short arrow* indicates region shown at higher power in *D*. (*B*) Mild perivascular/arachnoid inflammation. (*C*) Macrophages on inner surface of dura. (*D*) Mild leptomeningeal inflammation and focal cluster of macrophages. *B–D*, *bar* = 100 μm. (*E*) Moderate inflammatory infiltrates in dura, arachnoid, and Virchow-Robin spaces (*bar* = 3 mm). *Arrows* indicate regions shown at higher power in *F–H*. (*F*) Inflammatory infiltrates through full thickness of dura (*bar* = 100 μm). (*G*) Perivascular inflammatory infiltrates in dura (*bar* = 200 μm). (*H*) Inflammatory infiltrates in arachnoid, extending into Virchow-Robin spaces in spinal cord (*bar* = 100 μm).

Pathology

At necropsy, acutely sacrificed animals reliably displayed a white, acellular, solid deposit in the intrathecal space at or below the catheter tip on the spinal cord in all drug-treated animals. This deposit typically extended a distance of 1–3 cm in length and was believed to be MPA (fig. 5). At long-term sacrifice, such deposits were observed in four of six dogs. No other visibly evident pathologic signs were observed in any animal at acute or long-term necropsy.

Histopathology

Vehicle-treated animals showed minimal changes in dura and arachnoid, with mild infiltrates of lymphocytes, plasma cells, and macrophages at acute sacrifice and focal fibrosis and thickening of dura with minimal arachnoid inflammation at long-term sacrifice. No spinal cord changes were seen in vehicle-treated animals. The median total histology score for the vehicle-treated animals was 1.3 at acute and 1.0 at long-term sacrifice (figs. 6 and 7). Low-dose MPA-treated animals at both acute and long-term sacrifice had a dural reaction that consisted of a diffuse infiltrate of macrophages along the inner surface of the dura, with variable numbers of lymphocytes, plasma cells, and neutrophils in dura and arachnoid. The median total histology score was 2.0 at acute and 3.0 at long-term sacrifice (figs. 8 and 9). High-dose MPA-treated animals had more severe inflammatory responses, with large inflammatory masses (one with neutrophil aggregates in the center, suggesting abscess formation) on the inner surface of the dura and in arachnoid in two of three animals at each survival time. The median total histology score was 4.0 at acute and 7.0 at long-term sacrifice (figs. 10 and 11). The severity of the inflammatory reaction expressed as the total histology score, increased significantly with increasing dose at long-term sacrifice (acute $P = 0.167$, long-term $P = 0.014$).

Spinal cord pathology consisted of focal aggregates of activated microglia (in one of four animals of the low-dose MPA group at acute sacrifice, one of three animals in the high-dose MPA group at acute sacrifice and all three animals of the high-dose MPA group at long-term sacrifice) and focal, mild inflammation (in one of three animals of the low-dose MPA group at long-term sacrifice). (table 1) No evidence of neuronal injury (by hematoxylin and eosin or FluoroJade C stains), demyelination, or gliosis was seen in any animal. Specific examination of the adjacent nerve roots revealed no signs of Schwann cell injury or demyelination.

Discussion

This study aimed to provide a systematic assessment of the safety of repeated intrathecal administration of reformulated MPA, delivered in doses comparable to those reported to be used in humans. The model, the chronically catheterized canine, has been widely used for defining the potential spinal toxicity and kinetics of a large number of spinal agents.[11–14] In short, in the current studies using this model, we observed a dose-dependent inflammatory reaction proximal to the lumbar catheter delivery site. Issues pertinent to the interpretation of this observation are considered in the next paragraphs.

Acute Tolerability of Intrathecal MPA

Repeated intrathecal injections of vehicle or MPA solutions in the dog were well tolerated and did not have any deleterious effects upon neurologic function. The absence of effect upon an acute thermal threshold other than the acute block associated with the action of the lidocaine is not unexpected, because no anesthetic or acute analgesic properties have been ascribed to steroids after intrathecal delivery. Similarly, the absence of any reaction upon injection in the unanesthetized

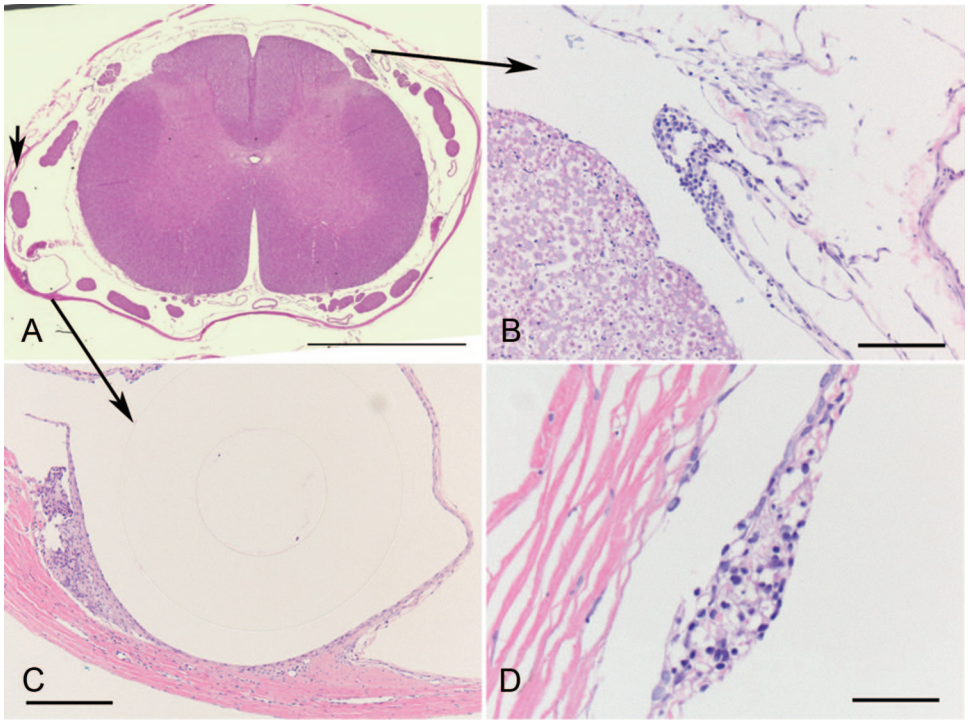

Fig. 9. Methylprednisolone acetate 20 mg/ml, long-term sacrifice. (*A*) Minimal inflammatory infiltrates and fibrosis in arachnoid and surrounding catheter site (*bar* = 3 mm). *Long arrows* indicate regions shown at higher power in *B* and *C*. *Short arrow* indicates region shown at higher power in *D*. (*B*) Mild arachnoid inflammation away from catheter site (*bar* = 100 μm). (*C*) Mild fibrosis and chronic inflammation around catheter (*bar* = 200 μm). (*D*) Focal aggregate of macrophages on inner surface of dura (*bar* = 50 μm).

animal or any change in the thermal escape threshold suggested no proalgesic action.

Pharmacokinetics of Intrathecal MPA

In dogs, peak methylprednisolone plasma concentrations were observed between 3 and 6 h after intrathecal MPA injection. Although plasma levels decreased after 6 h, methylprednisolone was still measurable after 7 days but went below the detection threshold 3 weeks after the last injection.

In humans, a similar timeframe was described; after 80 mg MPA, intrathecal peak plasma and CSF levels were observed after 1 day and were measurable for at least 2 weeks.[15] After intraarticular injection of MPA, methylprednisolone plasma levels decreased below the detection level after 24 h, much shorter than observed after intrathecal administration and likely suggesting more rapid clearance through lymphatic drainage.[16]

Twenty-four hours after intraarticular administration, when methylprednisolone plasma levels were undetectable, postmortem examinations showed a significant quantity of white material believed to be MPA precipitated at the bottom of the synovial cavity. In the current study, similar white deposits were observed 1 week after the last intrathecal delivery, when methylprednisolone plasma levels were still measurable. Although the deposits were not chemically identified, it seems likely based on the comparable results observed in joints that these deposits were methylprednisolone. Accordingly, it is probable that these white deposits were present in the intrathecal space for a longer period of time, a possible explaining cofactor for the inflammatory process observed at 6 weeks after the last intrathecal injection.

Histopathologic Effects of Intrathecal MPA

Despite the absence of clinical symptoms, there were evident histologic signs of inflammation in all drug-treated animals. An inflammatory meningeal reaction was seen, one accompanied with inflammatory masses suggesting abscess formation, the other with granuloma formation. In the spinal cord, focal aggregates of activated microglia were observed, but there was no evidence of neuronal injury or demyelination in adjacent nerve roots. These physical observations were in addition accompanied by increased protein in the CSF that appeared to be most evident in animals sacrificed within 7 days of the last injection.

This work thus reveals an inflammatory response in the intrathecal space after repeated administration of an antiinflammatory drug after the removal of its preservatives. Importantly, this effect was not seen in the vehicle-treated animals, emphasizing that the results were not secondary to either the chronic polyurethane catheter or to the vehicle (2% lidocaine). Although lidocaine has been previously reported to produce signs of demyelination, no evidence of

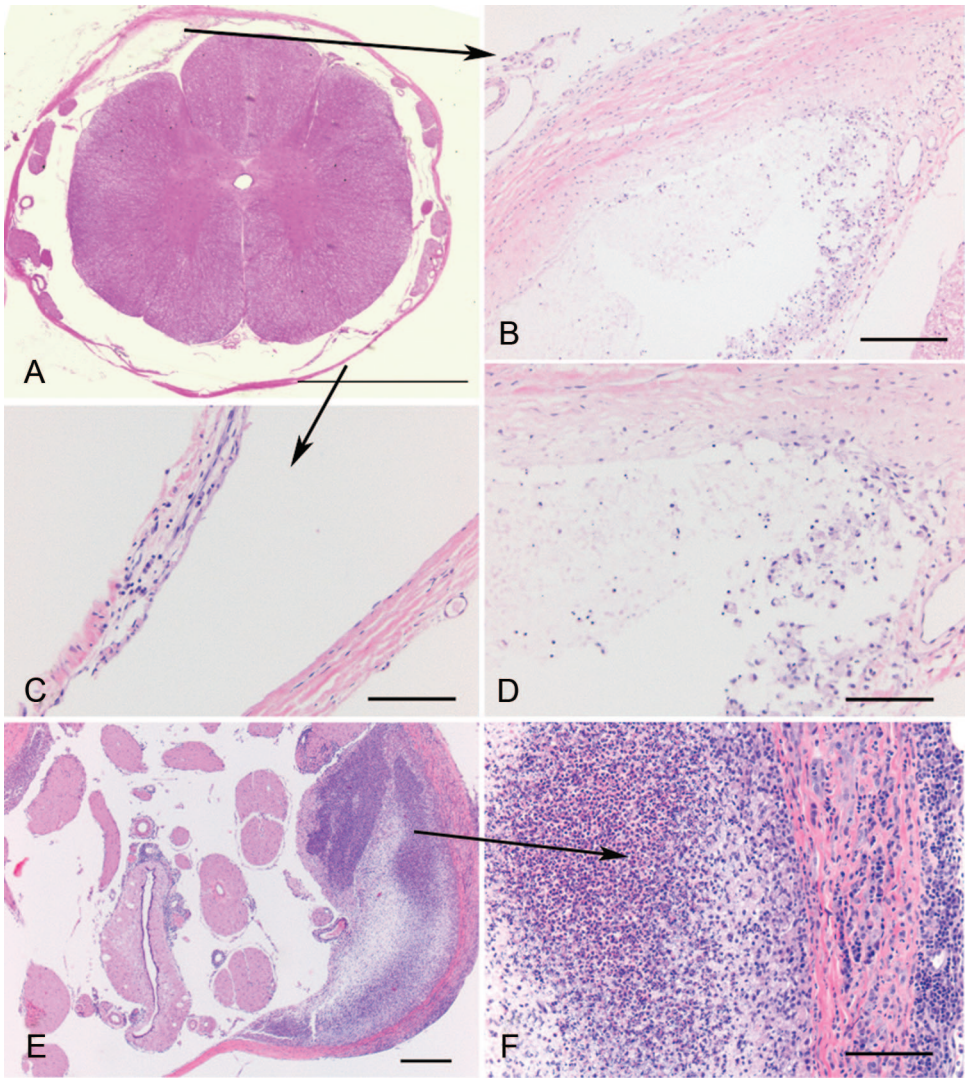

Fig. 10. Methylprednisolone acetate 80 mg/ml, acute sacrifice. (*A*) Focal subdural aggregates of macrophages surrounding foreign material (*bar* = 3 mm). *Arrows* indicate regions shown at higher power in *B* and *C*. (*B*) Subdural aggregates of macrophages surrounding foreign material (*bar* = 200 μm). (*C*) Mild arachnoid inflammatory infiltrates (*bar* = 100 μm). (*D*) Higher power of region shown in *B* (*bar* = 100 μm). (*E*) Caudal sacral section demonstrating severe inflammation in dura and arachnoid, with large inflammatory mass (*bar* = 300 μm). *Arrow* indicates region shown at higher power in *F*. (*F*) Higher power of inflammatory mass, with inflammation in dura and arachnoid; necrotic center with neutrophils resembling an abscess (*bar* = 100 μm).

such untoward signs were observed in the current study with the brief exposures and low concentrations used.[17,18]

The appearance of inflammatory responses described as chemical meningitis, transverse myelitis, and adhesive arachnoïditis have been observed in patients after intrathecal administration of MPA.[1,7] Unlike in the current study, however, these previous reports have used a MPA formulation that included the preservative myristylgamma-picolinium chloride upon which the untoward reaction was attributed. In addition, in animal studies, intrathecal MPA and other steroids have been shown to cause an inflammatory response.[8,9,19,20] Accordingly, we believe the current study points out that the intrathecal delivery of MPA, a particulate suspension, has a role itself in the inflammatory response.

The MPA Study Formulation

In the current study we attempted to obtain a preservative-free MPA formulation by centrifuging and resuspending the general formulation of MPA. To be able to truly assess the test article used by Kotani *et al.*[2] and which was to be used in a replication RCT in the Netherlands, we decided not to construct the test article from pure compound. Our drug analysis showed that less than 0.025 mg/ml myristylgamma-picolinium chloride (0.36 mg/ml in general formulation) remained in the test article. Myristylgamma-picolinium chloride, a bacteriostatic agent, is known to be neurotoxic.[21] In concentrations of approximately 0.4 mg/ml it has been shown to cause retinal damage in a rabbit eye.[21] The concentration measured in our drug assays was less than 0.025 mg/ml, nearly 20-fold lower. To the best of our knowledge there

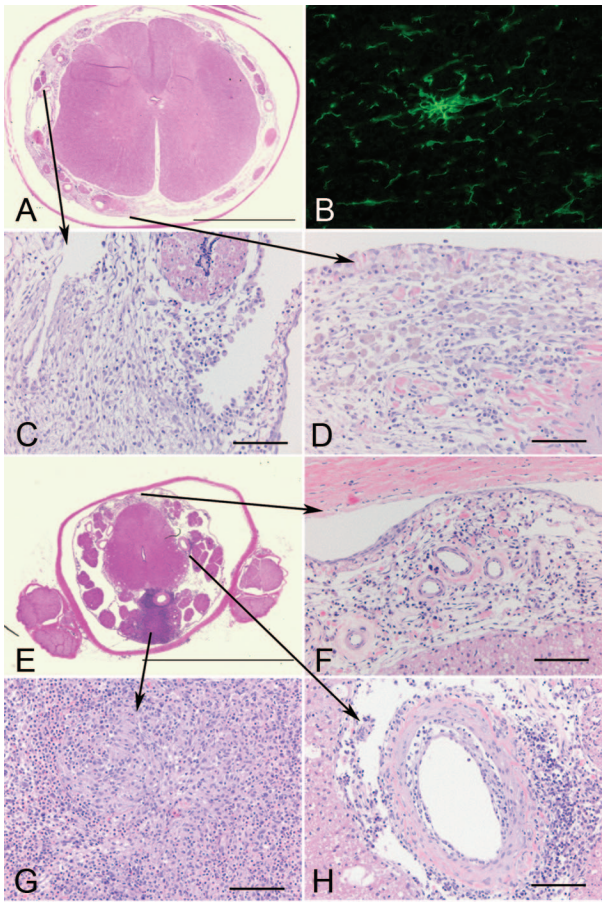

Fig. 11. Methylprednisolone acetate 80 mg/ml, long-term sacrifice. (*A*) Diffuse inflammatory infiltrates and fibrosis in arachnoid. *Arrows* indicate regions shown at higher power in *C* and *D* (*bar* = 3 mm). (*B*) Immunofluorescent staining for Iba1 (*green*). The large cell in the center is an activated microglia. There are multiple smaller resting microglia in the surrounding tissue. (*C*) Fibrosis and macrophage infiltration in arachnoid and inflammatory cell infiltration of a nerve root (*bar* = 100 μm). (*D*) Focal aggregate of pigment-laden macrophages in arachnoid (*bar* = 100 μm). (*E*) Large arachnoidal inflammatory mass. *Arrows* indicate regions shown at higher power in *F–H* (*bar* = 3 mm). (*F*) Mixed inflammatory infiltrate away from inflammatory mass. (*G*) Granuloma formation with epithelioid macrophages within inflammatory mass. (*H*) Perivascular inflammation and vasculitis. *B–D*, *bar* = 100 μm.

are no studies available testing the neurotoxicity of myristylgamma-picolinium chloride in concentrations less than 0.025 mg/ml. Although we cannot completely rule out that this low concentration contributed to the toxicity findings in the current study, we believe that the extent of the toxicity findings are not likely to be caused solely by the small concentrations of myristylgamma-picolinium chloride.

Polyethylene glycol has recently been studied in spinal cord injury models because it is reported to promote the restoration of functional and structural integrity of nerve tissue by direct application on the spinal cord.[22] In high concentrations, its prolonged focal application may induce a conduction block[23] but no inflammatory responses or neurotoxicity have been observed. In addition, polyethylene glycol is used as a spinal sealant after dural repair during neurosurgery in humans. No neurotoxicity after use of the polyethylene glycol sealant was reported.[24] Thus, although we are aware of no specific assessments of the long-term effects of intrathecal polyethylene glycol, these results suggest that polyethylene glycol does not possess evident signs of toxicity in the models thus far examined at the concentrations used in these studies.

Origins of Observed Toxicity

The observation of the intrathecal inflammatory reactions was surprising given the touted role of steroids as antiinflammatory agents. Specific studies on the mechanisms of this observed toxicity were not undertaken. In the current study, we would raise the possibility that the particulate nature of the formulation itself might account for some component of the inflammatory response. Two issues may be considered regarding mechanisms underlying this inflammatory reaction.

First, steroids acting through intracellular linkages have historically been appreciated to suppress the inflammatory response through an effect on several signaling cascades such as that for nuclear factor κ B and to accordingly diminish the release of proinflammatory and proalgesic cytokines, such as tumor necrosis factor and interleukin-8.[25] However, there are data suggesting that steroids may, under certain proinflammatory conditions, enhance the release of chemoattractants such as interleukin-8 and produce an increased transcription of adhesion molecules such as intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, agents known to mediate inflammatory cell migration.[26] The observations here suggest that the depot formulation has that proinflammatory action. This possibility is supported by the appearance of inflammatory cells in the mass perimeter. Given that soluble steroids do not initiate such migratory activity[27] or cytokine release, the physical nature of the MPA particle may contribute to this effect.

This leads to the second possibility, which is that the inflammatory reaction reflects the presence of the MPA particles. Systematic examination of MPA revealed that between 30–40% of the particles observed in MPA solution were larger than 20 μm in diameter.[28,29] An extensive literature search emphasizes that in other systems such as lung, particulate materials such as carbon particles as well as other environmental toxins including nanoparticles can initiate an activation of cytokine release that in turn leads to an activation of a variety of cell adhesion factors leading to macrophage and neutrophil migration.[30–32] Thus, in a number of different study systems, particulates have been shown to drive robust inflammatory reactions.[33–36] Whether this phenomenon is observed with nonparticulate steroids is not known and may reflect a peculiarity of the steroid effects with the stimulus provided by the particulate aggregation.

The role of such spinal particulates has yet to be systematically studied, but the current results suggest the speculative hypothesis that particulates in the intrathecal space may

Table 1. Overview of Histopathology Results

Treatment	Survival	N	Dura Scores	Arachnoid Scores	Spinal Cord Scores	Total Histology Score
Vehicle	1 wk	2	0/0	0.5/2	0/0	0.5/2
Vehicle	6 wk	2	0.5/1	0/0.5	0/0	0.5/1.5
MPA 20 mg/ml	1 wk	4	1/4/1/1	0.5/3/1/1	0/1/0/0	1.5/8/2/2
MPA 20 mg/ml	6 wk	3	1/1/2	0.5/1/1	0/1/0	1.5/3/3
MPA 80 mg/ml	1 wk	3	2/1/3	2/2/4	0/0/1	4/3/8
MPA 80 mg/ml	6 wk	3	2/4/2	4/4/2	1/1/1	7/9/5

The histopathology scores are based on the lumbar and sacral sections. Dura, arachnoid, and spinal cord are examined for the presence, location, and type of inflammatory reaction, including inflammatory cell infiltrates, granulation tissue, and fibrosis. Scoring system from 0 to 4: 0 being no inflammatory response and 4 being the maximal response observed in this cohort. Total histology score was the sum of the scores for dura, arachnoid, and spinal cord (possible score of 0–12).

MPA = methylprednisolone acetate; N = Number of dogs; wk = weeks after the last intrathecal MPA dose.

promote inflammatory cell migration. Further studies on this intriguing hypothesis are clearly warranted as they raise the question of whether particulate formulations in general may be contraindicated in the intrathecal space.

Conclusions

This preclinical study was performed to provide supporting information on a parallel pilot study of a RCT studying the efficacy of intrathecal MPA in patients suffering from PHN. That study was designed to verify the results of a previous large RCT.[2] Importantly, however, the current results suggest an untoward effect of intrathecal MPA and do not support performing additional human studies with even this reformulated material having minimal additives. This study shows again that the intrathecal implementation of a novel therapy requires robust preclinical safety assessment in well-validated models.[37,38]

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